Helen L. Henry

University of California, Riverside

Helen L. Henry received her Ph.D. in 1970 from Washington University, St. Louis, and did postdoctoral work in animal reproduction at Ohio State University. Following further postdoctoral work at the University of California, Riverside, she joined the faculty and is currently a professor of biochemistry. From 1990 to 1996, she served as Associate Dean of Biological Sciences in the College of Natural and Agricultural Sciences.

Dr. Henry's laboratory has made major contributions to the understanding of vitamin D metabolism, particularly regulation of the production of the active vitamin D hormone by the kidney. She pioneered the use of cell culture systems to study renal vitamin D metabolism. A related area of research focus is the regulation of gene expression in the kidney by 1,25-dihydroxyvitamin D_3. In addition to peer-reviewed research articles, Dr. Henry has authored chapters for the books *Vitamin D* and *Handbook of Physiology*, as well as the article "Vitamin D Metabolism" for this encyclopedia.

Dr. Henry was awarded a National Institutes of Health (NIH) Research Career Development Award in 1977. She received the Fuller Albright Award from the American Society for Bone and Mineral Research in 1984 and has served this society as a member of the council and on several scientific program committees. Dr. Henry has been a member of the NIH General Medicine B Study Section (1989–1993) and the National Science Foundation Panel on Integrative Biology (1994–1996). She has served on the editorial boards of several scientific journals, including *Endocrinology*, *American Journal of Physiology*, and *Journal of Bone and Mineral Research*. She is currently a member of the Endocrine Society, the American Society of Biochemistry and Molecular Biology, the American Society for Bone and Mineral Research, and the American Institute of Nutritional Sciences.

Anthony W. Norman

University of California, Riverside

Anthony W. Norman received an A.B. from Oberlin College in 1959, and an M.S. and Ph.D. in biochemistry in 1961 and 1963, respectively, from the University of Wisconsin, Madison. Following postdoctoral work in Paul D. Boyer's group at UCLA, in 1964 he joined the Department of Biochemistry at the University of California, Riverside, as an assistant professor. From 1976 to 1981, he served as chair of the department and currently holds a Presidential Chair and is a Distinguished Professor of Biochemistry and Biomedical Sciences. Dr. Norman has also been active for some 25 years in medical education on the UC Riverside campus and at UCLA through participation in the UC Riverside/UCLA Program in Biomedical Sciences, of which he was Dean and Director from 1986 to 1991.

Dr. Norman's biomedical research career has focused on the mechanism of action of the vitamin D family of steroids. His chief contributions to these areas of cellular and molecular endocrinology have played a pivotal role in defining the boundaries of this research domain via discoveries that have opened new areas of investigation. The first of these was the discovery in 1968, and chemical characterization in 1971, of the hormonally active form of vitamin D, $1\alpha,25(OH)_2$-vitamin D_3. Subsequent achievements include the discovery and characterization of the nuclear receptor for $1\alpha,25(OH)_2D_3$, the clinical evaluation of $1\alpha,25(OH)_2D_3$ in renal osteodystrophy, articulation of the concept of the vitamin D endocrine system, the importance of $1\alpha,25(OH)_2D_3$ to insulin secretion, and the discovery of a new rapid, nongenomic, signal transduction process for $1\alpha,25(OH)_2D_3$.

Dr. Norman has been the recipient of awards that include a Fulbright Fellowship, 1970; Public Health Service Career Development Award, 1970; Mead Johnson Award, American Institute of Nutrition, 1977; Ernst Oppenheimer Award, Endocrine Society, 1977; Visiting Lecturer, Australian Society of Endocrinology, 1978; Visiting Faculty Member, Mayo Clinic, 1981; Prix Andre Lichtwitz (INSERM, Paris, France), 1981; Faculty Research Lecturer, UC Riverside, 1982; MERIT Award from National Institutes of Health, 1986; David Curnow Plenary Lecturer, Australian Society for Clinical Biochemistry, 1989; Osborne and Mendel Award, American Institute of Nutrition, 1990; Visiting Professor, Catholic University of Leuven, Belgium, 1992; William F. Neuman Award, American Society for Bone and Mineral Research, 1995; Fellow of American Association for the Advancement of Science, 1995; and Visiting Professor, Department of Biochemistry, UC San Francisco/Presidential Chair in Biochemistry, UC Riverside, 1999.

Antony W. Burgess

Ludwig Institute for Cancer Research, Melbourne, Australia

Antony W. Burgess has had an interest in protein chemistry since his early studies on the conformational determinants of peptides and proteins. He received a Ph.D. at the University of Melbourne; after postdoctoral studies at Cornell University and the Weizmann Institute between 1972 and 1974, Dr. Burgess returned to Australia, to the Walter and Eliza Hall Institute, to study the growth factors that stimulate blood cell formation. He and his colleagues purified the first colony-stimulating factor (CSF) and discovered growth and differentiation factors controlling the development of blood cells. This work led to the molecular cloning of a CSF and the initial clinical studies with recombinant forms of these factors.

Dr. Burgess was involved in the detection and biology of the cell surface receptors for the CSFs before taking up studies on the epidermal growth factor (EGF)/receptor system. These studies have contributed to the understanding of the three-dimensional structure of EGF and its receptor, as well as the mechanisms associated with signal transduction from the different EGF receptor complexes. At present, Dr. Burgess is attempting to develop improved approaches to cancer therapy through the use of EGF receptor inhibitors. He has an active research program on the molecular basis of colon cancer, in particular the biology and molecular biology of the apc protein.

Presently on staff at the Ludwig Institute for Cancer Research in Melbourne, Dr. Burgess is a committee member of the ARC Selection Committee for Centres of Excellence, a past president of the Australian Society for Biochemistry and Molecular Biology, a former chairperson of the Board of the Biomolecular Research Institute, a former board member of the International Society of Differentiation, and a former World Committee member of the Society for Research into Comparative Leukemic and Associated Diseases. He has been awarded honors that include the Australian Academy of Science Gottschalk Medal, 1981; Australian Academy of Science Fellow, 1993; the Amgen Prize, 1996; Companion of the Order of Australia, 1998; and the MOG/AMRAD Cancer Achievement Award, 1999.

P. Michael Conn

Oregon National Primate Research Center, Beaverton, Oregon

P. Michael Conn is Associate Director and Senior Scientist of the Oregon National Primate Research Center and Special Assistant to the President and Professor of Physiology and Pharmacology at Oregon Health and Science University. After receiving a B.S. and teaching certification from the University of Michigan in 1971, an M.S. from North Carolina State University in 1973, and a Ph.D. from Baylor College of Medicine in 1976, Dr. Conn did a fellowship at the NIH/National Institute of Child Health and Human Development. He then joined the faculty in the Department of Pharmacology at Duke University Medical Center, where he was promoted to Associate Professor in 1982. In 1984, he became Professor and Head of Pharmacology at University of Iowa College of Medicine, a position he held for 11 years.

Dr. Conn is presently Editor-in-Chief of *Endocrine, Methods*, *Contemporary Endocrinology,* and *Contemporary Drug Therapy*; prior Editor-in-Chief of *Endocrinology*, *Molecular and Cellular Neurosciences*, *Methods in Neuroscience,* and *Recent Progress in Hormone Research*; and prior Editor of *Journal of Clinical Endocrinology and Metabolism*. He has edited texts in the fields of pharmacology (*Essentials of Pharmacology*), neuroscience (*Neuroscience in Medicine*), neuroendocrinology (*Neuroendocrinology in Physiology and Medicine*), endocrinology (*Endocrinology: Basic and Clinical Principles*), and molecular endocrinology (*Principles of Molecular Regulation),* as well as more than 100 volumes in endocrinology and neuroscience.

Best known for his research in the area of neuroendocrinology, Dr. Conn has focused on the cellular basis of action of gonadotropin-releasing hormone action in the pituitary and central nervous system. He has authored or coauthored nearly 300 publications in this area. The work of his laboratory has been recognized with the MERIT Award from the NIH, the J.J. Abel Award of the American Society for Pharmacology and Experimental Therapeutics, the Weitzman and Oppenheimer awards of the Endocrine Society, the National Science Medal of Mexico (the Miguel Aleman Prize), and the Stevenson Award of Canada. Dr. Conn has served on the National Board of Medical Examiners, including two years as

Chairman of the Reproduction and Endocrinology Committee, and is a previous member of council for the American Society for Cell Biology and a past president of the Endocrine Society. Conn is a member of the Mexican Institute of Medicine and an honorary investigator of the Mexican Institute of Social Security.

George H. Greeley, Jr.

University of Texas Medical Branch, Galveston, Texas

George H. Greeley, Jr. earned his Ph.D. in endocrinology at the Medical College of Georgia in 1974 and was recently recognized by the school as alumni of the year. Dr. Greeley did postdoctoral training in neuroendocrinology at the University of North Carolina in Chapel Hill. Presently, he directs an internationally recognized research program in physiology of gastrointestinal hormones in the Department of Surgery at the University of Texas Medical Branch in Galveston.

Dr. Greeley has authored or coauthored more than 190 peer-reviewed journal articles and serves on the editorial boards of *Endocrinology*, *American Journal of Physiology*, and *Regulatory Peptides*. His primary areas of research include feedback mechanisms underlying regulation of gut hormone secretion, a new stomach hormone called ghrelin, and luminal regulation of cholecystokinin (CCK) secretion. Honors awarded Dr. Greeley include the NATO Collaborative Research Grant, 1989 to present; Invited Participant, Second Galveston International Symposium, Galveston, Texas, 1989; Invited Participant, International Conference of Gut Hormones, Shizuoka, Japan, 1993; Mentor in the APS–NIDDK Travel Fellowship Program for minority students, Experimental Biology Meetings, 1995, 1996; and Mentor, American Gastroenterological Association, Endocrine Society, Summer Student Research Fellowship Recipients, 1995–1998.

Martin J. Kelly

Oregon Health and Science University, Portland, Oregon

Martin J. Kelly is a professor of physiology and pharmacology at Oregon Health and Sciences University (OHSU). He obtained his Ph.D. in 1976 from the University of Texas Southwestern Medical School in Dallas and did his postdoctoral training at the Max Planck Institute for Biophysical Chemistry (1976–1979). In 1980, Dr. Kelly joined the Department of Physiology at the University of Pittsburgh School of Medicine and moved to OHSU in 1982. His area of research is the cellular neurophysiology and neuropharmacology of hypothalamic neurons that control neuroendocrine functions, motivation, and reward in the female. Dr. Kelly has been the recipient of two research scientist development awards from the NIH, the Research Career Development Award from the National Institute of Child Health and Human Development (1987–1992), and the Research Scientist Development Award from the National Institute on Drug Abuse (1994–1999).

Dr. Kelly was the first to demonstrate rapid signaling of estrogen in the central nervous system and to show how it alters reproductive function in the female. In particular, he found that 17β-estradiol can directly alter the excitability of gonadotropin hormone-releasing hormone (GnRH) neurons, which are critical for the control of female reproduction. He also found that the rapid activation by estrogens of protein kinase activity in hypothalamic opioid and dopamine neurons alters the coupling of neurotransmitter receptors to their effector systems (e.g., channels) in the female. These results have significant ramifications in terms of stress responses, appetite control, fluid balance, and motivated behaviors, and may explain many of the gender differences in these functions.

Dr. Kelly and colleagues are also interested in characterizing the membrane properties of proopiomelanocortin (POMC), dopamine, and GnRH neurons in males and females, and the effects of neurotransmitters on these neurons. Using guinea pigs and transgenic mice as models, they are trying to identify the phosphorylated target proteins that are altered by neurotransmitters and steroids in hypothalamic neurons that are critical for the control of neuroendocrine functions, motivation, and reward in the female. Dr. Kelly's recent manuscripts include "Rapid Actions of Plasma Membrane Estrogen Receptors" with E. R. Levin in *Trends in Endocrinology and Metabolism* and "Rapid Membrane Effects of Estrogen in the CNS" with O. K. Rønnekleiv in the Academic Press book *Hormones, Brain and Behavior.*

Paul B. Larsen

University of California, Riverside

Paul B. Larsen received his Ph.D. from Purdue University in 1994, did postdoctoral work at Cornell University (1994–1997) and at the University of

Maryland (1997–2000), where he was also a U.S. Department of Agriculture Fellow. Dr. Larsen is presently an assistant professor of biochemistry in the College of Natural and Agricultural Sciences at the University of California, Riverside.

Dr. Larsen's laboratory focuses on two topics of plant biology. The first deals with the elucidation of the mechanism responsible for the signal transduction of ethylene, a plant hormone that regulates many physiological processes throughout plant growth and developement. Using *Arabidopsis thaliana* as a model genetic system, he has been responsible for identification of novel components of the ethylene signaling pathway, an approach that may ultimately give the means to better control such ethylene-regulated processes as fruit ripening, tissue senescence, and induction of pathogen defenses. Additionally, Dr. Larsen's group is exploring the mechanisms that plants utilize to cope with abiotic stress, particularly that of aluminum toxicity in acid soil. Aluminum toxicity in acid soils is a global problem limiting crop productivity for more than 30% of agriculturally available land. As for ethylene signaling, Dr. Larsen is also using *Arabidopsis* as a model system for identification of genes that are required for plant growth in aluminum toxic environments.

Dr. Larsen has presented papers in his areas of expertise at prestigious seminars at Cambridge University and at the American Society of Plant Physiologists and has authored or coauthored a number of articles published in scientific journals such as *Plant Physiology*, *Plant Molecular Biology*, and the *Proceedings of the National Academy of Science*. He also authored the article, "Ethylene" in this *Encyclopedia of Hormones*.

Warren J. Leonard

National Institutes of Health, Bethesda, Maryland

Warren J. Leonard is Chief, Laboratory of Molecular Immunology, at the National Heart, Lung, and Blood Institute of the National Institutes of Health. Dr. Leonard was a pioneer in the interleukin-2 field, having cloned the IL-2 receptor α chain in 1983. Dr. Leonard has published more than 200 articles and reviews, most of which are related to the IL-2 family of cytokines. His main research focus relates to IL-2 and other cytokines with receptors that contain the common cytokine receptor γ chain, γc, which Dr. Leonard's group demonstrated is the protein that is mutated in X-linked severe combined immunodeficiency. He demonstrated that this is a disease of defective cytokine signaling and that mutations in the Janus family tyrosine kinase, Jak3, which associates with γc, cause a similar clinical phenotype. Most of his research focuses on signal transduction and gene regulation related to γc-dependent cytokines.

Dr. Leonard is currently Vice President and President-elect of the International Cytokine Society, a member of the American Association of Immunologists, American Society for Clinical Investigation, and the American Association of Physicians, and a fellow of the American Association for the Advancement of Science. He is a current or past member of major editorial boards, including *Immunity*, *Journal of Immunology*, *Journal of Biological Chemistry*, and *Cytokine*. He has helped organize major international meetings in the cytokine field, including a Keystone Symposium and an annual meeting of the International Cytokine Society. Dr. Leonard has received a number of major awards, including the Outstanding Investigator Award of the American Federation for Clinical Research Foundation. (Note that Dr. Leonard's contributions to this book were performed in his private capacity, and the contents of this book do not necessarily reflect the views of NIH.)

Gerald Litwack

Thomas Jefferson University College of Medicine, Philadelphia, Pennsylvania

Gerald Litwack obtained his Ph.D. in biochemistry from the University of Wisconsin in 1953. After a postdoctoral year at the Biochemical Laboratories of the Sorbonne in Paris, he spent the early part of his academic career at Rutgers University and the University of Pennsylvania, where he was the recipient of a National Institutes of Health Career Development award. In 1964, Dr. Litwack became Professor of Biochemistry at the Fels Institute for Cancer Research and Molecular Biology at Temple University School of Medicine. In the 1980s, he became Deputy Director of the Institute and Laura H. Carnell Professor of Biochemistry and received the Faculty Research Award of Temple University.

In 1991, Dr. Litwack moved to the Jefferson Medical College as Chairman of the Department of Pharmacology and Deputy Director of the Jefferson Cancer Institute (now the Kimmel Cancer Institute), an appointment he continued when, in 1996, he became Chair of the newly fused Department of Biochemistry and Molecular Pharmacology and Associate Dean for Scientific Affairs. In 2000, he was appointed Vice Dean for Research.

Dr. Litwack's research has centered on regulation, particularly by hormones, and, during the past 15 years, the mechanism and regulation of apoptosis. The Litwack laboratory group characterized the mammalian glucocorticoid receptor and co-discovered "ligandin," subsequently found to be the glutathione *S*-transferase family of enzymes. Dr. Litwack has published more than 300 papers in these areas and is a co-discoverer on several patents covering many of the caspases in the apoptotic cascade. His service includes participation on scientific advisory boards (the Diabetes Center of the University of Pennsylvania) and on grant review panels of the National Science Foundation, National Institutes of Health, U.S. Army, Israel Cancer Research Fund, and American Cancer Society. His editorial board service includes *Endocrinology*, *Cancer Research*, *Anticancer Research*, *Oncology Research*, *Proceedings of Experimental Biology and Medicine*, *Journal of Nutrition, Growth and Cancer*, *ISI Atlas of Science*, *Cancer Communications*, *Chemtracts*, *Oncology Reports*, *Critical Reviews in Eukaryotic Gene Expression*, and *Apoptosis*.

The books and publications Dr. Litwack has authored, coauthored, or edited include *Experimental Biochemistry* (1960, John Wiley & Sons), *Biochemical Actions of Hormones* (1970–1987, Academic Press, a serial in 14 volumes), *Actions of Hormones on Molecular Processes* (1964, Academic Press), *Receptor Purification* (1989–1990, Humana Press), *Receptor* (renamed *Receptors & Signal Transduction*; 1990–1998, a journal founded by G. Litwack and Editor-in-Chief, Humana Press), *Hormones* (1987, 1997, Academic Press), and *Vitamins and Hormones* (Academic Press, the publisher's longest running serial).

Alexander S. Raikhel

University of California, Riverside

Alexander S. Raikhel is Professor at the University of California, Riverside. He received his Ph.D. in 1975 from the Zoological Institute of the Academy of Science in St. Petersburg, Russia. Dr. Raikhel is the leading authority in molecular endocrinology of insects. His research focuses on endocrine control of insect reproduction. In particular, he and his collaborators have elucidated a complex network of nuclear receptors that mediate the action of a steroid hormone ecdysone in gene activation and repression during egg maturation in mosquitoes.

Dr. Raikhel is the author of more than 100 research papers and reviews on molecular aspects of insect reproduction. He has served as a member of the World Health Organization Advisory Committee and has organized several symposia on the topic of insect reproduction and endocrinology, including several Keystone Symposia in Molecular Insect Science. He is the recipient of numerous awards for his research achievements. Dr. Raikhel is Editor-in-Chief of *Insect Biochemistry and Molecular Biology* and is a member of the editorial board of *Annual Reviews of Entomology*.

R. Paul Robertson

University of Washington, Seattle, Washington

R. Paul Robertson, M.D. is President, Scientific Director, and CEO of the Pacific Northwest Research Institute. He became Professor of Medicine and Pharmacology at the University of Washington in 1980, where he received most of his postgraduate training. He has been elected into membership of the American Society for Clinical Investigation and the Association of American Physicians.

Dr. Robertson has been Editor-in-Chief of *Diabetes*, the research journal of the American Diabetes Association, and is currently on the editorial board of the *Journal of Biological Chemistry*. Honors received by Dr. Robertson include the endowed Pennock Chair for Diabetes Research at the University of Minnesota, Banting and Best Lecturer at the Joslin Clinic, the Moses Barron Award of the Minnesota Affiliate of the American Diabetes Association, and the Albert Renold Award of the American Diabetes Association. He has published more than 260 manuscripts with primary emphasis on beta cell function in humans, animals, and clonal cell lines. His most recent research activities are centered on studies of glucose toxicity of the beta cell as seen in type 2 diabetic patients. He is also very active in studies of the metabolic consequences of successful pancreas and islet transplantation in patients with type 1 diabetes mellitus.

Charles Eugene Roselli

Oregon Health and Science University, Portland, Oregon

Charles Eugene Roselli is a professor in the Department of Physiology and Pharmacology at the Oregon Health and Science University, where he has

been on the faculty since 1985. He received his Ph.D. in 1981 from Hahnemann University in Philadelphia, Pennsylvania, and did his postdoctoral training at the Oregon Regional Primate Research Center (1981–1984). Dr. Roselli's research focuses on the neurobiological activity of androgens. His work has contributed to an integrated understanding of the subcellular signaling pathways, the steroid-sensitive brain circuitry, and the neurochemical mechanisms that are responsible for the behavioral and neuroendocrine actions of androgens.

A major emphasis of Dr. Roselli's research has been directed at the characterization of the aromatase (CYP19, or estrogen synthetase) signaling pathway in neural tissue, characterizing the distribution and regulation of CYP19, and defining its role in neural development, adult reproductive behavior, and gonadotropin secretion. Dr. Roselli and his colleague Dr. Resko were the first to demonstrate that androgens regulate their own efficacy in the mammalian brain through androgen receptor-dependent positive feedback of the aromatization pathway. His research also demonstrated for the first time that gender differences in neural responsiveness to androgens are expressed at the subcellular level through the differential expression of androgen receptors and aromatase. Dr. Roselli and collaborators recently identified a sexually dimorphic preoptic nucleus in the ovine brain and found that the volume of this nucleus correlates with sexual partner preference in rams. They are currently studying the neuroendocrine and neuroanatomical basis of naturally occurring variations in sexual partner preference.

Evan R. Simpson

Prince Henry's Institute of Medical Research,
Clayton, Australia

Evan R. Simpson began his career in 1964 and is presently Director of Prince Henry's Institute of Medical Research of Monash University, Australia. Historically, Dr. Simpson's research has been in three major fields: the regulation of steroid hormone biosynthesis in the adrenal cortex and in the ovary, the role of lipoprotein cholesterol as precursor for steroid hormone biosynthesis, and the study of estrogen biosynthesis, in particular the regulation of biosynthesis of the enzyme aromatase, responsible for the biosynthesis of estrogens. Most recently, Dr. Simpson's research group developed the aromatase knockout mouse as a model of estrogen insufficiency. This has provided insights into the role of estrogens in the physiology and pathophysiology of both males and females, revealing many unexpected and non-sexually dimorphic roles for estrogens unrelated to sexual differentiation or reproduction.

Dr. Simpson has authored more than 300 peer-reviewed articles and some 70 book chapters and nonreviewed publications. He has been a featured speaker at events ranging from closed workshops such as the Wyeth Ayerst sponsored symposium on Frontiers in Estrogen Action to international and national society meetings. Currently, he is Chairman of the International Organizing Committee of the International Congress of Hormonal Steroids and Hormones and Cancer, and until last year was Chairman of the International Aromatase Conference. In 1998, he was Chairman of the Program Organizing Committee of the U.S. Endocrine Society Annual Meeting. He is a member of the council of the Endocrine Society of Australia, Editor-in-Chief of the *Journal of Molecular Endocrinology*, and Associate Editor of *Endocrine Reviews*.

Dr. Simpson's honors and awards include the Transatlantic Medal Lecturer for the U.K. Society for Endocrinology in 1990, and in 2003 he is the society's Asia and Oceania Medal Lecturer; he received the President's Scientific Achievement Award from the Society for Gynecological Investigation (United States). He has been a guest lecturer at several institutions, including the University of Western Ontario, the University of Kansas, and Johns Hopkins University, and at Princess Takematsu's Annual Symposium on Breast Cancer in Tokyo.

Guido Verhoeven

Catholic University of Leuven, Leuven, Belgium

Guido Verhoeven was born in Antwerpen, Belgium, on March 26, 1945. In 1970, he completed medical studies at the Catholic University of Leuven, Belgium, and in 1974 obtained a Ph.D. from the same institution. He is a Registered Specialist in Clinical Chemistry with authorization for nuclear medicine *in vitro*. In 1974, he received a Biomedical Fellowship of the Population Council in New York and worked for one year in the laboratory of Professor Jean D. Wilson at the University of Texas Southwestern Medical School in Dallas on the problem of androgen insensitivity syndromes. Professor Verhoeven received several postdoctoral fellowships from the National Fund for Scientific Research from Belgium

and became Full Professor at the Catholic University of Leuven in 1986, where he teaches pathophysiology and general medicine both at the Medical School and at the School for Pharmacy.

Professor Verhoeven has been Chairman of the Department of Developmental Biology for 14 years. He is Full Member of the Belgian Royal Academy of Medicine and has served in a variety of capacities within the scientific community. These include Secretary of the Belgian Contact Group on Steroid Hormones and Secretary and Vice President of the Belgian Society for Endocrinology. He is a member of the Endocrine Society, an academician of the European Academy of Andrology, and a member of the Permanent Scientific Committee of the European Testis Workshops. In 1984, he organized the 8th European Workshop on the molecular and cellular endocrinology of the testis in De Panne, Belgium. Professor Verhoeven's main research interests are cell–cell interactions and androgen action in the testis, and androgens and the control of proliferation and differentiation in the normal prostate and in prostate tumor cells. In recent years, his research has increasingly focused on prostate cancer. Professor Verhoeven has published nearly 200 papers in international peer-reviewed journals.

Nancy L. Weigel

Baylor College of Medicine, Houston, Texas

Nancy L. Weigel earned undergraduate degrees at Cornell University and a Ph.D. from Johns Hopkins University. Dr. Weigel was named a National Institutes of Health Postdoctoral Fellow (1979–1981) and a Searle Scholar (1983–1986). Following postdoctoral work in steroid receptors, she joined the faculty of Baylor College of Medicine, where she is presently a professor in the Department of Molecular and Cellular Biology.

Dr. Weigel is engaged in a variety of research projects, including the regulation of human steroid receptors by phosphorylation, the role of androgen receptors in prostate cancer, and the effects of vitamin D on both bone loss and prostate cancer. She has authored or coauthored more than 100 scientific journal publications. Her manuscripts are also included in a number of books: *Gene Regulation by Steroid Hormones* (1982, Springer-Verlag), *Mechanisms of Steroid Action* (1981, Macmillan Press Ltd.), *Methods in Enzymology* (1982, Academic Press), *Steroid Hormone Receptors, Structure and Function* (1983, Elsevier), *Laboratory Methods Manual for Hormone Action and Molecular Endocrinology*, (1989, Houston Biological Associates), *Receptor Purification* (1990, Humana Press), *Endocrinology*, 4th Edition (2000, Saunders Company), and *Encyclopedia of Molecular Medicine* (2002, Wiley and Sons).

Dr. Weigel has served on a number of review panels and editorial boards and has participated in many invited lectures around the United States and Europe, most recently for the fifth consecutive year at Frontiers in Reproduction in Woods Hole, Massachusetts. She has chaired the American Cancer Society Tumor Biochemistry and Endocrinology Study Section, is an editorial board member for *Steroids*, *Endocrinology*, and *Journal of Biological Chemistry*, and is a member of the Endocrine Society, the American Association for Cancer Research, Women in Cancer Research, the American Society for Bone and Mineral Research, the American Society for Biochemistry and Molecular Biology, the American Association for the Advancement of Science, and Women in Endocrinology, serving as Secretary–Treasurer from 1998 until 2001.

Roy E. Weiss

University of Chicago, Chicago, Illinois

Roy E. Weiss is Professor of Medicine at the University of Chicago and is Associate Director of the Clinical Research Center. Since the mid-1980s, Dr. Weiss has been involved in efforts to understand the molecular basis of thyroid hormone action. As an active clinician, educator, and molecular biologist, he has studied the clinical and physiological abnormalities in patients with the syndrome of resistance to thyroid hormone, to understand mutant thyroid hormone receptor isoform and cofactor interaction. Dr. Weiss was the first to demonstrate that this syndrome could be diagnosed at birth and the effect of treatment on outcome. Dr. Weiss has also demonstrated the importance of nuclear coactivators in thyroid hormone action *in vivo*. Currently, he is working on understanding the basis for resistance to thyroid hormone in patients with normal thyroid hormone receptors.

FOREWORD

The discipline of endocrinology was born with the discovery of hormones, but the concept of endocrinology has been substantially expanded by the more recent discovery of paracrine and autocrine regulators. The field of hormone action was formed to understand the molecular mechanisms by which hormones act in cells, and continues to expand explosively. In the late 1960s, the prevailing view of hormone action ranged from effects on membrane transport of nutrients and precursors for RNA and protein synthesis to effects on the translation of mRNA at the level of ribosomes. Nevertheless, a cadre of voices predicted a possible nuclear action on mRNA synthesis. These voices were correct in that steroid hormones, acting via their receptors, indeed were proved to regulate gene transcription. To the best of my knowledge, the first paper to be presented at the national endocrine meetings in a new field of hormone action was in 1967, and it dealt with hormonal stimulation of oviductal protein synthesis. It was about this time that a small group of scientists interested in hormone effects in cells attended a Gordon Conference in New Hampshire; this was one of the first conferences to focus an entire program on hormone action and mechanisms. The attendees were primarily involved in aspects of steroid hormone and thyroid hormone actions; peptide hormone action was yet to experience its own birth and a similar expansive growth. Only a short time previously, Elwood Jensen had discovered the estrogen-binding protein that eventually became the "estrogen receptor," thus it was logical that the conference dealt mainly with steroid receptors; there were also a few papers presenting data that steroid hormones could induce specific enzyme/protein synthesis in target cells. The mechanisms of these effects were the subject of great debate at this first conference on steroid hormone action, a meeting that persists to this day in New England each summer.

Following the monumental discovery of peptide immunoassays, workers in the peptide field were immersed in the work of measuring hormones, ranging from insulin, to luteinizing hormone, to follicle-stimulating hormone, to thrytropin-releasing hormone, to growth hormone, to name a few. For over a decade, little attention was given to the more difficult task of understanding the functions of their receptors and intracellular signaling pathways. Nevertheless, the advent of this assay methodology, including the ability to synthesize radiolabeled peptide hormones, eventually allowed the identification and quantification of cell surface receptors for peptide and amine-containing hormones. The time of this application was about 1970. Researchers demonstrated that cAMP levels were induced in concert with ligand occupation of certain membrane receptors, and the second messenger cAMP was postulated to initiate intracellular phosphorylation of unknown targets. At this point, the field of peptide hormone action also was born.

The two distinct but related fields, steroid hormone action and peptide hormone action, developed together for much of the next decade. Hormone action conferences invariably contained talks on both types of receptors and progress was rapid and in concert with the development of molecular biology. In the steroid field, progress was more rapid initially, but by the mid 1980s, the peptide field attained equal mechanistic status.

Investigators of steroid hormone action concentrated on first understanding the "pathway of action" for their hormones. Scientists looked for model systems showing large responses to steroids. One approach was to assess changes in enzyme levels in cultured cells. The intact chicken oviduct was another of the more notable systems, in that regard because of the ability of sex steroids (estrogen, progesterone) to induce large increases both in certain egg-white proteins and in their respective mRNAs. Viral proteins also were shown to be induced by glucocorticoids. The finding that purified steroid receptors could bind to DNA directly led to a new understanding of the pathway of steroid, to intracellular receptor, to DNA, to mRNA, to protein, and to function. Still, many complexities remained to be sorted out when the receptor cDNAs were cloned in the 1980s.

Our concept of hormones expanded considerably with the advent of growth factors and cytokines. Arguably, the myriads of growth factors only represent an additional list of peptidelike hormones that often act within the tissue of their origin; they have a strong predilection for growth and cell cycle control. Cytokines have both local and distal actions and are particularly oriented to processes such as smooth muscle function and inflammation and apoptosis.

For a decade, it seems as if the peptide action researchers were unduly fixated on cAMP induction and protein kinase A activation. The complexity of signaling pathways emanating from membrane receptors increased logarithmically with the discovery of the numerous protein kinases that phosphorylated serine and tyrosine, the kinase-kinases, the phosphatases, the calcium and diacylgycerol regulators, and the regulators of all of the phosphorylation intermediates. The types of receptors that proliferated ranged from seven membrane (protein kinase A), growth factors (tyrosine kinase, protein kinase C), cytokine, and eventually even chemokine in nature. The appreciation of G-proteins as upstream targets of the cAMP pathway was key to eventual solutions of the signaling cascade. The discoveries of the ras/raf pathway for mediating the effects of mitogens, and the JAK/Stat pathway as mediator for cytokines and certain peptide hormone effects, were also important milestones in unraveling signal transduction in eukaryotic cells. The realization that CREB and Stat proteins were regulatable transcription factors that eventually acted on DNA united the steroid–peptide fields, in part, at the level of the nucleus. That order was made out of apparent chaos is a striking tribute to the intellectual prowess and perseverance of the workers in this field. Most importantly, the signaling pathways emanating from the membrane have brought new insights to pathologies such as cancer, and have led to an explosive development of new pharmaceutical stimulators and inhibitors, with good promise for therapies of neuropsychiatric disorders, cancer, and other human disorders.

After a consolidation period in the 1970s, the steroid action field heated up again with the cloning of the steroid, thyroid, vitamin D, and retinoic receptors. Certain unpredictable events occurred. Investigators began to clone (by cross-screening) numerous molecules that were similar to steroid receptors, but that were not known to be activated by an existing ligand. The term "orphan receptors" was born and the deduction was made that the steroid receptors were part of a giant superfamily of nuclear receptor transcription factors, numbering 48 in humans. The availability of cloned cDNAs and other reagents allowed mutational analyses of receptors, followed by their reintroduction into cells to monitor effects on synthetic reporter genes; structure–function relationships proved the existence of receptor domains for transcriptional activation, nuclear translocation, and DNA binding. Now at a frenetic pace, information on dimeric DNA binding and heterodimeric partners (retinoic acid X receptor), receptor crosstalk with peptide pathways, ligand-independent receptor activation, and receptor phosphorylation was accumulated. More definitive appreciation of the biology of classical and orphan receptors was accomplished by the emerging transgenic technologies and gene knockout strategies. The ability to screen for new ligands for orphan receptors extended the range of hormones to lipids (peroxisome proliferator-activated receptors) and other previously unsuspected metabolic regulators. The yearly stream of publications on the physiology of orphan receptors and their novel ligands continued to bring excitement and more expansion to the field. Pharmaceutical companies salivated at the possibilities of new drugs acting at the nuclear level. The tamoxifen paradox (it acts in one tissue as an agonist and in another as an antagonist) provided encouragement for the generation of a successful search for selective receptor modulators that contain tissue- and function-specific profiles.

Still, the field clamored for a greater molecular understanding of how the nuclear receptors worked at the level of DNA. The discovery of receptor-associated regulatory proteins provided this missing link and changed the field of hormone action further. We moved from a situation wherein many believed that intracellular receptors carried out the transcriptional regulation inherent to the actions of steroid/thyroid hormones and vitamins, to an understanding that the receptor-associated "coregulators" are the primary mediators of this genetic response. We now know that the receptor co-activators act as powerful transducers of hormone action, either through inherent enzyme activities or by serving as a scaffold for recruitment of additional co-activator proteins. The coregulators can be divided loosely into two camps: co-activators and co-repressors. Taken together, these molecules mediate the two main tasks of receptors: stimulation and repression of gene expression.

It is perhaps fitting that a burst of recent attention has focused again on the membrane, where both steroids and their receptors have been postulated to have biologically important effects. Actions of nuclear

receptors, and also of free hormones binding to traditional membrane receptors or ion channels, are likely to be of increasing future interest to workers investigating hormonal function. In this respect, we have traveled full circle. I have no doubt that the future will see many additional examples of membrane reinforcement of nuclear gene regulation, of important ligand-independent activities of receptors initiated at the membrane, and of pathway crosstalk among the varieties of hormones and their intracellular pathways.

These volumes of the *Encyclopedia of Hormones* represent one of the most ambitious projects completed to date in the field of hormones and their actions. The Editors have assembled articles that address a full spectrum of the biology and cellular physiology of numerous hormones and their actions in many species. This effort allows the reader the opportunity to survey the state of both membrane receptor-initiated signaling and nuclear receptor-initiated signaling from the viewpoints of a wide variety of leading investigators. The history and breadth of this field are evident within the articles of these volumes. The ambitious project will stand as a major reference source for the field, and I predict that readers will have no problem savoring the current rapid progress and heightened excitement that exists in this vast field of molecular endocrinology.

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PREFACE

The publication of the *Encyclopedia of Hormones* is intended to provide a comprehensive reference work on all known hormones in vertebrate animals, insects, and plants. The list of classical hormones that had been discovered and characterized over the interval from 1914 to 1985 numbered approximately 55; however, as a consequence of the application of modern chemical characterization techniques and molecular biology methodology, this list now exceeds 150 and is still expanding. In fact, during the production interval for the *Encyclopedia,* several new hormones were discovered and new activities for existing hormones were clearly defined. In addition, enormous strides have been made in our understanding of the detailed actions of hormones at the molecular and cellular levels. There have been dramatic applications of this new knowledge in the medical arena with respect to both diagnosis and treatment of diseases; for the plant and insect hormones, new applications have arisen in the realms of agricultural biotechnology and biological control.

Some comment is appropriate concerning the definition of a hormone that has been utilized in compiling entries in the *Encyclopedia*. Of course, the classical definition of a hormone is that it is a chemical messenger in the body: it is secreted by an endocrine gland and is delivered through the circulatory system to target cells that possess receptors specific for the hormone. Occupancy of the receptor by its cognate hormone leads to the initiation of signal transduction processes that result in generation of specific biological responses. But in this post-human-genome era, and with the rich array of technologies used to study and define at the cellular and molecular levels the enormous array of signal transduction pathways employed by cells, the Editors have adopted a broader definition of hormone. Hormones can now be considered to include not only chemical messengers in the classical sense, but also local paracrine and autocrine signals. Thus, the *Encyclopedia* includes articles on many growth factors, interleukins, and intracellular mediators of signal transduction.

The *Encyclopedia of Hormones* is intended to serve as a useful and comprehensive source of information spanning all aspects of the general subject of hormones. It consists of nearly 300 articles that collectively describe hormones from several key perspectives: (1) the cellular and subcellular sites of functioning of the hormone, (2) the major physiological system(s) in which it is operative (e.g., reproductive, immune, neuroendocrine, digestive, and developmental), (3) the nature of the receptor and signal transduction pathway(s) used by the hormone (e.g., nuclear or membrane signal transduction), and (4) for the vertebrate hormones, the important diseases of deficiency or excess or other instances for which there is unusual molecular insight available. We expect that the *Encyclopedia of Hormones* will be as useful to the scientific expert concerned with cutting-edge questions as it will be to students and interested nonscientists.

Given the broad scope of such a major reference work, it was essential to assemble a team of Associate Editors. Each of these 14 individuals has dedicated his or her professional career to researching scholarly endeavors in a specific domain of hormones and, as a consequence of the breadth and depth of achievement in this area, is an acknowledged leader in their field. These interests include the hormone domains of adrenal cortex, calcium-regulating hormones, cytokines, female reproduction, male reproduction, gastrointestinal hormones, growth factors, thyroid, membrane signal transduction, neuroendocrinology, nuclear signal transduction, pancreas, plant hormones, and insect hormones.

The *Encyclopedia* was launched at a two-day meeting of the Editors, Associate Editors, and Elsevier–Academic Press representatives in La Jolla, California, in April, 2001. Here the preliminary list of article titles prepared by the Editors was refined and a list of potential authors created. Each Associate Editor was then responsible for the crucial process of recruiting authors for the individual entries. As the manuscripts were received by Academic Press,

they were critically reviewed by the Associate Editors and Editors, as well as by the editorial staff at Academic Press. The final total of 296 articles entered production in only 16 months.

All of the articles are formatted according to the same blueprint and each is intended to be a self-contained presentation. Each article begins with a brief topical content outline that provides the reader with a listing of the major topics presented in the article. The article body begins with an introductory paragraph that defines the topic under discussion and summarizes the content of the article. Following the article are reference citations to provide the reader with access to further in-depth consideration of the topic at hand and a cross-reference to related entries in the *Encyclopedia*. A glossary list defines key terms that may be unfamiliar to the reader and are important to an understanding of the article. A compilation of all glossary terms appearing in the complete multivolume *Encyclopedia* is presented in the final volume as a dictionary of subject matter relevant to hormones.

If the *Encyclopedia* has merit, it is due largely to the contributions of the authors of all the articles, and as well to the dedication of the Associate Editors. Shortcomings are, of course, the responsibility of the Editors and we would appreciate having them brought to our attention. The completion of this large project in the relatively short time, from launch meeting to the actual printing of the *Encyclopedia* (only 23 months), is the result of much hard work and dedication. Certainly the primary credit must go to the some 500 authors who prepared their contributions in a timely fashion. The board of Associate Editors also provided exceptional leadership and service. The Editors thank them all.

Finally, thanks are due to the staff of Elsevier–Academic Press, including Tari Paschall, Judy Meyer, Chris Morris, and Carolan Gladden, who each provided skillful and friendly ongoing management of the project.

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Abscisic Acid

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The development of strategies that enable growth without excessive consumption of water was vital to the evolution of terrestrial plants. It is now well established that phytohormones, in particular, abscisic acid, regulate plant water status.

I. INTRODUCTION

During the 1960s, two independent groups identified a compound active in the initiation of bud dormancy in sycamore and cotton boll abscission, naming it dormin and abscisin II, respectively. Following its purification from cotton fruits, the chemical structure of this compound was determined in 1965 and it was renamed abscisic acid (ABA). Shortly after this, it was discovered that ABA levels increase considerably when plants wilt and that ABA causes stomatal closure. These two discoveries highlighted the importance of ABA in mediating responses of vegetative tissues to environmental stresses such as drought, high salinity, and low temperature. ABA is also required for the accumulation of seed nutrient reserves, the acquisition of desiccation tolerance, and the arrest of embryonic development during seed maturation. Despite its name, ABA is not a major regulator of absicission, which is primarily controlled by ethylene.

II. STRUCTURE AND OCCURRENCE

Like all hormones, ABA responses depend not only on the sensitivity of the tissue to ABA, but also on local ABA concentration. This is regulated by the biosynthesis, degradation, inactivation, transport, and subcellular compartmentation of the hormone.

A. Structure

The 15 carbon atoms of the sesquiterpene ABA configure an aliphatic ring with one double bond, three methyl groups, and an unsaturated chain containing the carboxyl group (Fig. 1). The *cis* and *trans* isomers differ in the orientation of the carboxyl group, and the asymmetric carbon at the 1′ position of the ring distinguishes between the *S*(+) and *R*(−) enantiomers. The different forms of ABA occur in different proportions in plants and can have different activities. The *S-cis* form is the most abundant naturally occurring form and is the active form in fast responses such as stomatal closure. Both enantiomers are active in long-term responses such as changes in gene expression and protein synthesis. In contrast to the *cis-trans* isomers, the *S* and *R* forms cannot be interconverted *in planta*. The exact ABA chemical structure is essential for its physiological activity, and the loss of a carboxyl group, a tertiary hydroxyl group, a 2-*cis* 4-*trans*-pentadienoic side chain, a 4′-ketone, or a double bond in the cyclohexane ring greatly reduces activity.

B. Occurrence

ABA is widespread in vascular plants, occurring in mosses, ferns, liverworts (where a similar compound, lunaric acid, plays a similar role), and all algal classes, including photosynthetic prokaryotes such as cyanobacteria. Some pathogenic fungi make ABA, but the biosynthetic pathway appears to be quite different from that of higher plants. ABA is also reported to occur in the mammalian brain, although its role there is not known.

III. SYNTHESIS

The endogenous ABA concentration can rise and fall dramatically in response to environmental or developmental cues. It appears that ABA is synthesized in almost all cells containing chloroplasts or amyloplasts (i.e., plastids), but the regulatory controls appear to differ between tissues. Not only do absolute ABA concentrations increase dramatically during embryogenesis, but the ABA content of leaves and roots increases 10- to 50-fold when water potentials fall below −1.0 MPa (approximately −10.0 bar).

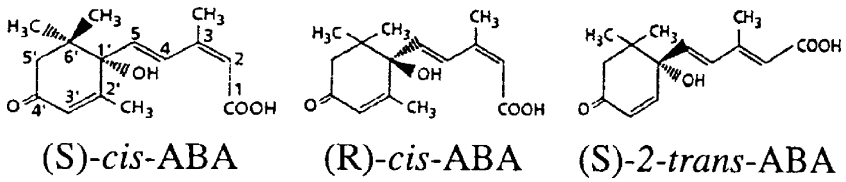

FIGURE 1 Chemical structures of the *S* and *R* forms of *cis*-ABA and the (*S*)-2-*trans* form of ABA.

The concentration of ABA in the xylem sap of well-watered plants is 1.0–15.0 nM and can increase to 3.0 μM after water stress. The main rise in ABA caused by water loss occurs some 2–3 h after the onset of wilting. The ability of cycloheximide to block this process indicates a requirement for *de novo* protein synthesis and thus implicates an up-regulation of ABA biosynthesis in stressed tissues.

The plant ABA biosynthetic pathway represents a minor branch of the carotenoid pathway and begins in plastids. In contrast with isoprene biosynthesis in animal cells, the main precursor of ABA, isopentenyl diphosphate (IPP), is generated by the methyl erythritol phosphate pathway and not from mevalonic acid. Eight IPP residues are combined to form geranylgeranyl diphosphate, the precursor for the biosynthesis of the C40 compound β-carotene. Both rings of β-carotene are hydroxylated to form the xanthophyll zeaxanthin, which can be regarded as the first intermediate in ABA synthesis (Fig. 2). The role of xanthophylls as intermediates in ABA biosynthesis is supported by the reduced ABA content of maize *vp* (*vivi*parous) mutants that are blocked in early steps of carotenoid synthesis. Zeaxanthin is then oxidized to antheraxanthine and to all-*trans*-violaxanthin by zeaxanthin epoxidase (ZEP), which is absent in the *Nicotiana plumbaginifolia aba2* mutant. ZEP contains a putative N-terminal transit sequence for targeting to chloroplasts. *ABA2/ZEP* expression is detected in stems, leaves, roots, and seeds and it is strongly induced by drought stress in roots but not leaves.

The oxidative cleavage of the 9-*cis*-epoxycarotenoid precursor generates the 15C skeleton of ABA. The maize *vp14* mutant is deficient in the chloroplastic 9-*cis*-epoxycarotenoid dioxygenase (NCED) responsible for the cleavage of the 9-*cis*-isomers, but not the all-*trans*-isomers, to xanthoxin. Therefore, the precise order of isomerization-type reactions remains uncertain. The gene is expressed constitutively in embryos and roots and in contrast with *ABA2/ZEP* transcripts, *NCED* transcripts accumulate to high levels in water-stressed leaves. Thus, ABA accumulation in wilted leaves is primarily regulated at the level of plastidic xanthoxin production, which appears to be rate-limiting in ABA biosynthesis. Transgenic experiments indicated that ZEP is primarily involved in the regulation of ABA synthesis during seed development.

The final steps are not yet completely defined. Xanthoxin is converted to ABA in the cytosol via either AB-aldehyde or xanthoxic acid. Genetic evidence suggests that xanthoxin is first oxidized to AB-aldehyde, although the involvement of xanthoxic acid as a precursor has not been completely eliminated. *Arabidopsis thaliana aba3* and tomato *flacca* and *sitiens* mutants are defective in the last oxidation step and are thus unable to oxidize AB-aldehyde to

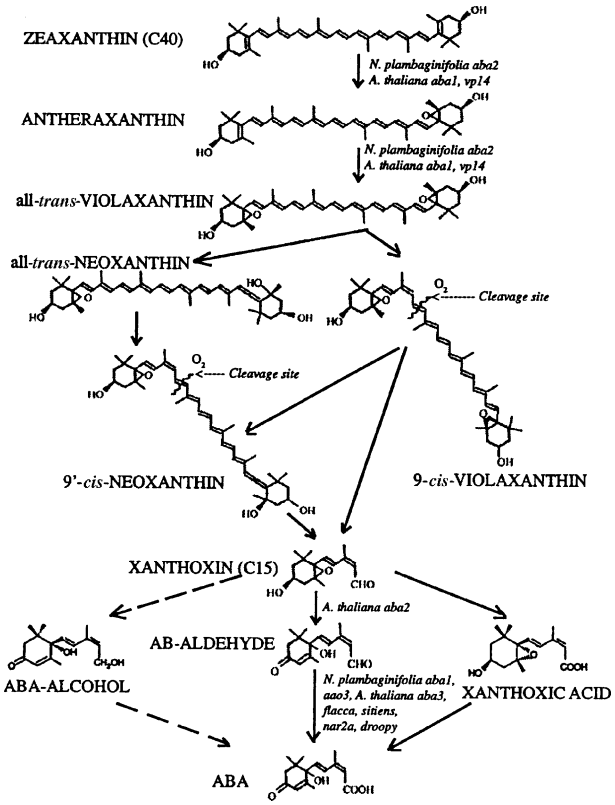

FIGURE 2 The ABA biosynthetic pathway. The metabolic blocks in various ABA-deficient mutants are indicated in italics. Adapted from Taylor *et al.* (2000).

ABA. This last step involves an enzyme that requires a molybdenum cofactor. *Arabidopsis aba3* and *N. plumbaginifolia aba1* mutants cannot produce the functional molybdate cofactor required by AB-aldehyde oxidase (AO). This last enzyme of ABA synthesis is not highly substrate-specific and interestingly, AO can also catalyze synthesis of another plant growth regulator, the auxin indole-3-acetic acid. In *Arabidopsis*, a multigene family comprising at least four members encodes AO, only one of which appears to act specifically in ABA synthesis.

A. Alternative Pathways

Although *ABA2* is a single-copy gene, the ABA content of the *N. plumbaginifolia aba2* null mutant is 23–48% that of the wildtype (WT). Moreover, ABA is present in tomato *flacca* and *sitiens* mutants that lack an effective AO. These results suggest either that there is more than one biosynthetic pathway or that there is some redundancy in part of the primary biosynthetic pathway. The 2-*trans*-ABA-alcohol accumulated during water stress in *flacca* and *sitiens* mutants could be formed via a P450 mono-oxygenase able to add a second oxygen atom to the C1 position and can be further slowly converted to ABA. This reaction may also occur to a small extent in WT plants. Unlike plants, fungi are able to synthesize ABA directly from the 15C compound farnesyl pyrophosphate.

IV. DEGRADATION

After wilted leaves regain turgor, ABA can be inactivated by oxidation to phaseic acid and dihydrophaseic acid or by conjugation to glucose to form a glucose ester. In the first case, catabolic inactivation proceeds via hydroxylation at the 8′ position to form an unstable intermediate that subsequently forms phaseic acid. The ABA-8′-hydroxylase considered as the pivotal enzyme in ABA degradation is a membrane-associated cytochrome P450 mono-oxygenase. It is expressed at high levels in plant tissues recovering from hyperosmotic stresses. Although phaseic acid is still able to trigger stomatal closure in some species, its activity is much weaker than that of ABA. In contrast, dihydrophaseic acid, which is the reduced form of phaseic acid, has no detectable activity. Conjugation of ABA to glucose not only renders ABA inactive but also changes its distribution in the cell. Whereas free ABA is mainly cytosolic, ABA-β-D-glycosyl ester accumulates in vacuoles and could be a storage form of the hormone. Until now, neither the enzymes involved in ABA catabolism nor the genes that encode them have been isolated.

V. TRANSPORT

ABA is secreted by cells into the apoplast (i.e., intercellular space) and is easily transported in both xylem and phloem sap to most plant parts, especially stems, leaves, roots, and ripening fruits. Since roots are the primary sites of perception of water deficit, ABA synthesized in roots can be transported to shoot tissues via the transpiration stream, where it triggers stomatal closure to reduce water loss from leaves.

Within leaves, ABA is redistributed as a function of pH. In a well-watered plant, the xylem sap is more acidic (approximately pH 6.3) and ABA occurs in its protonated form (ABAH). During drought stress, the sap becomes slightly alkaline (approximately pH 7.2), favoring the deprotonation of ABAH to ABA. As a result, less ABA is taken up by mesophyll cells and more is diverted to guard cells. Therefore, even though absolute ABA levels may not change, the pH-dependent redistribution of root-derived ABA to guard cells can induce stomatal closure.

A similar redistribution may exist within cells. When photosynthesis is active, the pH of the chloroplast stroma increases as protons are pumped into the thylakoid lumen. A prevalence of deprotonated ABA limits its ability to cross the chloroplast membrane, causing the accumulation of ABA in the stroma. During drought stress, photosynthetic rates decrease. The resulting drop in stromal pH increases levels of ABAH, which can traverse membranes and be released for transport to guard cells.

VI. ROLES OF ABA

ABA is unquestionably involved in a plant's response to stress and in the initiation and maintenance of seed dormancy. However, it also influences many other aspects of plant physiology, often by interacting synergistically/antagonistically with hormones such as ethylene, gibberellins, cytokinins, auxin, jasmonic acid, and brassinosteroids or by modulating metabolic sensing pathways such as those monitoring cellular sugar status.

A. ABA Triggers Stomatal Closure During Water Stress

Stomata are pores, found on the aerial surfaces of plants, that allow CO_2 uptake for photosynthesis and

at the same time the loss of water, which drives the transpiration stream. Stomatal pore diameter is regulated by turgor changes of the two surrounding guard cells. Unlike most other cells in higher-plant tissues, the absence of plasmodesmata in mature guard cells renders them independent of surrounding cells and enables them to respond autonomously to stimuli such as CO_2, water status, temperature, red/blue light, and plant pathogens. Applied ABA inhibits the opening and promotes the closure of stomata. The increased transpiration rates observed in ABA-biosynthetic mutants and the accumulation of ABA in stressed leaves with reduced transpiration are consistent with the view that endogenous ABA normally plays an important role in the reduction of water loss by transpiration. Expression of an anti-ABA antibody in transgenic tobacco plants retained ABA in the endoplasmic reticulum and caused leaves to wilt by impairing stomatal closure.

The molecular mechanism by which ABA induces stomatal closure has been studied using genetic, biochemical, single-cell, and electrophysiological approaches. Opening and closing of stomata is thought to provoke turgor and volume changes in guard cells. During water stress, the increase in cellular ABA or in apoplastic ABA at guard cell surfaces mediates guard cell closure by triggering a net efflux of K^+ and Cl^- from the vacuole to the cytoplasm and from the cytoplasm to the apoplast. Additionally, sucrose and malate are metabolized to osmotically inactive starch, all of which function to reduce the osmolarity in the guard cells. During stomatal opening, guard cells swell following the accumulation of K^+, anions, and sucrose. The resulting out-bowing of the guard cell pair increases pore aperture and allows reestablishment of transpiration.

B. ABA Promotes Seed Maturation and Dormancy

One of the clearest effects of ABA is to prepare the seed for desiccation and to impose embryo dormancy to prevent premature germination. Seeds of ABA-deficient mutants or transgenic plants depleted of endogenous ABA by expression of an ABA-specific antibody fail to mature fully and acquire dormancy.

Seed development can be divided into two phases of equal duration. The first includes growth and development of the embryo and the endosperm. The second phase begins with the arrest of cell division and the accumulation of storage reserves and is followed by preparation for desiccation, which occurs in the last stages of seed maturation. Seeds prepare for desiccation by accumulating nutritive reserves and proteins that allow the cell to survive the ensuing loss of up to 90% of its water content. As a consequence of dehydration, seeds become dormant. The ABA content of seeds increases during the first half of seed development and decreases during the second phase involving seed maturation.

ABA strongly induces genes that encode abundantly expressed seed storage proteins (e.g., zein, conglycinin, and lectin proteins) as well as proteins involved in desiccation tolerance. The highly conserved water-soluble and basic late-embryogenesis abundant (LEA) proteins are rich in glycine/lysine and low in hydrophobic residues and are thought to stabilize other proteins when the cell is dehydrated. They are related to members of the DHN (dehydrin) and RAB (Responsive to ABA) protein families.

C. ABA Inhibits Germination and Seedling Growth

Seed germination can be defined as the resumption of growth of the embryo following dormancy. As possibly the most critical developmental transition in the plant life cycle, germination is contingent on suitable environmental conditions. However, dormant seeds will not germinate even under normally permissive conditions of temperature or water, light, and oxygen availability. Seed dormancy introduces a delay in germination that provides additional time for geographical dispersal and also maximizes seedling survival by preventing germination under adverse conditions.

ABA appears to be the most important mediator of seed dormancy. The ability of exogenous ABA to prevent seed germination in many species has been used to isolate several *abi* (ABA-*i*nsensitive) *Arabidopsis* mutants (Table 1). Exogenous ABA can also inhibit the precocious germination of immature embryos in culture. ABA-deficient *Arabidopsis* (*aba*) mutants are nondormant at maturity, and embryos of maize *vp* mutants germinate directly on the cob while still attached to the mother plant. This precocious germination, named vivipary, suggests that ABA normally constrains developing embryos in an early developmental stage. In contrast with maize, ABA deficiency in *Arabidopsis* does not cause vivipary because the rigid seed coat prevents embryo growth while the seed is in the seed pod. Nevertheless, vivipary occurs when *Arabidopsis abi3* mutant embryos are dissected out of the seed coat before complete desiccation.

TABLE 1 Mutations Affecting ABA Biosynthesis and Signal Transduction Pathway in Plants

Plant species	Mutations[a]	Gene product/function
ABA-deficient mutants		
Zea mays	*vp2,5,7–9*	*Carotenoid biosynthesis*
Z. mays	*vp14*	9-*cis*-Epoxycarotenoid dioxygenase
Chlamydomnas reinhardtii	*M526*	ABA xanthophyll biosynthesis
Arabidopsis thaliana	*aba1*	Zeaxanthin epoxidase
A. thaliana	*aba2*	Xanthoxin oxidase
A. thaliana	*aba3*	Molybdenum cofactor biosynthesis
A. thaliana	*aao3*	Aldehyde oxidase
Nicotiana plumbaginifolia	*aba2*	Zeaxanthin epoxidase
N. plumbaginifolia	*aba1/ckr1*	Molybdenum cofactor biosynthesis
N. plumbaginifolia	*aba2*	ABA xanthophyll biosynthesis
N. plumbaginifolia	*cnxA*	Molybdenum cofactor biosynthesis
Lycopersicum esculentum	*notabilis*	9-*cis*-Epoxycarotenoid dioxygenase
L. esculentum	*flacca*	Molybdenum cofactor biosynthesis
L. esculentum	*sitiens*	Aldehyde oxidase
Solanum phureja	*droopy*	Aldehyde oxidase
Hordeum vulgare	*nar2a*	Molybdenum cofactor biosynthesis
Pisum sativum	*wilty*	ND
ABA-insensitive mutants		
A. thaliana	*abi1* (SD)	Type 2C protein phosphatase
A. thaliana	*abi2* (SD)	Type 2C protein phosphatase
A. thaliana	*abi3*	Seed-specific putative transcription factor
A. thaliana	*abi4*	Transcription factor
A. thaliana	*abi5*	bZIP transcription factor
A. thaliana	*axr2 (D)*	Auxin response factor (allelic to the auxin mutant *iaa7*)
A. thaliana	*gca1–8*	ND
A. thaliana	*gpa1*	Heterotrimeric G-protein α-subunit
Z. mays	*vp1*	Seed-specific bZIP transcription factor
Z. mays	*rea*	ND
Hordeum vulgare	*cool* (ND)	ND
Craterostigma plantagineum	*cdt-1* (D)	Regulatory RNA or short peptide
ABA-hypersensitive mutants		
A. thaliana	*abh1*	Subunit of a nuclear RNA cap-binding complex
A. thaliana	*bri1*	Steroid receptor kinase
A. thaliana	*era1*	Farnesyltransferase β-subunit
A. thaliana	*era2*	ND
A. thaliana	*era3*	Novel transmembrane protein (allelic to the ethylene mutant *ein2*)
A. thaliana	*fiery1*	Inositol polyphosphate 1-phosphate
A. thaliana	*jar1*	ND
A. thaliana	*jin4*	ND
A. thaliana	*hyl1*	Double-stranded RNA-binding protein
A. thaliana	*sax*	ND

[a]Unless indicated, all mutations are recessive (SD, semidominant; D, dominant; ND, not determined).

Germination is regulated by an antagonism between ABA, which promotes dormancy, and gibberellic acid (GA), which counteracts the effects of ABA by promoting growth and the mobilization of storage reserves. An elegant demonstration of this antagonism is the recovery of mutants defective in ABA synthesis in a screen for revertants of GA-deficient mutants.

D. ABA Controls Root and Shoot Growth

ABA shows different effects on root and shoot growth depending on plant water status. Under water stress, ABA depresses both shoot and root growth, but the overall effect is a dramatic increase in the root:shoot ratio, which facilitates water conservation.

E. ABA Mediates Wound Responses

After mechanical wounding, a specific set of defense-related proteins, such as protease inhibitors I and II, cathepsin D inhibitor, and threonine deaminase, accumulate both at the site of injury and systemically throughout the plant. ABA, together with jasmonic acid, appears to play a role in the induction of these genes.

VII. THE ABA SIGNAL TRANSDUCTION PATHWAY

Clues as to how the ABA signal is transduced to mediate its physiological and developmental processes are now beginning to emerge. It should be emphasized that although many individual components have been identified mainly by molecular genetic approaches, the complete network has not yet been elucidated.

A. Receptor(s)

ABA is thought to initiate its effects by binding to a receptor(s) that triggers the signal transduction cascade. Currently, the identity of the receptor(s) is unknown. Cells may possess at least two sites of ABA perception, one of which is located at the plasma membrane and is triggered by extracellular ABA. Biophysical studies indicate that ABA effects in stomatal guard cells also involve intracellular receptors accessible to the protonated form, ABAH, which readily permeates membranes.

B. Downstream Signaling Events

Recently, considerable insights have been gained into the identities of molecular components of the complex signaling network that mediates the actions of ABA. In particular, ion channels and fairly ubiquitous small second messengers have been implicated in ABA action.

1. Ion Channels Regulated by ABA Control Stomatal Aperture

Electrophysiological studies, either by whole cell impalement or by patch clamping of the plasma membrane of guard cell protoplasts or isolated vacuoles, have identified a number of membrane ion channels. The sequence of events in ABA-induced stomatal closure is thought to be the following: (1) ABA induces release of Ca^{2+} into the cytosol from an internal store, e.g., the vacuole. (2) The resultant increase in cytosolic Ca^{2+} inhibits plasma membrane H^+ pumps and inward K^+ (K^+_{in}) channels, but activates two types of plasma membrane anion-efflux channels. One of these shows voltage-dependent slow activation (S-type), whereas the other shows rapid transient activation (R-type). The two types may reflect different states of a single channel. (3) The conjugate actions of these channels lead to a transient or sustained depolarization and the alkalinization of the guard cell cytoplasm, which (4) deactivates (K^+_{in}) channels and also contributes to the opening of voltage-gated K^+_{out} channels. The ensuing long-term efflux of both anions and K^+ from guard cells contributes to loss of turgor and to stomatal closure.

2. Ca^{2+} Channels

Considerable evidence indicates that ABA produces repetitive, transient increases or oscillations in intracellular Ca^{2+} levels. These encode information required for stomatal closure. Ca^{2+}-induced $[Ca^{2+}]_{cyt}$ oscillations include a repetitive Ca^{2+} influx across the plasma membrane coupled to Ca^{2+} release from an intracellular compartment. Stomatal closure is abolished in guard cells when a nonoscillating Ca^{2+} plateau is imposed experimentally. Many other stimuli responsible for stomatal closure (cold shock, oxidative stress, and increases in CO_2) also cause $[Ca^{2+}]_{cyt}$ elevations. Anion channel regulation and stomatal movement phenotypes of *Arabidopsis abi1-1* or *abi2-1* mutants are suppressed by experimentally elevating $[Ca^{2+}]_{cyt}$.

The mechanisms by which ABA activates guard cell plasma membrane Ca^{2+} channels remain unknown. In *Arabidopsis* guard cells, ABA causes a rapid increase of reactive oxygen species (ROS) that activate hyperpolarization-activated Ca^{2+}-permeable channels. ROS-induced stomatal closure and Ca^{2+} activation are abolished in the ABA-insensitive mutant *gca2*. The origin of the Ca^{2+} required to elevate $[Ca^{2+}]_{cyt}$ in response to ABA is unclear, but is probably mediated by inositol 1,4,5-trisphosphate ($InsP_3$) and/or cyclic ADP-ribose (cADPR). *RAB18* expression in *Arabidopsis* suspension culture cells requires rapid ABA-induced Ca^{2+} influx and S-type anion channel activation. ABA-induced membrane depolarization in radish seedlings and tobacco epidermal and mesophyll cells indicates that these mechanisms are of general importance for ABA signaling in different cell types. A Ca^{2+}-independent pathway also appears to exist.

3. H^+ Channels

Inhibition of the plasma membrane H^+-ATPase mediated by both cytosolic alkalinization and the increase in cytosolic Ca^{2+} may also contribute to

membrane depolarization. The origin of ABA-induced cytosolic alkalinization is unknown.

4. Cyclic Nucleotides (cAMP, CGMP, CADPR)

Cyclic ADP-ribose (cADPR) plays a central role in ABA responses. Microinjection of hypocotyl cells of the tomato *aurea* mutant with both potential intermediates in the ABA signaling cascade and fusions of the *Arabidopsis RD29A* and *KIN2* promoters to a reporter gene suggested that ABA triggers a transient accumulation of cADPR, which induces a release of Ca^{2+} from internal stores such as vacuoles and the endoplasmic reticulum. Microinjection of mutant *abi1-1* protein inhibited ABA-, cADPR-, and Ca^{2+}-induced gene expression, and these effects were reversed by an excess of WT ABI1 protein.

Other cyclic nucleotides may also act in a Ca^{2+}-dependent stomatal opening pathway. For example, cAMP or the membrane-permeable cyclic GMP analog 8-Br-cGMP stimulates stomatal opening. cGMP-induced stomatal opening is inhibited by chelation of external Ca^{2+} or by inhibitors of intracellular Ca^{2+} release.

5. Lipid-Derived Second Messengers

Various lines of evidence suggest that ABA stimulates phosphoinositide metabolism. ABA-treated guard cell protoplasts showed a slight increase in $InsP_3$. The release of caged $InsP_3$ into the cytosol of guard cells caused $[Ca^{2+}]_{cyt}$ increases, inhibition of K^+_{in} channels, and stomatal closure. The *Arabidopsis fry1* (*fiery*1) mutant, which is defective in an inositol polyphosphate 1-phosphatase, accumulates more $InsP_3$ than WT plants after ABA treatment and is hypersensitive to ABA in germination and gene expression assays. Similarly, overexpression of a different $InsP_3$ phosphatase blocked the inhibition of germination and seedling growth in *Arabidopsis*. Overexpression of a stress- and ABA-inducible phosphatidylinositol-specific phospholipase C (PI-PLC) in *Arabidopsis* suggests that although increased $InsP_3$ levels are necessary for maximal ABA-induced gene expression in vegetative tissues, the AtPLC1 isoform is normally latent and probably participates in secondary ABA responses. A reduction in $InsP_3$ levels in transgenic lines expressing antisense *AtPLC1* correlated with their insensitivity to ABA in germination and seedling growth assays.

ABA also stimulates production of myo-inositol-hexakisphosphate ($InsP_6$) in guard cells to a greater extent than $InsP_3$. $InsP_6$ inhibits K^+_{in} channels in a Ca^{2+}-dependent manner with greater efficiency than $InsP_3$. Whether $InsP_6$ causes $[Ca^{2+}]_{cyt}$ elevations and whether both messengers function in the same or separate signaling branches is unknown.

Phosphatidic acid (PtdOH) generated from phospholipase D (PLD) increases transiently following ABA treatment of *Vicia faba* guard cells. PtdOH promotes stomatal closure and inactivates K^+_{in} channel currents but does not elicit a $[Ca^{2+}]_{cyt}$ increase, suggesting that PLD acts either in a parallel Ca^{2+}-independent pathway or downstream of Ca^{2+} release. Both U-73122 (a PI-PLC inhibitor) and 1-butanol (a PLD inhibitor) only partially inhibit ABA-dependent stomatal closure, and simultaneous application of both inhibitors does not have additive effects. Thus, PLC and PLD appear to act in the same pathway that requires the cooperation of an additional pathway(s) to attain the complete effects of ABA. This may be mediated by cADPR, since simultaneous application of 1-butanol with the cADPR antagonist nicotinamide increases the extent to which ABA-induced stomatal closure is reduced.

Sphingosine-1-phosphate (S1P) is another lipid-derived Ca^{2+}-mobilizing agent capable of inducing stomatal closure. An increase in S1P levels occurs in leaves following drought stress, but disruption of S1P production causes only partial inhibition of ABA-induced stomatal closure.

6. Protein Kinases and Protein Phosphatases

Phosphorylation/dephosphorylation events are central mediators in ABA signaling. In guard cells, the Ca^{2+} signal is possibly relayed by specific protein kinases and phosphatases. Protein kinase inhibitors abolish the activation of S-type anion channels and thus block ABA-induced stomatal closure. Reciprocally, the protein phosphatase inhibitor okadaic acid (OKA) maintains guard cell S-type channels in the active state. In-gel phosphorylation assays demonstrated that ABA rapidly activates a Ca^{2+}-independent 48 kDa Ser/Thr protein kinase in *V. faba* guard cells. ABA fails to activate anion channels or induce stomatal closure in guard cells that express a dominant loss-of-function allele of this kinase.

Several stress- and ABA-inducible protein kinases have been identified. In epidermal peels of *Pisum sativum*, the ABA-induced accumulation of a *DHN* transcript was reduced by K-252a (an inhibitor of Ser/Thr protein kinases) and also by OKA or cyclosporin A (an inhibitor of Ser/Thr protein phosphatases type 2B). In barley aleurone protoplasts, the stimulation of a MAP kinase activity appeared to be correlated with the induction of *RAB16* transcript. OKA inhibited the induction

of *HVA1* and *RAB16* transcripts by ABA, and phenylarsine oxide (an inhibitor of Tyr protein phosphatases) blocked *RAB16* induction.

The analysis of *abi1* and *abi2* has shed new light on the involvement of phosphorylation events in ABA signaling. These two dominant ABA-insensitive mutants, originally isolated in a screen for mutants able to germinate and grow in nonpermissive ABA concentrations, have phenotypes reminiscent of ABA deficiency viz. reduced seed dormancy, improper regulation of stomatal aperture, and decreased expression of various ABA-inducible genes. *ABI1* and *ABI2* encode Ser/Thr protein phosphatases type 2C (PP2C). The dominant mutant alleles *abi1-1* and *abi2-1* have point mutations that substitute a conserved Gly with Asp, probably disrupting the conformation of a site required for Mg^{2+}-binding or phosphatase activity. Several downstream responses to ABA are impaired in *abi1-1* and *abi2-1*, including K^+_{out} and K^+_{in} channel regulation, anion channel activation, and increases in $[Ca^{2+}]_{cyt}$. Because the mutations are dominant, it remains unclear whether the ABI1 and ABI2 are positive or negative regulators of ABA signaling or, indeed, whether they affect ABA signaling at all in WT plants. However, because intragenic revertants of *abi1-1* and *abi2-1* have reduced or no phosphatase activity *in vitro* and a double mutant of both revertants is hypersensitive to ABA, ABI1 and ABI2 are probably negative regulators of ABA signaling. Accordingly, overexpression of WT ABI1 in maize mesophyll protoplasts blocks ABA regulation of gene expression. The precise roles of kinases and phosphatases in ABA signaling and the identities of their protein substrates have not been clearly established.

7. Farnesylation

Although researchers have focused mainly on positively acting components of the ABA signaling pathway, inactivation of negative regulators of ABA signaling should result in an enhanced response to ABA. An *Arabidopsis* mutant *era1* (*e*nhanced *r*esponse to *A* BA) was isolated based on its inability to germinate in the presence of low concentrations of ABA (0.3 μM) that do not inhibit germination of WT seeds. The *era1* mutation markedly increases seed dormancy and ABA hypersensitive activation of S-type anion currents in this mutant increases stomatal closure, reducing water loss during drought. The *ERA1* gene encodes the β-subunit of a heterodimeric farnesyltransferase. Farnesyltransferases catalyze the attachment of a 15-carbon farnesyl lipid to C-terminal target sequences, which localizes specific soluble signaling proteins to membranes. In addition to enhancing ABA signaling, loss of *ERA1* function affects several other signaling pathways and developmental programs, including meristem development. Thus, although *ERA1* targets are not restricted to ABA action, a factor that normally suppresses ABA responses requires farnesylation. The exact relationship between *ERA1* and the *ABI* loci remains unknown.

8. RNA Binding and ABA

Arabidopsis abh1 mutants are hypersensitive to ABA-mediated inhibition of germination as well as induction of stomatal closure and increases in $[Ca^{2+}]_{cyt}$. *ABH1* is expressed in stomata and encodes a nuclear transcript cap-binding protein that apparently functions in a heterodimeric complex. *ABH1*, by analogy to yeast and mammalian RNA cap-binding proteins, is proposed to regulate the strength of ABA signaling by transcript modification of early signaling components.

9. Heterotrimeric G-Protein Action

Heterotrimeric G-proteins are central to many signaling processes. *Arabidopsis* contains only a single gene (*GPA1*) encoding a prototypic Gα subunit. ABA-mediated inhibition of stomatal opening, but not ABA-controlled promotion of stomatal closure, is impaired in *gpa1* null mutants. *GPA1* is required for negative regulation of K^+_{in} channels and the pH-independent activation of anion channels.

10. Role of the Actin Cytoskeleton in Stomatal Movements

A reorganization of the actin cytoskeleton of guard cells has been observed after ABA treatment. Cytochalasin D (an actin filament-depolymerizing agent) activates K^+_{in} channels, while phalloidin (an actin filament stabilizer) inhibits K^+ channel currents. ABA treatments reorganize actin cytoskeleton architecture from a radial arrangement to a randomly oriented and fragmented pattern. A small *Arabidopsis* GTP-binding protein, AtRac1, is a negative regulator in ABA-induced actin reorganization. The inactivation of AtRac1 by ABA is impaired in *abi1-1*.

C. Regulation of Gene Expression by ABA

ABA regulates the expression of numerous genes during embryogenesis and seed maturation as well as under stress conditions such as heat shock, low temperature, drought, and high salinity.

1. ABA-Inducible Genes

The use of ABA-deficient and ABA-insensitive mutants has demonstrated that ABA contributes to the regulation of numerous genes involved in seed maturation and/or the response of vegetative tissues to hyperosmotic stress. Characterization of the promoters of ABA-responsive genes has enabled identification of the *cis*- and *trans*-acting elements that act at the termini of branches in the ABA signaling cascade. Considerable evidence indicates the existence of ABA-independent dehydration and cold-induced signaling pathways.

2. Cis-Acting Elements

Gene activation is mediated by the binding of transcription factors to ABA-responsive elements (ABREs) located in the promoters of ABA-induced genes. To date, more than 20 functional ABREs have been found in ABA-inducible genes that are abundantly expressed in desiccating seeds and/or are responsive to drought stress and ABA in vegetative tissues.

The first type of ABRE defined was a sequence of 8–10 bp that shares a conserved ACGT core motif, named the G-box. The sequence flanking the ACGT core is important for *in vivo* and *in vitro* function. Some ACGT elements confer developmental and tissue-specific expression on a minimal promoter. In a natural promoter context, an ABRE functions with a coupling element (CE). ABA-responsive complexes comprising an ABRE and a CE can confer ABA-inducible transcription upon a minimal promoter. The sph element, first identified in the promoter of the *C1* gene involved in anthocyanin synthesis in maize endosperm, is a second category of *cis*-acting element distinct from the G-box.

3. Trans-Acting Factors

Yeast one-hybrid assays to identify ABRE-binding proteins (AREBs) have enabled cloning of several homologous transcription factors of the basic leucine zipper (bZIP) family. ABA-regulated transcription factors of the homeodomain leucine zipper, basic helix-loop-helix leucine zipper, and MYB classes have also been identified. AREBS are capable of activating reporter genes fused to ABREs and their induction by ABA at the transcript level frequently precedes the induction of other ABA-responsive genes. *Arabidopsis* ABI5, the only bZIP AREB recovered in a genetic screen, is also subject to posttranscriptional modification by ABA. Maize VP1 and *Arabidopsis* ABI3 appear to be orthologous seed-specific transcriptional activators, the loss of which affects several aspects of seed maturation, including the expression of storage proteins and *LEA* genes. VP1/ABI3-like proteins appear to activate transcription by distinct mechanisms depending on the target *cis* elements. VP1 interacts directly with the sph element of the C1 promoter and acts on ABREs via association with a distinct *trans*-acting factor(s).

D. Novel Genetic Screens

Quantitative and mechanistic characterization of new signaling mutants is necessary for a complete molecular understanding of the ABA signaling cascade. Several mutants have been isolated in screens for deregulated ABA control of ABREs fused to reporter genes. Eight *gca* (growth control by ABA) mutants are characterized by reduced sensitivity to the inhibition of seedling growth by exogenous ABA and aberrant stomatal regulation. An elegant screen that uses small differences in leaf temperature to distinguish transpiration rates in mutants and WT plants is likely to identify new elements which mediate ABA action in guard cells. Screens for enhancer or suppressor mutations offer one approach to identify genes which interact genetically with known participants in ABA signaling.

VIII. BIOTECHNOLOGICAL FEATURES

Fresh water scarcity is currently one of the principal threats to global food security. Plants account for approximately 65% of global fresh water use. Losses in agricultural yields resulting from the desiccation of crops and horticultural plants during periods of drought have severe social and economic repercussions. Unfortunately, because of the high cost of synthesis and its instability in UV light, there are no practical uses of ABA. However, synthetic ABA analogs such as the acetyleneacetal-type compounds LAB 173 711 and LAB 144143 reduce crop water use and increase cold-hardiness. Engineering the ABA signal transduction network in guard cells to control CO_2 intake and water loss could contribute substantially to more sustainable water use under adverse environmental conditions. The manipulation of seed maturation and dormancy in certain species by modification of ABA-regulated developmental programs may also be of considerable agricultural significance.

IX. CONCLUSIONS

Recent advances have filled in many gaps concerning the biochemistry and subcellular localization of ABA synthesis as well as demonstrating the unquestionable involvement of ion channels, cytosolic pH, protein (de)phosphorylation, and cADPR- and phosphoinositide-mediated increases in $[Ca^{2+}]_{cyt}$ in transducing the ABA signal. Substantial progress has been made in characterization of the terminal signaling elements involved in ABA-mediated transcriptional regulation. Nonetheless, the mechanism(s) of ABA perception and early signaling events that result in cADPR synthesis or $InsP_3$ release remain to be resolved. Considering the multitude of physiological responses modulated by ABA, it will be interesting to assess the extent of overlap in the signaling events involved in well-characterized ABA effects such as the regulation of stomatal closure, the inhibition of seed germination, and the induction of stress-responsive gene expression. The striking degree of phenotypic pleiotropy observed in many mutants recovered in screens for altered sensitivity to ABA indicates extensive overlap between ABA action and other signaling pathways. Better insight into the regulation of ABA concentrations and the cellular capacity for response to ABA will provide a more complete picture of its importance to growth and development throughout the plant life cycle.

Glossary

abscission The rejection of plant organs at an abscission zone where hydrolytic enzymes reduce cell adhesion.

dormancy A resting condition with reduced metabolic rate found in ungerminated seeds and nongrowing buds.

stomata Small openings located in the epidermal layers of plants allowing uptake of CO_2 and loss of water. Stomata are surrounded by two guard cells that control the pore size.

stress The consequence of suboptimal environmental conditions that significantly decrease plant growth and/or reproductive capacity below potential.

vivipary The ability of a plant embryo to bypass dormancy and proceed directly from embryogenesis to germination if rescued from the normal dehydration that occurs during seed maturation.

See Also the Following Articles

Auxin • Brassinosteroids • Cytokinins • Ethylene • Gibberellins • Jasmonates • Salicylic Acid

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ACTH

See *Adrenocorticotropic Hormone*

Activating and Inactivating Receptor Mutations

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Human hereditary diseases can be divided according to the Mendelian inheritance pattern into three categories: autosomal dominant, autosomal recessive, and X-linked recessive. The same division applies to the mutations that are known today in hormone receptor genes. The autosomal dominant mutations of receptor genes cause *activating* or *gain-of-function*

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mutations. In these cases, the structure of the receptor protein is usually altered in such a fashion that its signal transduction is constitutively activated in the absence of ligand hormone, and one altered allele is sufficient to cause the phenotype. The autosomal recessive mutations are usually of the *inactivating* or *loss-of-function* type; i.e., the mutant allele encodes a functionally inactive receptor protein. In most cases, the normal allele is sufficient to maintain normal hormonal function, but in some situations the heterozygous carrier has a phenotype, usually in the form of mild hormone resistance. X-linked recessive mutations cause a phenotype in all males and in homozygous females, and in some cases heterozygous females have a milder phenotype. A special case of inactivating receptor mutations is the *dominant negative* mutations, where the mutated receptor inhibits the function of that encoded by the wild-type allele. This is the only situation where an inactivating mutation causes the phenotype in dominant fashion. Besides the above hereditary (germ line) mutations, somatic mutations are also known in hormone receptors. They are invariably found in tumors, are of the activating type, and play a role in tumor formation or growth. In addition, numerous polymorphisms are known in receptor genes; they either cause no phenotype or may alter susceptibility to certain diseases.

I. INTRODUCTION

A large number of hormone receptor mutations are known today, and this article presents appropriate examples of the different mechanisms by which the mutated receptor brings about either receptor inactivation or constitutive activation. Hormone receptors can be subdivided into two functional categories on the basis of their cellular location, i.e., those present on the plasma membrane and those present inside the cell, in the nucleus and/or cytoplasm. Because of differences in the function of these two receptor types, they are presented separately, under the headings of activating and inactivating mutations. Table 1 summarizes the phenotypes, types of functional alteration, and modes of inheritance of most of the currently known hormone receptor mutations.

II. ACTIVATING MUTATIONS

Activating or gain-of-function mutations can be classified, according to the functional alteration, into four categories: (1) constitutive receptor activation in the absence of ligand hormone, (2) increased sensitivity of the receptor to its normal ligand, (3) relaxed specificity of the receptor to ligands, and (4) acquired novel functions of the mutated receptor. Category 1 is most common among the activating receptor mutations. Some of these mutations are inherited via the germ line, but some, in particular those encountered in tumors, are somatic (Table 1).

A. Nuclear Receptors

Only a few cases of gain-of-function mutations of nuclear receptors are currently known, although it is possible to produce constitutively activated nuclear receptors through site-directed mutagenesis *in vitro*. The reason for their paucity may be the fundamental role that many of the nuclear receptors play in cellular functions and because their inappropriate activation may be embryo-lethal. In fact, almost the only germline mutations known in nuclear receptors are those of the androgen receptor (AR), not necessary for life. The extensions of the polyglutamine (CAG) repeat in the N-terminal part of AR (see below) in Kennedy's disease apparently bring about novel neurotoxic functions for the mutated receptor protein. Conversely, if this repeat is shortened, as is found in somatic mutations in advanced forms of prostatic cancer, the mutated AR either is constitutively activated or has relaxed ligand specificity. Activating mutations of retinoic acid receptors may play a role in the pathogenesis of certain leukemias and hepatomas, due to chromosomal rearrangements within the retinoic acid receptor genes, resulting in fusion proteins with novel functions. The only other piece of information on constitutively activated nuclear receptors concerns estrogen receptors that may contribute to the formation of estrogen-independent cell clones in breast cancer.

B. Cell Membrane Receptors

The receptors for the glycoprotein hormones, i.e., luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (TSH), constitute a good example of gain-of-function mutations of cell membrane receptors. They all belong to the family of G-protein-associated seven-transmembrane domain receptors. Normal receptor activation entails binding of the ligand to the extracellular receptor domain, whereby a conformational change occurs in the transmembrane domain, allowing activation of the intracellular second-messenger generation and ultimately the functional response of the target cell to hormonal stimulation. Accordingly, most of the activating mutations in these

TABLE 1 Currently Known Mutations in Hormone Receptor Genes

Receptor	Type of mutation	Type of inheritance	Phenotype
Nuclear receptors			
Thyroid hormone	Dominant negative	D	Thyroid hormone resistance
	Inactivating	R	Thyroid hormone resistance
Estrogen-α	Inactivating	R	Male: unfused epiphyses, poor sperm, osteoporosis, insulin resistance
	Activating	S	Receptor positive, hormone-resistant breast cancer
Androgen	Inactivating	XR	Lack of male sexual differentiation (testicular feminization)
	Activating	XR	Kennedy's disease
		S	Advanced prostate cancer
Glucocorticoid	Inactivating	R	Variable symptoms of glucocorticoid resistance
		S	Nelson's syndrome (pituitary adenoma)
Mineralocorticoid	Inactivating	D	Autosomal dominant pseudohypoaldosteronism I
Vitamin D	Inactivating	R	Hypocalcemic vitamin D-resistant rickets
Retinoic acid	Activating	S	Various malignant tumors
	Dominant negative	D	
Plasma membrane receptors			
TSH	Activating	S	Toxic thyroid adenomas
		D	Toxic thyroid hyperplasia (hereditary)
	Inactivating	R	Euthyroid, elevated TSH (mild)
		R	Hypothyroidism (severe)
LH	Activating	D	Males: gonadotropin-independent precocious puberty Females: no phenotype
		S	Leydig cell tumors
	Inactivating	R	Males: lack of male sexual differentiation Females: anovulatory infertility
FSH	Activating	D	Normal spermatogenesis in the absence of gonadotropins
	Inactivating	R	Females: infertility with arrest of follicular maturation Males: suppressed spermatogenesis
ACTH	Inactivating	R	Familial glucocorticoid deficiency
	Activating	D	Normal cortisol, low ACTH
MSH	Inactivating	R	Red hair and light skin
Vasopressin	Inactivating	XR	Nephrogenic diabetes insipidus
GnRH	Inactivating	R	Hypogonadotropic hypogonadism
Ca^{2+}-sensing	Inactivating	R	Familial hypocalciuric hypercalcemia, neonatal severe hyperparathyroidism
	Activating	D	Hypoparathyroidism, hypocalcemia
PTH/PTHrP	Activating	D	Jansen's metaphyseal chondrodysplasia
GH	Inactivating	R	Dwarfism
	Dominant negative	D	Dwarfism
GHRH	Inactivating	R	Dwarfism
Insulin	Inactivating	R	Leprechaunism, Rabson–Mendenhall syndrome
	Dominant negative	D	Type A insulin resistance
Erythropoietin	Activating	D	Primary polycythemia
Leptin	Inactivating	R	Obesity, pituitary dysfunction

D, dominant; R, recessive; S, somatic; X, X-linked; TSH, thyroid-stimulating hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; ACTH, adrenocorticotropic hormone; MSH, melanocyte-stimulating hormone; GnRH, gonadotropin-releasing hormone; PTH, parathyroid hormone; PTHrP, parathyroid hormone-related peptide; GH, growth hormone; GHRH, growth hormone-releasing hormone.

receptors have been discovered in the third intracellular loop or the sixth transmembrane region of the receptor, assumed to be the hot spot for activating mutations. The mutation apparently brings about a conformational change in the transmembrane receptor domain, allowing activation of signal transduction without preceding ligand binding. The functional alteration can be demonstrated by transfecting cDNA that encodes the mutated receptor into a cell line, which then displays the cellular response,

for instance, cAMP production, in the absence of ligand hormone (Fig. 1).

Both activating germ-line and somatic mutations have been observed in the TSH receptor (R), and the latter mutations explain the molecular pathogenesis of a large proportion of toxic thyroid adenomas; they have also been implicated in some follicular thyroid carcinomas. Tens of this type of mutations are currently known in the TSHR, but why this receptor in particular is prone to somatic mutations remains unclear. Activating germ-line mutations of the TSHR are found in hereditary and sporadic toxic thyroid hyperplasia. The hereditary form is characterized by autosomal dominant transmission, hyperthyroidism with variable age of onset, hyperplastic steadily growing goiter, and absence of stigmata of autoimmunity. The sporadic cases present with congenital hyperthyroidism, due to neomutations in the TSHR gene. The mutations found in toxic adenomas and neomutations overlap, but another set of mutations are found in hereditary hyperthyroidism. The latter present with milder phenotypes and, hence, the autonomous receptor activation is milder than in the other two cases with more severe phenotypes. Natural selection may explain this difference. The sporadic cases display such strong TSHR activation that carriers of the same mutations in the germ line would apparently not have survived until fertile age in the past.

The other examples of activating mutations in cell membrane receptors are those of the LHR. Approximately 15 activating mutations of the LHR are currently known, but interestingly, not a single unequivocally documented case of activating FSHR mutation is known. The latter may be due to the fact that activating FSHR mutations do not cause a clear phenotype or to the fact that the phenotype is unexpected and the right types of patients have not been investigated. Gain-of-function mutations of the LHR cause in males gonadotropin-independent early onset precocious puberty (testotoxicosis), but in women, for reasons not clearly understood, there is no phenotype. Most of the activating LHR mutations bring about a clear, approximately 10-fold increase in basal cAMP production in cell lines expressing the mutated receptor. This explains why testosterone production in affected boys is activated well before the normal age of puberty. An interesting special case is an activating somatic LHR mutation that was recently discovered in Leydig cell tumors. This mutation activated, in addition to cAMP, the inositol phosphate signaling pathway, providing an example of an activating mutation with acquired novel function.

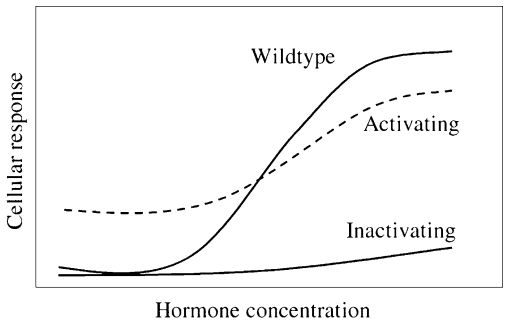

FIGURE 1 Schematic presentation of hormone-stimulated response, for instance. cAMP production, in cells transfected with cDNA encoding the wild-type receptor and one carrying an activating or inactivating mutation. The elevated response in the absence of hormonal stimulation is typical for activating mutation. In inactivating mutation the response is totally or nearly completely missing.

III. INACTIVATING MUTATIONS

An inactivating mutation can cause the following functional aberrations in the receptor protein: (1) decreased synthesis, (2) aberrant intracellular processing, (3) impaired or missing ligand-binding activity, (4) impairment or lack of signal transduction, (5) inability to anchor to the plasma membrane, (6) inability to dimerize, if needed for signal transduction, or (7) increased degradation.

A. Nuclear Receptors

A good example of a nuclear receptor with inactivating mutations is the AR, a member of the steroid hormone/thyroid hormone/retinoic acid receptor family. It is extremely polymorphic and more than 250 AR mutations are known today. Because the AR gene is located on the X chromosome, mutational AR defects exist only in men (X-linked dominant inheritance). A homozygous female is impossible due to obligatory infertility of men carrying a mutated AR gene. The AR protein is a single polypeptide chain, containing, like all nuclear receptors, three functional domains, i.e., the transactivation domain, DNA-binding domain, and ligand-binding domain. The phenotypes with androgen insensitivity vary from mild defects of virilization (Reifenstein's syndrome) to complete female phenotype (testicular feminization), and the extent and location of the AR mutation often predict the phenotype. Splicing defects, frameshifts, immature termination codons,

and partial or complete gene deletions invariably result in total androgen insensitivity. Single amino acid substitutions, in contrast, result in a wide variety of phenotypes, with severity depending on location of the mutation. Substitutions in the DNA-binding domain are a relatively homogenous group in which ligand binding is not altered, but the capability of the AR to modulate androgen-responsive genes is impaired. Mutations in the hormone-binding domain have more varying effects. The mutated receptor is seldom totally unable to bind hormone, whereas the ligand binding may be qualitatively altered or has low affinity. The phenotype of these mutations is often difficult to predict. Mutations in the transactivation domain are relatively rare. This part of the AR is the least conserved and it possibly allows greater variability in structure without hampering receptor function. In addition, no abnormalities in ligand binding can be detected in a large proportion of the patients despite their clear phenotype of androgen insensitivity. Some other genes involved in androgen action, e.g., those of AR co-activators, may be mutated in these cases.

AR has been found to have an intriguing type of structural polymorphism, a polyglutamine repeat (CAG at the DNA level), in exon 1 of its N-terminus. Short repeats (20 residues) are detected in advanced forms of prostatic cancer, apparently as somatic mutations, in which case the ligand specificity of the AR is relaxed and various steroidal and nonsteroidal ligands can activate it. The normal length of the repeat is 23–27 residues. Slight androgen insensitivity is apparent when the repeat length is 28, as can occur in men with oligo-azoospermia. Finally, clearly extended polyglutamine repeats (40 repeats) exist in spinal and bulbar muscular atrophy due to degeneration of motor neurons (Kennedy's disease) where the receptor function is more drastically altered, and it may have acquired novel toxic effects on motor neurons.

Inactivating mutations are also known in other steroid receptors. Only one case of estrogen receptor-α mutations has been described. This was detected in an adult male with tall stature, incomplete closure of epiphyses, osteoporosis, and insulin resistance. The receptor inactivation was due to a point mutation that induced a premature stop codon and hence formation of nonfunctional receptor protein. No progesterone receptor mutations have been described, possibly since they would be effectively eliminated from the genetic pool due to their adverse effect on reproduction. Glucocorticoid receptor (GR) mutations present a variable range of phenotypes from asymptomatic to isolated chronic fatigue and hypertension with or without hypokalemic alkalosis and/or hyperandrogenism. All GR mutations detected cause partial hormone resistance, since complete inactivation of glucocorticoid function would probably be lethal. Differences in impact of the various mutations on glucocorticoid sensitivity of the different target organs probably explain the variability of phenotypes encountered in this syndrome. A mild form of autosomal dominant hypoaldosteronism (type I) has been found to be due to inactivating mutations in the mineralocorticoid receptor. Inactivating mutations of thyroid hormone receptors cause, as expected, thyroid hormone resistance. The vitamin D receptor mutations cause hypocalcemic vitamin D-resistant rickets. In this case, the mechanism of receptor inactivation falls into three categories: (1) suppressed DNA binding/nuclear localization, (2) suppressed hormone binding, and (3) inhibition of heterodimerization with retinoic acid X receptor (RXR). The latter effect is incompatible with the mechanism of vitamin D action, where the vitamin D-receptor complex must heterodimerize with RXR to evoke a biological response.

B. Cell Membrane Receptors

An inactivating mutation of a cell membrane-associated receptor causes hormone resistance usually in the recessive mode of inheritance. Large deletions of the gene often predict the inactivation mechanism of the receptor, but especially if a point mutation is discovered, it is difficult to decipher from the small structural alteration the type of functional impact of the mutation. Functional analysis of synthesis, intracellular processing, and function of the mutated receptor protein in cell culture transfections is therefore needed to prove its functional significance and to solve the molecular basis of the inactivation mechanism. A typical finding with an inactivating receptor in functional analysis is schematically presented in Fig. 1. Similar functional tests are available for nuclear receptors.

Mutations of the growth hormone receptor (GHR) provide good examples of the various mechanisms of receptor inactivation. More than 30 GHR mutations are currently known, mostly in the area encoding the extracellular receptor domain, including exonic deletions and nonsense, frameshift, splice-site, and missense mutations. As is well known, the phenotype of this condition is GH resistance (Laron dwarfism), which is usually inherited in an autosomal recessive manner. The various categories of GHR inactivation

in this syndrome can be grouped according to their effects on circulating GH-binding protein (GHBP), which is a proteolytic cleavage product of the plasma membrane GHR. In some of the cases, the GHBP level is suppressed, as a sign of failure of the mutated GHR protein to bind GH or to be expressed at the cell membrane. In other types of this syndrome, the GHBP levels are either normal or elevated. One of them, with normal GHBP levels and a point mutation in the extracellular domain, was found to be caused by inactivated GHR due to impairment of expression and signaling. The third type of GHR mutation has increased GHBP levels, and in this case a point mutation resulted in alternative splicing and a premature stop codon, with the resulting receptor protein lacking the transmembrane domain and most of the intracellular domain. The truncated receptor was unable to anchor to the cell membrane, but was instead secreted in large amounts into the circulation, thus causing the elevated GHBP levels and lack of GH response. In addition, there are cases of GH insensitivity with normal GHR function, explained by abnormalities in postreceptor signaling mechanisms. A fourth mechanism of GHR inactivation, through a dominant negative mutation, will be discussed below.

Another example of inactivating receptor mutations is that of the TSHR. A number of mutations are known, and depending on the extent of receptor inactivation, their phenotypes vary greatly. The mildest cases represent euthyroidism with elevated TSH levels; the mutation lowers only the affinity of the TSHR-ligand complex, and higher TSH levels can maintain euthyroidism, since otherwise the receptor function is normal. In more severe cases, the affected individual is hypothyroid, and the mutation causes sequestration of the mutated receptor inside the cells and near total abolition of its function.

A third example of inactivating receptor mutations concerns those of the gonadotropins LH and FSH. The inactivating LHR mutation in the male causes, depending on the extent of receptor inactivation, an array of phenotypes ranging from micropenis and hypospadias to total lack of male sexual differentiation (XY, pseudohermaphroditism). The female phenotype is much milder, entailing only anovulatory infertility. In functional analyses of the LHR mutations there is a good correlation between the severity of phenotype and the completeness of receptor inactivation, as demonstrated by the variably suppressed functional responses to gonadotropin stimulation in cells expressing the various forms of mutated receptors. In most cases, the real mechanism of receptor inactivation, whether a defect in synthesis, intracellular processing, transport to the plasma membrane, ligand binding, or signal transduction, has not been studied.

As with the LHR, inactivating mutations of the FSHR show correlation between the severity of phenotype and the completeness of receptor activation. Complete receptor inactivation in women causes primary hypergonadotropic amenorrhea with impaired pubertal development, lack of follicular maturation, and infertility, whereas the phenotype of incomplete receptor activation is secondary amenorrhea with arrest of follicular maturation. In men, a surprisingly mild phenotype with variable derangement of spermatogenesis, but no obligatory infertility, is observed with complete FSHR inactivation. The impairment of function of the mutated FSHR is primarily due to sequestration of the synthesized receptor protein inside the cell. The few mutated receptors expressed at the plasma membrane of transfected cells seem to display a normal cAMP response to hormonal stimulation.

IV. DOMINANT NEGATIVE MUTATIONS

The concept of dominant negative mutation means that a heterozygously inherited mutated receptor has the capability of blocking the action of functional receptors encoded by the wild-type allele. Abolition of hormone action occurs thus even though only one allele is mutated, and therefore, the mode of inheritance is dominant despite a loss-of-function mutation. This type of inactivation mechanism is encountered with receptors that need to be homo- or heterodimerized in order to achieve their biological effects. The different modes in which a dominant negative mutation brings about inactivation of the wild-type receptor are presented in Fig. 2.

In thyroid hormone resistance, typical loss-of-function mutations with recessive inheritance are rare. Usually, the mutation causes receptor inactivation in a dominant negative fashion. In most cases, the patients have an inactivating mutation in one of their thyroid hormone receptor (TR) β alleles. The mutated receptor interferes negatively with functional receptor proteins encoded by the two normal TRα alleles and one TRβ allele. Unliganded TR is bound as a homodimer, or as a heterodimer with RXR, to the thyroid hormone response element of a target gene and is associated with a complex of corepressor proteins, being transcriptionally inactive. Mutations usually abolish ligand-binding activity

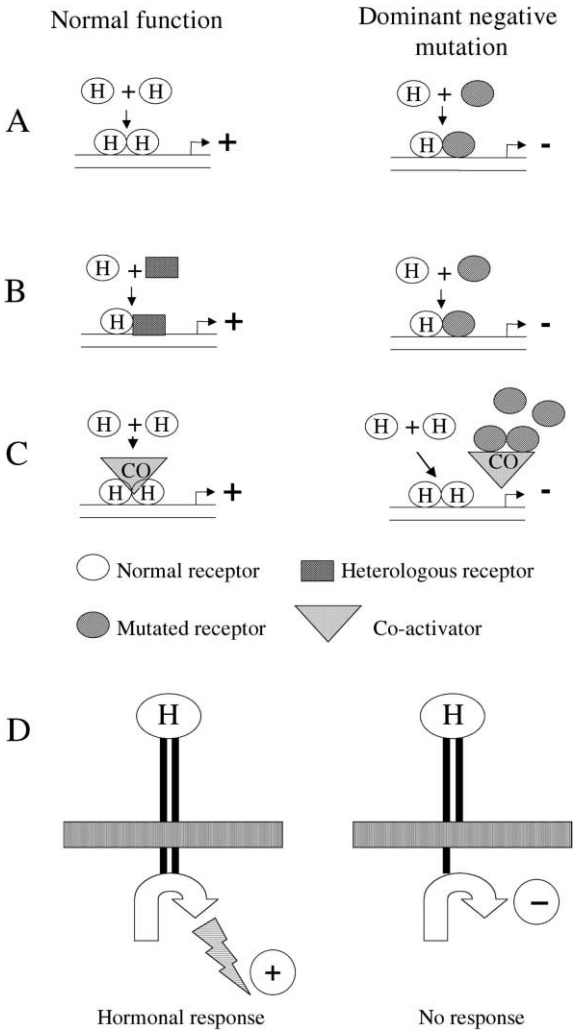

FIGURE 2 Models for mechanisms of receptor inactivation by dominant negative mutations of nuclear (A–C) and cell membrane (D) receptors. In each case, this type of receptor inactivation requires that the wild-type receptor forms homo- or heterodimers in order to exert its biological action.

of the TR. Normally, ligand binding promotes replacement of co-repressor with co-activators, which results in receptor activation. Since the mutated receptor dimerizes with wild-type TR, it keeps the complexes transcriptionally inactive, explaining the dominant negative effect. Mutated TR may also make stable dimers with RXR at the TR recognition site, thus blocking the binding of wild-type TR, needed for activation of the heterodimer.

In retinoic acid receptors, chromosomal rearrangements and other types of mutations have been described in connection with various malignancies; some of the mutated genes function in dominant negative fashion and some in gain-of-function fashion.

Dominant negative mutations can explain hormone resistance in the molecular pathogenesis of a rare syndrome with insulin resistance, acanthosis nigricans and polycystic ovary syndrome (type A insulin resistance). Another example of a dominant negative mutation in cell membrane receptors concerns GHR. In this case, a particular single base substitution can cause skipping of exon 9 of the GHR upon transcription with consequent formation of a receptor without the intracellular domain, which is an essential part for signal transduction. The mutated receptor was unable to transmit signal, but it effectively heterodimerized with a wild-type receptor to form dimers devoid of signal transduction capability (Fig. 2D).

V. SUMMARY

The hormone receptor mutations can be divided in two categories: (1) the constitutively activating mutations that cause inappropriate activation of a hormonal effect in the absence of ligand hormone and (2) the inactivating mutations that are the most common cause of hormone resistance syndromes. Mutations have already been detected in a number of hormone receptor genes, and new mutations are expected to be found. However, it should be kept in mind that the receptor mutations may represent only the tip of the iceberg. All genes encoding the various components of a hormonal regulatory cascade, from synthesis of ligand to specific cellular responses downstream of receptor activation, are prone to mutations. Conditions with a clear phenotype of activating or inactivating receptor mutation, but intact receptor function, are candidates for mutations of the other genes involved in hormone action. Due to the large number of such candidate genes it will be a long time before all aspects of the molecular pathogenesis of inappropriate hormone action and hormone resistance are fully understood.

Glossary

activating (gain-of-function) mutation Structural alteration of the receptor makes it constitutively activated, i.e., in the absence of ligand hormone. Activating mutation can also increase the receptor's affinity for the ligand hormone, alter its binding specificity, or permit new functions.

cell membrane receptor A hormone-binding transmembrane protein. Binding of the ligand hormone triggers a cascade of responses: conformational change of the receptor → activation of the intracellular second-messenger response(s) → secondary intracellular

response(s) → functional target cell response(s) to hormone stimulation.

dominant negative mutation A mutation in a receptor that must be dimerized to evoke the functional response. When the mutated receptor dimerizes with wild-type receptor, the heterodimer remains functionally inactive.

hormone resistance Disease condition in which a specific hormone is unable to exert its biological actions; it is usually caused by an inactivating receptor mutation.

inactivating (loss-of-function) mutation A mutation that inactivates the function of a receptor by one of the following mechanisms: decreased synthesis, aberrant intracellular processing, impaired or missing ligand binding, impaired or missing signal transduction, inability to anchor to plasma membrane (cell membrane receptors), inability to dimerize (if needed for action), or increased degradation.

nuclear receptor A hormone receptor present inside the cell, in either the cytoplasm or the nucleus, binding hormones that are able to pass through the cell membrane (e.g., steroid and thyroid hormones). The hormone-receptor complex binds in dimerized form to specific promoter sequences of hormone-responsive genes, thus functioning as a transcription factor.

See Also the Following Articles

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Activin Receptor Signaling

PETER C. K. LEUNG* AND CHUN PENG†

University of British Columbia, Vancouver • †York University, Toronto

Activins are dimeric proteins consisting of two inhibin β-subunits. Homo- and heterodimerization of two isoforms of β subunits, βA and βB, result in three forms of activins, activin-A, -B, and -AB. It is now recognized that activins function mainly as autocrine/paracrine factors to regulate the proliferation, differentiation, and apoptosis of many types of cells and are involved in a variety of physiological processes. Activins also play important roles during embryonic development. Activins elicit their biological effects through interaction with a receptor complex that contains two types of structurally related transmembrane serine/threonine kinases referred to as type I and type II receptors, respectively. The signal is then propagated from the receptors at the cell surface to target genes in the nucleus by intracellular mediators, the Smad family of proteins. This signaling cascade can be modulated at the cell surface by interfering with the interaction between activins and their receptors and in the cytoplasm by facilitating or blocking the activation

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of Smads. Finally, activin signaling can be terminated through ubiquitin-mediated degradation of Smads.

Activins share structural similarities with transforming growth factor-β (TGF-β) and are recognized as members of the TGF-β superfamily. In addition to activins and TGF-βs, bone morphogenetic proteins (BMPs), growth and differentiation factors, inhibins, Müllerian inhibiting hormone, glial cell line-derived neurotrophic factors, and Nodal are also members of this superfamily.

Originally, activin-A was isolated from porcine follicular fluids and identified as a stimulator of pituitary follicle-stimulating hormone (FSH). This molecule was found to be identical to a protein that exhibited potent differentiating effects on erythroleukemia cells and was designated erythroid differentiation factor.

I. ACTIVIN RECEPTORS

Initial affinity labeling and cross-linking studies using activin-A as a ligand revealed the presence of two activin receptor complexes with molecular weights of approximately 65 and 85 kDa, designated type I and type II activin receptor complexes, respectively. Subsequently, cDNAs and genes for type I and type II activin receptors were cloned. Receptors that can bind to activin in transfection assays include activin type I (ActR-I, also known as ActR-IA or activin receptor-like kinase 2, ALK-2), ActR-IB (or ALK-4), ActR-II (also named ActR-IIA), and ActR-IIB. However, as discussed below, these receptors can also bind to and mediate the actions of other ligands of the TGF-β superfamily.

A. Type II Receptors

Using an expression cloning strategy, in 1991 Mathwes and Vale cloned the first activin receptor from AtT20 mouse corticotropic cells. The cDNA encodes a protein of 513 amino acids. The mature peptide has 494 amino acids and contains an extracellular ligand-binding domain, a single transmembrane domain, and an intracellular region containing a juxtamembrane region, a kinase domain predicted to be a serine/threonine kinase, and a C-terminal tail (Fig. 1). Based on its ability to bind activin and its size, the receptor was identified as the type II activin receptor and is now referred to as ActR-IIA. Another activin receptor that showed a structure similar to that of ActR-IIA was subsequently

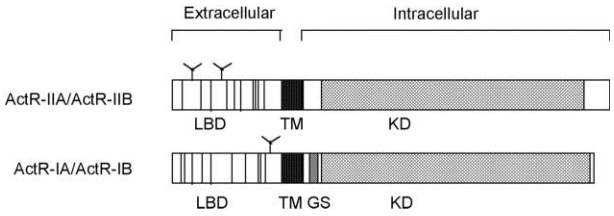

FIGURE 1 Schematic representation of activin receptors. Both type I and type II receptors have an extracellular region where the ligand-binding domain (LBD) is located, a transmembrane (TM) region, and an intracellular region containing a juxtamembrane region, a serine/threonine kinase domain (KD), and a C-terminal tail. The LBD has 10 cysteine residues (indicated by vertical lines) and one or two glycosylation sites (ϒ). Type I receptors have a GS domain in the juxtamembrane region and a shorter C-terminal tail.

cloned and designated ActR-IIB. The human ActRII-A gene is mapped to 2q22.2–q23.3 while the ActR-IIB gene is located in 3p22. When type II receptors are expressed alone in mammalian cells, they are able to bind to activins with high affinity. The K_d value of activin type II receptors ranged from 0.1 to 0.7 nM.

The extracellular domain of activin type II receptors has two N-glycosylation sites and 10 cysteine residues (Fig. 1). The spacing between each cysteine is identical between ActR-IIA and ActR-IIB and is conserved in different groups of vertebrates. Studies on the ActR-IIA have shown that deglycosylation of the receptor resulted in only a moderate decrease in receptor affinity, suggesting that it is not critical for receptor binding. On the other hand, disulfide bonds formed by cysteine residues are important in maintaining the proper confirmation required for activin binding. In ActR-IIA, five disulfide bonds are formed from the cysteines in the following arrangement: C1–C3, C2–C4, C5–C8, C6–C7, and C9–C10. The crystal structure of the extracellular ligand-binding domain has been solved. This region exhibits a three-finger toxin-like fold formed by seven disulfide-cross-linked β sheets. Further analyses have revealed that three conserved hydrophobic residues (Phe42, Trp60, and Phe83) interact with one another to form a binding site on ActR-IIA for activin-A.

The kinase domain of ActR-IIA and ActR-IIB contains sequence similar to that of serine/threonine protein kinases. Studies have shown that they indeed have kinase activities. Substrates of type II receptor kinases include the receptors themselves and type I activin receptors. Type II receptors are constitutively phosphorylated, even in the absence of ligands. The phosphorylation of type II receptors is, at least

in part, the result of autophosphorylation. The phosphorylation occurs predominantly on serine residues and to some extent on threonine.

Gene knock-out studies in mice have shown that deletion of the ActR-IIA gene resulted in severe suppression of FSH secretion and impaired reproductive functions. Female mice showed defects in folliculogenesis and were infertile. Male mice showed decreased sperm production and delayed fertility. These findings demonstrate that ActR-IIA is a functional receptor that mediates the action of activin on FSH secretion. On the other hand, the ActR-IIA-deficient mice had several developmental defects that are quite distinct from those found in activin-deficient mice, suggesting that other ligands, rather than activins, signal through ActR-IIA to regulate embryonic development. Targeted disruption of the mouse ActR-IIB gene caused abnormal left–right axis formation and lateral asymmetry. Mice deficient in the ActR-IIB gene died shortly after birth due to complicated cardiac defects. The developmental abnormality of axial formation observed in ActR-IIB knockout mice is a characteristic nodal activity, suggesting that ActR-IIB mediates Nodal signaling during embryonic development.

Type II activin receptors have a broad specificity. In addition to binding to activins, they have been shown to bind to BMPs, such as BMP2 and BMP7, and Nodal when co-expressed with appropriate type I receptors. Studies using truncated ActR-IIA and IIB have shown that these receptors mediate both activin and BMP signaling. As discussed above, gene knock-out experiments also support the notion that other ligands signal through type II activin receptors to regulate vertebrate embryonic development.

B. Type I Receptors

Following the cloning of type II receptors, the sequences of type I activin receptors were also obtained. Similar to type II receptors, the type I receptors also have an extracellular ligand-binding domain, a transmembrane domain, and an intracellular kinase domain. The extracellular domains of type I receptors also have 10 cysteine residues; however, the spacing among these residues is different between ActR-IA and ActR-IB and also differs from that in the type II activin receptors. A unique feature of the type I receptors is that they contain a segment of about 30 residues that is rich in serine, threonine, and glycine, located between the transmembrane and kinase domains. This segment has a characteristic sequence of SGSGSG and therefore was named the GS domain. The GS domain is present in all type I receptors of the TGF-β superfamily. The C-terminal tail in the type I receptors is shorter than that in the type II receptors. The human ActR-IA is mapped to 2q23–q24 and the ActR-IB gene is located at 12q13.

Unlike type II receptors, type I receptors do not bind to activins on their own. They can bind to ligands only in the presence of activin type II receptors. On the other hand, type I receptors are essential for receptor signaling to downstream mediators. It is well established now that activins first bind to type II receptors, which then recruit type I receptors to form a complex that contains two or more of each of the type I and type II receptors. Type II receptors subsequently phosphorylate type I receptors, primarily, but not exclusively, at the GS domain, which then in turn propagate the signals to downstream targets, namely, Smads.

Although initial studies demonstrated that ActR-IA, when co-expressed with type II activin receptors, can bind to activins, increasing evidence has indicated that the physiological ligand for this receptor is not an activin. In *Xenopus laevis* embryos, ActR-IA mimics the role of BMP-4, but not activins. Many other reports have documented that ActR-IA binds to and/or mediates the signaling of other members of the TGF-β superfamily, including Müllerian inhibiting substance, BMP-2, BMP-4, and BMP-7. Also, ActR-IA activates Smad1, which is known to mediate BMP, but not activin, signaling. On the other hand, the role of ActR-IB in activin signaling has been confirmed. ActR-IB mediates the growth-inhibitory effects of activin in several cell lines. Also, the regulatory effects of activins on target gene expression, such as plasminogen activator inhibitor-1, are conferred by ActR-IB.

II. SMADS AS INTRACELLULAR MEDIATORS

Once type I receptors are phosphorylated by type II receptors, they become active kinases and can phosphorylate downstream signaling molecules. Smad proteins are intracellular mediators of the TGF-β superfamily. They function as effector molecules to transduce the signals of the TGF-β superfamily from the cell surface to the nucleus. The first member of the Smad family was discovered in *Drosophila melanogaster* through genetic screenings and was named Mad (mother against *dpp*). A related protein was also identified in *Caenorhabditis elegans* and is referred to as Sma. Subsequently, homologues of Mad and Sma were found in various vertebrates and are now called Smads.

A. Structural Features of Smads

To date, eight Smads have been identified in vertebrates and they are classified into three subfamilies based on their structural and functional characteristics: receptor-regulated Smads (R-Smads), common Smads (Co-Smads), and inhibitory Smads (I-Smads). The R-Smads, Smad1, 2, 3, 5, and 8, are activated by specific type I receptors of the TGF-β superfamily through phosphorylation. Smad4, also referred to as "deleted in pancreatic carcinoma" (DPC4), is the only Co-Smad in vertebrates and can form a complex with phosphorylated R-Smads. The I-Smads (Smad6 and 7) function as inhibitors of the receptor-regulated Smads. Figure 2 shows the major structural features of Smads. In R-Smads and Co-Smads, there are two highly conserved regions, MH1 and MH2. These two domains, located at the N-and C-terminals, respectively, are linked by a less conserved linker region. The MH1 domain is much shorter in the inhibitory Smads than in the R-Smads and Smad4. R-Smads also have a characteristic phosphorylation site, SSXS, at their C-termini. All Smads lack intrinsic enzyme activity. They function primarily through regulation of protein–protein and protein–DNA interactions.

Smads that have been identified as being involved in activin receptor signaling include Smad2, 3, 4, and 7. This set of Smads also mediates TGF-β signal transduction. In human, genes for Smad2, Smad4, and Smad7 are all mapped to the region 18q21.1, and the Smad3 gene is found at 15q21–q22. The MH1 domain of Smad3 can bind to DNA directly, but the MH1 domain of Smad2 lacks DNA-binding activity. This is apparently due to an insertion of 30 residues in the MH1 domain of the Smad2. This insertion is thought to alter the conformation of the MH1 domain, thereby preventing the direct interaction of Smad2 with DNA. The MH1 domain of Smad4 also has DNA-binding activity. The MH2 domain of Smad2, Smad3, Smad4, and Smad7 is mainly responsible for protein–protein interactions. Proteins that are found to associate with this region include Smad themselves to form oligomers and many transcription factors. In the case of Smad2 and Smad3, this domain also contains phosphorylation sites for ActR-IB and a binding site for a Smad anchor for receptor activation (SARA), which recruits Smad2 and 3 to activin and TGF-β receptors. The linker region, although poorly conserved, also has important functions. This region is known to contain the phosphorylation sites for mitogen-activated protein kinases. The linker region of Smad4 has a Smad activation domain that is important for transcriptional activation.

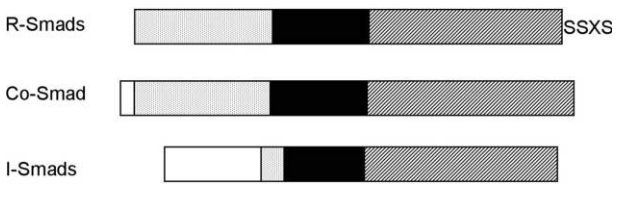

FIGURE 2 Structural features of Smads. R-Smads and Co-Smad (Smad4) have two conserved domains, MH1 (gray) and MH2 (vertical rules), connected by a linker region (black). I-Smads have a much shorter MH1 domain.

B. Signal Transduction via Smads

Upon activin binding, Smad2 and (or) Smad3 are transiently associated with activin receptor complexes. Both Smad2 and Smad3 can be phosphorylated by ActR-IB. Subsequently, the activated Smad2 and (or) Smad3 form a complex with Smad4 and are translocated into the nucleus. This Smad complex then interacts with DNA to regulate target gene expression. Although the Smad complex can bind to DNA directly, the affinity and specificity of such binding are low and therefore DNA-binding partners are required for regulation of specific target gene expression. The best-characterized transcription factor that interacts with Smads to regulate activin-induced transcription is the forkhead activin signal transducer (FAST), a winged helix forkhead transcription factor. Activin induces the transcription of homeobox genes *Mix*. 2 in *Xenopus* and *goodecoid* in mouse during embryonic development. Activation of these genes by activin involves the formation of a transcription complex comprising Smad2, Smad4, and FAST. The protein complex binds to specific elements in the promoter regions of these genes through both FAST- and Smad-binding sites. Following the binding of Smad and DNA-binding partners to the DNA, Smads can further recruit transcription co-activators or co-repressors to positively or negatively regulate gene expression.

III. MODULATION OF ACTIVIN SIGNALING

A. Inhibition of Signaling at the Cell Surface

Activin signaling can be blocked at the receptor level by two types of molecules, follistatins and inhibins. Follistatins are binding proteins of activins that neutralize activin actions in many biological systems

by controlling the accessibility of activins to their receptors. There are two major forms of follistatins, FS-288 and FS-315, generated from alternative splicing of the same gene. Each β-subunit can bind to one follistatin and therefore, the activin–follistatin complex consists of one activin molecule and two follistatin molecules. The affinity of follistatin binding to activins is similar to that between activins and their receptors. Follistatins neutralize activin bioactivity by inhibiting the binding of activins to their type II receptors. In addition, FS-288 inhibits activin action by increasing the endocytotic degradation of activin through the association with cell surface heparan sulfate.

Inhibins are structurally related to activins by sharing the same β-subunit. It is well documented that inhibins block many actions of activins and function as activin antagonists. Several studies have shown that inhibins can bind to ActR-IIA and, therefore, antagonize activin actions. The binding affinity of inhibins to ActR-IIA is 10-fold lower than that between activins and ActR-IIA; however, it has been recently shown that betaglycan, a proposed TGF-β type III receptor, can bind to inhibins with high affinity and that the binding of inhibins to betaglycan results in an increased affinity of inhibin binding to ActR-IIA. It was therefore suggested that inhibins bind to betaglycan and ActR-IIA and block activin signaling by competing with activins for their type II receptors.

B. Modulation in Cytoplasm

The interaction between activin receptors and Smads in the cytoplasm is facilitated by some proteins with a FYVE (Fab1p, YOTB, VAC1p, EEAI) domain, such as SARA and Hgs. It has been shown that SARA and Hgs (Hrs) cooperate to recruit Samd2 and Smad3 to

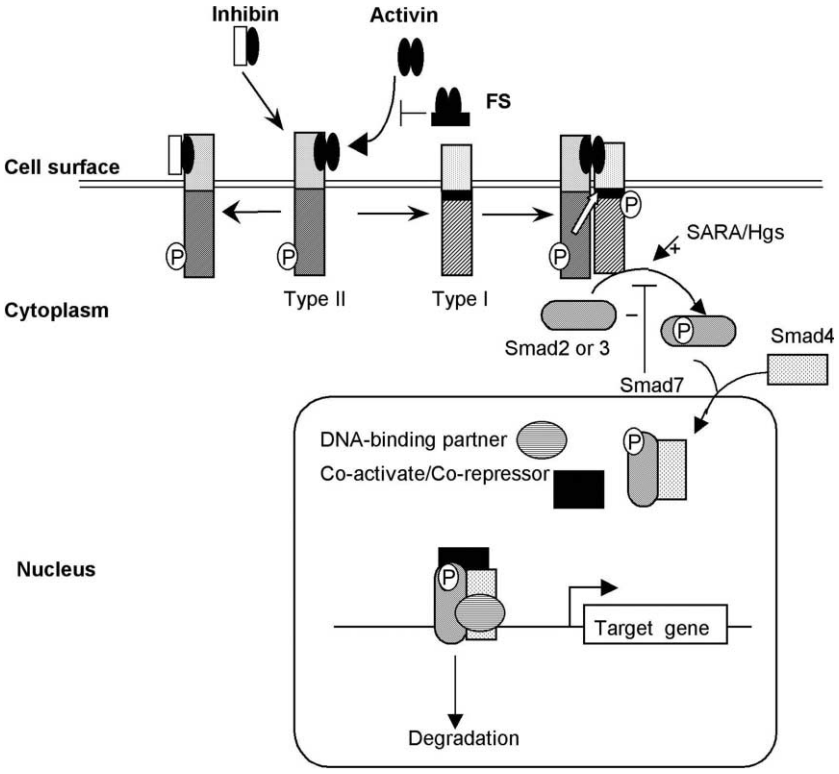

FIGURE 3 Activin signaling via the Smad pathway. Activin first binds to the type II receptors, which in turn recruit and phosphorylate type I receptors. The type I receptors then phosphorylate Smad2 and/or Smad3. The phosphorylated R-Smad forms a complex with Smad4 and is then translocated to the nucleus. The R-Smad–Smad4 complex interacts with other DNA-binding co-factors to regulate gene expression. Follistatin inhibits activin signaling by binding to activin and making it unavailable to its receptor. Inhibin blocks activin signaling by competing with activin for type II receptors. SARA and Hgs enhance activin signaling by recruiting R-Smads to the activin receptors while Smad7 inhibits activin signaling by preventing phosphorylation of R-Smads by the activin receptor. Finally, Smad signaling may be turned off via ubiquitin-mediated degradation.

the activin receptor complex. Overexpression of SARA and Hgs enhances activin signaling by increasing the phosphorylation and activation of Smad2 and Smad3 by ActR-IB. On the other hand, overexpression of a nonfunctional mutant of SARA or Hag decreases activin signaling.

Smad7 inhibits activin signaling by binding stably to the activated receptor complex and thereby blocking the association of Smad2 and/or Smad3 with the receptors and preventing the phosphorylation and activation of R-Smads. Smad7 may also facilitate the degradation of the active receptor complex. Interestingly, the Smad7 gene is induced by activin. Therefore, Smad7 likely plays a role in the negative feedback control of activin signaling. Smad7 has been shown to attenuate the actions of activin on cell growth, apoptosis, and *Xenopus* embryonic development.

C. Termination of Signaling in the Nucleus

The activated Smad2 has been shown to be degraded through the ubiquitin-dependent 26S proteasome pathway. Although the precise involvement of this pathway in activin signaling requires further studies, degradation of activated Smads in the nucleus could provide a mechanism to switch off activin signaling.

IV. SUMMARY

Although activin receptors were discovered almost a decade ago, the signaling mechanisms underlying activin actions have been uncovered only in recent years. As shown in Fig. 3, activins bind to cell surface serine/threonine kinase receptors via two activation steps. First, activins bind to constitutively active type II receptors and this complex then recruits type I receptors. Second, type II receptors phosphorylate type I receptors at the GS domain. Following receptor activation, the activin signal is transmitted into the nucleus via intracellular mediators, Smads. The type I receptor, ActR-IB, phosphorylates Smad 2 and/or Smad 3. The phosphorylated Smad2 and (or) Smad3 then form(s) a complex with Smad4, and the complex enters the nucleus. The Smad complex then interacts with other transcription factors, such as FAST-1, and recruits transcription co-activators or co-repressors to regulate the expression of target genes. Activin signaling can be blocked at the receptor level by their binding proteins, follistatins, and by their antagonists, inhibins. In the cytoplasm, SARA and Hgs facilitate activin signaling by recruiting R-Smads to activin receptors while Smad7 inhibits activin signaling by blocking the interaction between R-Smads and receptors. Finally, Smad signaling can be terminated through the proteolytic degradation of Smads via the ubiquitin-dependent pathway.

Glossary

activins Protein hormones belonging to the transforming growth factor-β superfamily that regulate many developmental and physiological processes, particularly reproduction. They are either homo- or heterodimers of two related inhibin β-subunits, βA and βB.

receptor serine/threonine kinase A membrane receptor that phosphorylates serines and/or threonines on target proteins. The receptor has an extracellular domain, a transmembrane region, and an intracellular kinase domain.

Smad proteins A group of intracellular proteins that mediate signaling by members of the TGF-β superfamily. They are vertebrate homologues of *Drosophila* Mad (mother against *dpp*) and *Caenorhabditis elegans* Sma. They include receptor-mediated Smads that are phosphorylated and activated by type I receptors, common Smads that form complexes with receptor-mediated Smads, and inhibitory Smads that antagonize signaling by members of the TGF-β superfamily.

type I activin receptor A component of the activin receptor complex. It is a serine/threonine kinase that phosphorylates intracellular mediators after being phosphorylated by a type II activin receptor.

type II activin receptor A component of the activin receptor complex. It binds to activins and in turn phosphorylates type I receptors via its intracellular serine/threonine kinase domain.

See Also the Following Articles

Activins • Angiotensin II Receptor Signaling • Follicle Stimulating Hormone (FSH) • Inhibin Receptor Signaling • Inhibins, Activins, and Follistatins • Luteinizing Hormone Receptor Signaling • Protein Kinases

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Activins

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Activins, acting in opposition to inhibins, are involved in diverse physiological activities, including stimulating pituitary, hypothalamic, and gonadal hormones, insulin secretion, germ cell development and differentiation, erythroid differentiation, nerve cell survival, and embryonic development. Activin β-subunit types A–E form homodimers and heterodimers that play different roles in health and disease in different tissues throughout the body. Assays that allow comparisons of specific subunit activities are necessary to understand the complex roles of each of the activin subunits.

I. INTRODUCTION

Activin subunits are present in numerous human tissues of both endocrine and nonendocrine organs. Homo- or heterodimers of activin β_A or β_B subunits are members of the transforming growth factor-β (TGF-β) superfamily of growth and differentiation factors. Since these proteins were first described as regulators of the release of follicle-stimulating hormone (FSH) from the pituitary, multiple actions have been assigned to them in a variety of tissues. An additional three activin β-subunit proteins have been described, i.e., β_C, β_D, and β_E, and there is a growing family of recognized activin- and inhibin-binding proteins, receptors, and signaling molecules (Table 1).

II. EXPRESSION AND BIOSYNTHESIS OF ACTIVINS

The TGF-β superfamily of growth factors includes bone morphogenetic proteins (BMPs) and Müllerian inhibitory substance (MIS), and currently over 45 members of this family have been identified. Structural similarities between activins and other members of the TGF-β superfamily are based on the conservation of the number and spacing of the cysteines within each subunit and the disulfide linkages between the two subunits that form the characteristic cysteine knots. Other similarities relate to dimer formation, the location of the bioactive peptide in the carboxy-terminal region of the precursor molecule, and the similarities in intracellular signaling mechanisms.

The biosynthesis of activins requires the formation of homo- or heterodimers of disulfide-linked β_A- or β_B-subunit proteins that share 63% amino acid identity. The assembly of different combinations of activin subunits β_A and β_B is reflected in their nomenclature; thus, activin A is a dimer composed of $\beta_A\beta_A$, activin B is composed of $\beta_B\beta_B$, and activin AB is composed of $\beta_A\beta_B$. There is an independent pattern of synthesis of the two subunit genes, which are located on different chromosomes; the activin β_A-subunit gene is located on 7p15–7p14 and the activin β_B-subunit gene on 2qcen–2q13.

Another subset of activin β-subunits (β_C, β_D, and β_E) was identified more recently, based on their homology to subunits β_A and β_B; this subset of subunits shares 62% amino acid identity with each other. The activin β_C-subunit shows close similarity to the activin β_E-subunit in terms of genomic organization and chromosomal localization on 12q13.1, and subunits β_C, β_D, and β_E are considered to be a separate subset of activins. The β_C-subunit dimerizes with itself *in vitro* and with subunits β_A and β_B to form the putative activins, activin C, AC, and BC. Activin subunits β_D and β_E have been isolated from *Xenopus* and mouse cDNA libraries, respectively, but their capacity to form activin heterodimers

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TABLE I Activin Ligands: Purified Homo- or Heterodimers of Activin β-Subunit Proteins

Subgroups of activin β-subunits	Purified proteins	Evidence of putative dimeric proteins
β_A	Activin A[a]	—
β_B	Activin B[a], activin AB[a]	—
β_C	Activin C[b]	Activin AC[c], activin BC[c]
β_D	?	—
β_E	Monomeric activin β_E	—

[a]Bioactive and detected in biological fluids.
[b]No known bioactivity.
[c]Heterodimeric proteins formed *in vitro*; currently there is no evidence for their existence *in vivo*.

with subunits β_A and β_B or between themselves is unknown.

Homo- and heterodimers of activin subunits β_A and β_B were originally isolated from follicular fluid as inactive full-length precursor proteins. Similar to other growth and differentiation factors, the precursor activins require intracellular processing for full bioactivity. Precursor activins are 110-kDa dimers and consist of a signal peptide, a glycosylated prodomain (Pro-β), and a mature C-terminal domain (β) that is bioactive. Proteolytic cleavage releases the pro and signal peptides, resulting in mature, unglycosylated activin subunits.

Dimers of the activin β-subunits form activin ligands, but heterodimers of activin β-subunits with the inhibin α-subunit result in the formation of inhibin proteins (inhibin A or B). The inhibin α-subunit gene is located on 2q33–q36 and the pattern of expression of the inhibin α-subunit is independent of the activin β-subunits. As is the case for activins, the inhibins are synthesized as precursor proteins and proteolytic cleavage occurs intracellularly as well as in serum.

III. SIGNALING OF ACTIVIN LIGANDS

The high degree of homology between the ligands extends to the elements in their signaling pathways. At the cell surface, activin ligands interact with a dual receptor system involving a family of transmembrane serine/threonine kinase receptors classified as type I or type II receptors. Activin binding to the type II receptor (ActRII) leads to the recruitment of the type I receptor (ActRI) and formation of a heteromeric complex. Formation of this complex induces phosphorylation of the type I receptor, leading to activation of the receptor-regulated Smad (R-Smad). R-Smads are ligand specific, with Smad2 and Smad3 mediating activin and TGF-β signaling and Smad1, Smad5, and Smad8 mediating BMP signaling. The interaction between the R-Smads and the receptor complex involves a membrane-bound protein named Smad anchor for receptor activation (SARA). After phosphorylation, the R-Smads are released and form heteromeric complexes with Smad4, a common mediator (Co-Smad). The R-Smad and Co-Smad complexes then translocate to the nucleus to regulate gene expression. A third class of Smads, the inhibitory Smads (I-Smads; Smad6 and Smad7), antagonize the signaling events just described and prevent access and phosphorylation of the R-Smads or interfere with the formation of the R-Smad/ Co-Smad complexes.

In the nucleus, Smads target specific gene promoters with low binding affinity for DNA, and Smad binding alone is insufficient for gene activation. Smads use members of the forkhead activin signal transducer (FAST; also called FoxH1) family as DNA-binding partners to regulate gene transcription. Other transcription factors likely to be involved in the Smad pathway include *fos*, *jun*, and the vitamin D receptor. However, in contrast to FAST/FoxH1, many of the other DNA binding partners can function independently of Smads, whereas FAST/FoxH1 target genes require Smad function for transcriptional activation. Thus, the binding of activin ligands to the membrane-bound receptor initiates a cascade of protein–protein interactions that controls gene expression and specific biological responses.

IV. ACTIVINS IN PHYSIOLOGY

A. Biological Actions of Activins

Activins were originally isolated based on the stimulation of FSH production and secretion by rat pituitary cells *in vitro*. It is now recognized that activins have a range of biological activities that include, but are not limited to, mesoderm induction in *Xenopus laevis* embryos, reproduction through

the regulation of pituitary FSH production, bone growth, nerve cell survival, wound healing, and tissue differentiation in pancreas, kidney, and heart.

In some instances, the actions of activins are antagonized by inhibins. The most well-known opposing actions of activin and inhibin relate to effects on the reproductive system, including the regulation of pituitary FSH and gonadal steroidogenesis. Both ligands also oppose each other during chondrogenesis in chick limb bud mesoderm and in T-cell proliferation. However, there are other instances when the effects of activins are not opposed by inhibin, such as during mesoderm induction, in neuronal cell survival, and in some developmental processes. In these circumstances, other factors, such as activin-binding proteins (e.g., follistatins) are powerful antagonists of activins. In general, inhibins are regarded as endocrine factors that function primarily in the regulation of FSH, whereas activins are local paracrine and/or autocrine growth factors.

As predicted by their wide pattern of expression throughout the body and during all stages of life, the bioactivities of activins have been recorded in many cells and tissues (Table 2). *In vitro* model systems of cell growth (using cell lines and organ cultures) are common means to demonstrate significant effects that involve proliferative or antiproliferative actions, as well as those that alter cell differentiation. Most studies have been performed with activin A, less with activin B, and even fewer have reported the actions of activin AB; there are no reports of bioactivity of other putative activin dimers or heterodimers. Many of the effects of activins *in vivo* are inferred by expression of subunit mRNA or protein; due to the limited supply of purified ligands, very few studies have directly demonstrated *in vivo* actions of dimeric ligands. However, significant progress in understanding the roles for activins has been made using other approaches. Knockout and knock-in mouse models have been critical in the evaluation of the functional effects of activins, and many of these were developed in the Matzuk laboratory. As well, the development of specific immunoassays for the different forms of activins, follistatins, and inhibins has been essential in evaluating the expression and roles for specific ligands or binding proteins in physiology and in disease.

B. Knockout and Knock-in Mouse Models

A number of activin β-subunit knockout mice that exhibit unique phenotypes have been generated (Table 3). These studies confirm an essential role for activins in normal development of reproductive organs as well as other tissues. Activins are mesoderm-inducing factors, but mice deficient in subunits β_A or β_B develop to term. Activin β_A-subunit-deficient mice lack whiskers and lower incisors, have defects in their secondary palates, and die within 24 h of birth. Mice deficient in activin β_B are viable and fertile but have defects in eyelid development. The defects are additive in mice deficient in both subunits β_A and β_B and suggest that the subunits do not have overlapping functions up to this age of development. To determine if the unique phenotypes of these mice are related to differences in the temporal and spatial expression of the two subunits or to specific ligand–receptor signaling interactions, mice were generated in which activin β_B was expressed in the spatial and temporal pattern of activin β_A (i.e., activin β_B knock-in to the activin β_A locus). The craniofacial malformations

TABLE 2 Examples of Biological Fluids and Tissues in Which Activins A or AB or Follistatins Have Been Detected or Measured

Protein	Fluid or tissue
Activin A	Sera, e.g., from pubertal boys and girls, women with pre-eclampsia, or patients with chronic hepatitis
	Extracts, e.g., from gonadal, placental, choriodecidual, and allantoic tissues or fluids
	Media from cell lines, e.g., human prostate tumor cells, gonadotropin-releasing hormone-secreting neurons, and pituitary-derived folliculo-stellate cells
	Fluids, e.g., follicular fluid, seminal plasma, and amniotic fluid in normal and Down syndrome pregnancies
Activin AB	Follicular fluid from human, bovine, ovine, and porcine tissues
	Media from granulosa cells
Follistatin	Sera from pubertal girls, pre-eclamptic pregnancies, hypertensive pregnant women, postmenopausal women with epithelial ovarian cancer, and patients with chronic hepatitis
	Extracts from amnion, choriodecidual, and placental tissues
	Supernatants from prostate tumor cell line cells
	Amniotic fluid in normal and Down syndrome pregnancies

TABLE 3 Summary of Some of the Transgenic Mouse Model Phenotypes Bearing Modifications to Activin Subunits or Intermediates in Activin Signaling

Genetic modification	Phenotype
β_A knockout	Craniofacial defects, e.g., lack whiskers and lower incisors; cleft palate; die < 24 h after birth
Overexpression of β_A	Sterility in male and females; testicular degeneration, but no testicular tumors
β_B knockout	Viable and fertile but defects in eyelid development
β_A and β_B combined knockout	Additive defects; mice develop to term with defects in whiskers, incisors, and secondary palate formation
β_B knock-in to β_A locus	Rescues craniofacial malformations and neonatal lethality of β_A deficiency, but defects in hair, gonads, and external genitalia
Activin type II receptor knockout	Gonadal defects and altered fertility; some mice die due to mandible defects
Follistatin knockout	Craniofacial defects, growth retardation, and skin defects; neonatal lethal
Follistatin overexpression	Defects in testes, ovary, and hair
Inhibin α knockout	Gonadal and adrenal tumors develop, followed by cachexia-like wasting syndrome
Inhibin α + activin type II receptor knockout	Tumors develop, but without cachexia
Inhibin α + overexpression of follistatin	Reduced rate of tumor development and reduced symptoms of cachexia

and neonatal lethality were rescued and the results implied that activin subunits signaled through the same receptors in these tissues; further analyses revealed a greater level of complexity. In older mice, during testicular growth, $_B$-subunit does not replace the $_A$-subunit function and the knock-in mice show delayed testicular maturation and onset of fertility. Similarly, there are other defects in hair, ovarian growth, development of external genitalia, and somatic growth. Thus, although some actions of the $_A$ subunit are replaced by $_B$, others are not and the importance of the spatial and temporal patterns of expression of the subunits has been confirmed. The success of the approach of generating null mice for functional analyses of the activin subunits is less informative for the other activin subunits, $_C$ and $_E$. Mice lacking these subunits alone or in combination are viable and have no obvious abnormalities. Unequivocal evidence for a functional role for activin homo- or heterodimers of $_C$ or $_E$ or combinations with the $_A$ or $_B$ subunits has yet to be revealed.

C. Assays for Activins

The development of specific assays to measure the activin ligands in biological fluids and tissues, including serum, has significantly increased our understanding of the role of these ligands. Many, but not all, of the assays were developed in the Groome laboratory. The activin A assay measures total activin A, i.e., bound and unbound forms of the ligand, and is used to measure activin A in sera and other biological fluids (e.g., follicular fluid) in a number of species, including humans. A specific assay for activin B is unavailable, but an enzyme-linked immunosorbent assay (ELISA) for activin AB detects the homodimer in some biological fluids. A specific antibody for subunit β_C has been developed by Mellor *et al.*, who have also shown that β_C dimerizes with subunit β_A or β_B. The field awaits the development of new activin assays to expand our understanding of the role and expression of the different activin ligands in diverse tissues and systems. By analogy, the use of specific assays for inhibin A and B has been fundamental to understanding the roles of the inhibins in men and women, particularly in the field of reproductive physiology.

D. Overlapping Functions of Activins and Other Ligands in the TGF-β Superfamily

The relative redundancy between the ligands relates to the demonstration that β_B can replace β_A in some circumstances. The degree of overlap in functions of subunits that are less similar to subunits β_A and β_B is unknown, although activin subunit β_C can dimerize with β_A and β_B *in vitro*. The degree of overlap in the functions of activins and other members of the

TGF-β family is also unclear. TGF-β and activins signal through type I and type II receptors and utilize Smad2 and Smad3, but it is uncertain if the biological responses to the ligands are the same or different. Apparently, not all of the actions of activins are mimicked exactly by TGF-β or vice versa. For example, separate studies have shown that TGF-β inhibits cell proliferation in human prostate tumor cell lines PC3 and DU145, whereas the same cell lines fail to respond to activin A. Conversely, the LNCaP androgen-dependent human prostate tumor cells are growth inhibited by activin A, but are unresponsive to TGF-β. Relatively few studies have directly compared the ligands in the same biological systems, and the question of the redundancy between activins and TGF-β remains unexplored.

E. Follistatin-Binding Proteins

Prior to any signaling event, activins can be bound to binding proteins such as follistatins (FSs). In many tissues, the interplay between activins and FSs provides a potent mechanism to regulate the access of the ligands to their receptors. Follistatins are encoded from a single gene located on chromosome 5q11.2 and alternative splicing of the mRNA generates different molecular weight isoforms. The FS proteins include an "FS domain" of 10-cysteine structures and an extracellular calcium-binding domain that makes them structurally homologous with epidermal growth factor (EGF), a group of enzyme inhibitors of the Kazal family, and other proteins such as SPARC, agrin, testican, and follistatin-related protein (FSRP). The function of the FS domains is not known but binding to activins differentiates follistatins from other members.

The biological activity of FS is mediated via its high-affinity binding to activins. The binding is nearly irreversible and prevents ligand access to receptors on the cell surface, making FS binding a critical regulator of activin bioactivity in cells in which FS is expressed. FS also contains a heparin-binding domain that permits high-affinity association with cell surface heparin-sulfated proteoglycans (HSPGs). When activins are bound to FS–HSPG complexes at the cell surface or in the extracellular matrix, access to the receptors is restricted and hence the paracrine actions of locally or systemically derived ligands are inhibited. As well as activins, FS binds and neutralizes the mesoderm-inducing actions of other members of the TGF-β superfamily, such as BMP-2, -4, and -7, although the affinity of FS for the BMPs is significantly lower than that for the activins.

V. ACTIVINS IN PATHOLOGY AND DISEASE

The role and regulation of activin ligands in disease have received little attention in comparison to almost a decade of research into the use of inhibins for the detection of ovarian cancer or Down syndrome. Almost exclusively, the investigations center on activin A.

A. Pregnancy

In pregnancy, activin A (as well as inhibin A) is a product of the healthy placenta, but changes that have been reported to occur in pathological conditions might lead to diagnostic applications. Follistatins are also consistently higher at all stages of pregnancy, suggesting that activin A is present in bound form during a healthy pregnancy. Higher levels of activin A have been reported in women with pre-eclampsia, gestational hypertension, and chronic hypertension. In established cases of pre-eclampsia, several groups have shown that activin A (and inhibin A) levels are ~10 times higher, but it is not clear that changes in activin A are useful to predict the onset of pre-eclampsia in asymptomatic women. Research continues to assess if activin A, in combination with other markers, may be useful. The normal elevation in activin A in late pregnancy is related to the onset of parturition, but might also be used for the detection of preterm labor. There is no evidence of the usefulness of any activins as markers for Down syndrome, similar to that reported for inhibin A.

B. Inflammation

Activin A and FS have been implicated in inflammatory processes. In synovial fluid from patients with the inflammatory condition of rheumatoid arthritis, levels of activin A are elevated compared to those patients with degenerative osteoarthritis. An increased expression of activins related to change in β_A-subunit expression is implied in patients with inflammatory bowel diseases such as Crohn's disease and in ulcerative colitis. Indirect evidence for a role of activins has emerged from the detection of elevated levels of FS in patients with sepsis and meningitis, although activin levels in these conditions are unknown.

C. Kidney/Renal Ischemia

Evidence for a role of activin A in renal regeneration after ischemic injury has emerged from animal studies. Activin A, produced in the tubules, delays tubular

regeneration by inducing apoptosis, and FS antagonizes this effect. However, the interplay between activins and FS in the pathophysiology of renal diseases such as chronic glomerulonephritis remains unknown.

D. Bone

The osteogenic actions of activin A in bone have been demonstrated *in vivo* and *in vitro*. In animal models, activin A promotes fracture healing, and the detection of significant levels of activin A in bone matrix indicates an involvement in bone resorption and formation. Thus, activins (as well as FS) have potential roles to play in osteoporosis.

E. Tissue and Wound Repair

Mechanisms involved in tissue repair often closely resemble those involved in embryonic development. A number of studies have implicated activins in repair or regeneration of kidney, skin, and hair. During cutaneous wound repair in mice, activin A regulates formation of granulation tissue and extracellular matrix deposition. In animal models of acute brain injury, basic fibroblast growth factor (bFGF) is neuroprotective and neurotropic and induction of activin A appears to be a part of the signaling cascade for bFGF in this process. A potential role for activins in the prevention of neuronal loss during ischemic and traumatic brain injury in humans remains unknown.

F. Cancer

Activin, like TGF-β, can inhibit or stimulate tumor cell growth. For example, activins have growth inhibitory effects on breast, liver, and prostate cancer cells and pituitary adenomas, whereas growth stimulatory actions are described in ovary and testis. The role of activins in cancers is unclear and may be different in different organs and tissues or may vary even within the same tissue type. In tissues in which the effects are growth inhibitory, it is relevant to ask if the tumors become resistant to activins due to mutations in the signaling pathway, but very little is known about mutations in activin receptors or signaling factors specific for activin in cancer, compared to TGF-β. In tissues in which growth-promoting effects of activins have been documented, the sustained synthesis of the ligands in malignancy may contribute to tumorigenesis due to other effects of activins, e.g., on immune suppression, tissue remodeling, and angiogenesis. Apart from local effects on tumor tissues, activins are implicated in the promotion of cachexia. Mutant mice bearing deletions of both the inhibin α-subunit and the ActRII receptor develop gonadal tumors, but cachexia is reduced relative to animals with a single gene deletion.

VI. CLINICAL APPLICATIONS

In contrast to the assays for inhibins or the α-subunit, assays for activins remain to be validated for use as new diagnostic tools in clinical medicine.

VII. SUMMARY

There is substantial evidence for a role for activins in normal physiological systems and in disease. However, much of the information about these ligands relates to activin A (not activin B or activin AB), and even less is known about the biological roles of the other subset of activins (C and E) or the putative ligands formed between the groups of subunits. The overlap or redundancy between activins and other members of the superfamily is largely unexplored and few studies have made direct comparisons of the different members of the superfamily. For example, the bioactivities of TGF-β and activin A or B are seldom compared in the same models, and the regulatory actions of binding proteins such as follistatins, which bind both activins and BMPs, are unknown. Development of new assays and purified ligands to study these ligands and direct comparisons of activin and other TGF-β family members using new mouse models and new systems will facilitate and promote our understanding of the wide and diverse roles for activins in biology and disease.

Glossary

bone morphogenetic protein (BMP) Member of the transforming growth factor-β superfamily.
follistatins (FS) Activin-binding proteins.
Müllerian inhibitory substance (MIS) Member of the transforming growth factor-β superfamily.
transforming growth factor-β (TGFβ) Superfamily of growth and differentiation factors.

See Also the Following Articles

Activin Receptor Signaling • Anti-Müllerian Hormone • Bone Morphogenetic Proteins • Inhibins • Inhibins, Activins, and Follistatins

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Adipokinetic Hormones and Carbohydrate Metabolism

WIL J. A. VAN MARREWIJK
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Insect adipokinetic hormones (AKHs) belong to a large AKH/red pigment-concentrating hormone family, whose members are involved in the mobilization of energy substrates for flight activity. This article provides a survey of the way in which adipokinetic and other related hormones play a key role in the mobilization of carbohydrate reserves as flight fuels. Special emphasis is put on the hormonal control of the enzymes involved and on the mechanism of intracellular transduction of the hormonal signals.

I. CARBOHYDRATE MOBILIZATION

Carbohydrate is the major fuel in insects that fly only short distances, such as flies and bees, and it also provides most of the energy for the initial period of flight in long-term flying insects, such as locusts. Reserves of carbohydrate are stored in the fat body in the form of glycogen, which upon flight is degraded by the action of the enzyme glycogen phosphorylase. Unlike glucose in vertebrates and most invertebrates, the carbohydrate transport form in insects is trehalose, a nonreducing disaccharide that contains two D-glucose residues. The mobilization of fat body glycogen and the subsequent release of trehalose into the hemolymph is controlled by adipokinetic hormone/red pigment-concentrating hormone (AKH/RPCH) peptides, which are released by the corpus cardiacum upon the onset of flight. The involvement of these hormones in carbohydrate metabolism was demonstrated *in vivo* by the activation of fat body glycogen phosphorylase or by increases of trehalose in hemolymph after hormone injection and in fat body *in vitro* by activation of the enzyme or by increased trehalose release into the medium when isolated fat body tissue was incubated in the presence of hormone. The hormonal stimulation of carbohydrate mobilization in an insect does not necessarily lead to increased trehalose concentrations in the hemolymph under the appropriate physiological conditions. In the locust, for instance, in which the trehalose utilization rate is increased during prolonged flight, the enhanced rate of trehalose release does not lead to hypertrehalosemia.

II. HORMONAL ACTIVATION OF GLYCOGEN PHOSPHORYLASE

The rate of glycogenolysis in the fat body depends on the activity of glycogen phosphorylase. Hormonal stimulation of glycogenolysis leading to an increased

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I. CARBOHYDRATE MOBILIZATION

Carbohydrate is the major fuel in insects that fly only short distances, such as flies and bees, and it also provides most of the energy for the initial period of flight in long-term flying insects, such as locusts. Reserves of carbohydrate are stored in the fat body in the form of glycogen, which upon flight is degraded by the action of the enzyme glycogen phosphorylase. Unlike glucose in vertebrates and most invertebrates, the carbohydrate transport form in insects is trehalose, a nonreducing disaccharide that contains two D-glucose residues. The mobilization of fat body glycogen and the subsequent release of trehalose into the hemolymph is controlled by adipokinetic hormone/red pigment-concentrating hormone (AKH/RPCH) peptides, which are released by the corpus cardiacum upon the onset of flight. The involvement of these hormones in carbohydrate metabolism was demonstrated *in vivo* by the activation of fat body glycogen phosphorylase or by increases of trehalose in hemolymph after hormone injection and in fat body *in vitro* by activation of the enzyme or by increased trehalose release into the medium when isolated fat body tissue was incubated in the presence of hormone. The hormonal stimulation of carbohydrate mobilization in an insect does not necessarily lead to increased trehalose concentrations in the hemolymph under the appropriate physiological conditions. In the locust, for instance, in which the trehalose utilization rate is increased during prolonged flight, the enhanced rate of trehalose release does not lead to hypertrehalosemia.

II. HORMONAL ACTIVATION OF GLYCOGEN PHOSPHORYLASE

The rate of glycogenolysis in the fat body depends on the activity of glycogen phosphorylase. Hormonal stimulation of glycogenolysis leading to an increased

biosynthesis and release of trehalose has been investigated extensively in cockroaches, which rely mainly on carbohydrate as a fuel for flight activity. Hypertrehalosemic hormones (HTHs), representatives of the AKH/RPCH family, were shown to activate fat body glycogen phosphorylase and to stimulate trehalose synthesis in *Periplaneta americana* and *Blaberus discoidalis*. Activation of fat body phosphorylase by AKH peptides has been demonstrated in locusts, the moth *Manduca sexta*, and the beetle *Pachnoda sinuata*. In the locust *Locusta migratoria*, all three known AKHs (AKH-I, -II, and -III) are able to activate glycogen phosphorylase in a bioassay, although with different potencies. In vertebrates, phosphorylase is regulated by reversible phosphorylation/dephosphorylation between nonphosphorylated inactive

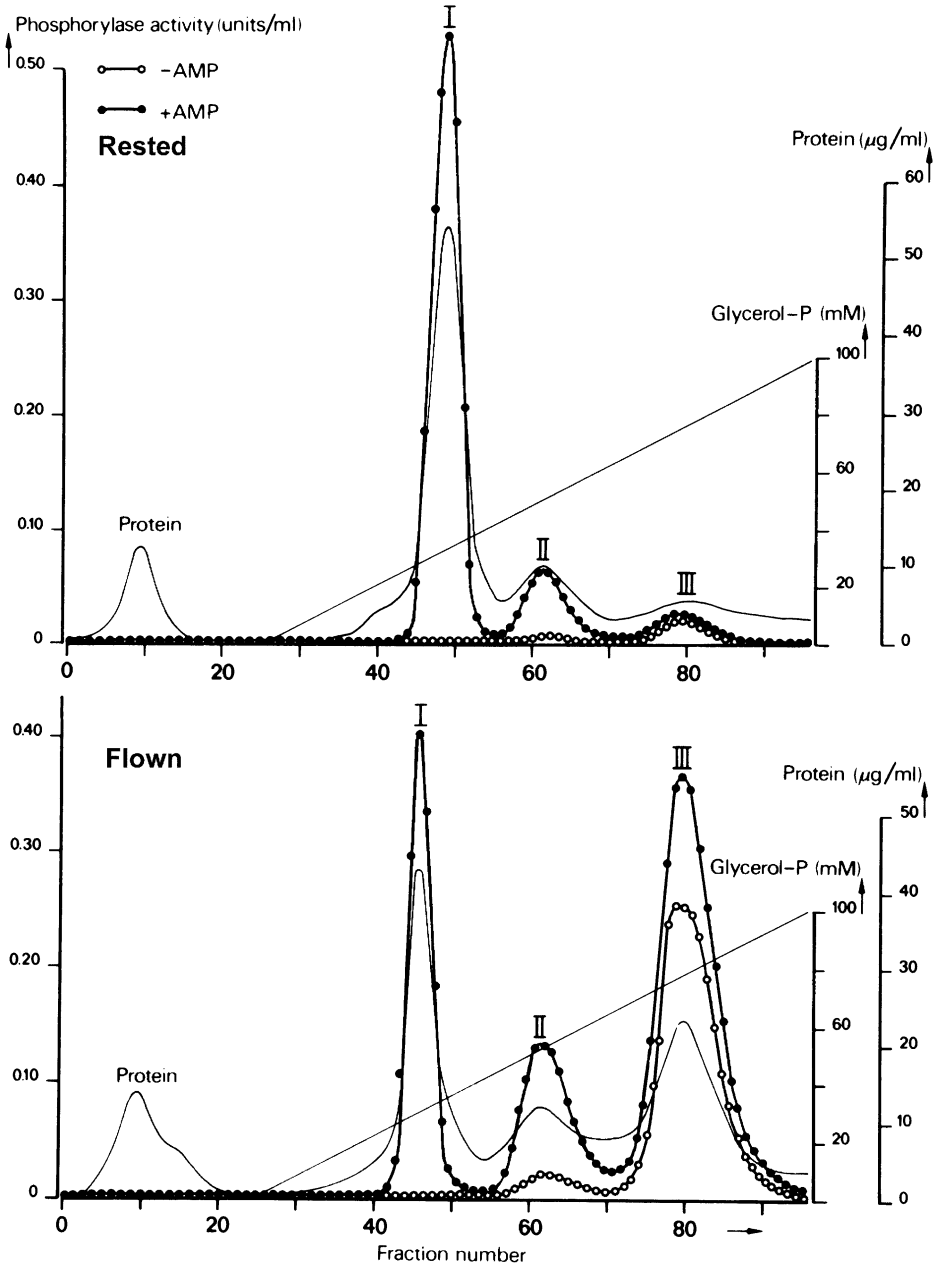

FIGURE 1 Fractionation of locust fat body phosphorylase by DEAE–Sephacel chromatography. Elution profiles were obtained with partially purified enzyme from the pooled fat bodies of 80 locusts at rest (Rested) and of 120 flown locusts (Flown).

phosphorylase *b* and phosphorylated active phosphorylase *a*. Also in locusts, flight activity as well as AKH injection induces the conversion of fat body phosphorylase *b* into the *a* form. This activating conversion indicates phosphorylation of each of the two subunits of the enzyme, which takes place in two steps, thereby giving rise to an intermediate hybrid *ab* form (Fig. 1). Activation of fat body phosphorylase by flight activity or hormone injection, combined with the occurrence of three forms of the enzyme including the partially phosphorylated phosphorylase *ab*, has also been demonstrated in *M. sexta* and *P. americana*. It has been suggested that the presence of this hybrid phosphorylase would provide for a sophisticated way of regulating carbohydrate metabolism in insects.

III. HORMONAL SIGNAL TRANSDUCTION

Binding of the AKH/RPCH peptides to their plasma membrane receptor(s) in the insect fat body results in the induction of a variety of signal transduction events that ultimately lead to the activation of target enzymes. As discussed above, the target enzyme in the mobilization of carbohydrate reserves is glycogen phosphorylase, which initiates the conversion of glycogen into trehalose. Signal transduction mechanisms have been investigated mainly in locusts and cockroaches, with focus on GTP-binding (G) proteins, cyclic AMP (cAMP), Ca^{2+}, inositol phosphates, signaling cross-talk, and hormone receptors. Some major results are discussed in this article.

A. Cyclic AMP and G-Proteins

In the fat body of *L. migratoria*, an accumulation of the second messenger cAMP, brought about by AKH-I has been demonstrated both *in vivo* and *in vitro*. Moreover, this accumulation of cAMP leads to the activation of glycogen phosphorylase, which is evidence in favor of a role of cAMP in AKH signal transduction. Each of the AKHs stimulates cAMP production in a dose-dependent manner in the fat body within 1 min. At a physiological dose of 4 nM, AKH-III is the most potent and AKH-I the least potent peptide hormone in stimulating cAMP production, and the same order of potency holds for the activation of glycogen phosphorylase by this hormonal dose. The observation that AKH-II is somewhat stronger than AKH-I in activating glycogen phosphorylase is in line with suggestions that the second AKH would be the major trigger for carbohydrate mobilization from the fat body and that the action of cAMP is directed more toward carbohydrate mobilization than lipid mobilization. The involvement of cAMP in the stimulation of glycogen mobilization is not a general feature of insects, since the HTHs of several cockroach species studied do not utilize this second messenger to activate trehalose synthesis in the fat body.

Experiments on locust fat body using cholera toxin (CTX) and pertussis toxin (PTX) suggest that the AKH receptor(s) is coupled to the G_s-protein (and not to G_i). The demonstration that AKH-I-, AKH-II-, and AKH-III-stimulated phosphorylase activation is ablated by the universal G-protein inhibitor guanosine-5′-*O*-(2-thiodiphosphate) strongly substantiates this suggestion.

B. Calcium

The relative importance of Ca^{2+} in signal transduction in the fat body is not equal in several closely related insect species. For example, the influx of extracellular Ca^{2+} into the fat body of *L. migratoria* has a much stronger stimulating effect on glycogen phosphorylase activity than the release of calcium ions from intracellular stores, but in *B. discoidalis* the opposite result holds. The presence of extracellular Ca^{2+} ions has been shown to be indispensable for the induction of fat body phosphorylase by AKH in *L. migratoria* and by HTH in *P. americana*. In the absence of Ca^{2+} in the medium, none of the three locust AKHs is capable of enhancing cAMP production and inducing glycogen phosphorylase activation in the fat body, but 1.5 mM Ca^{2+} (which is the Ca^{2+} concentration in hemolymph) is sufficient for complete activation (Fig. 2). Since the induction of

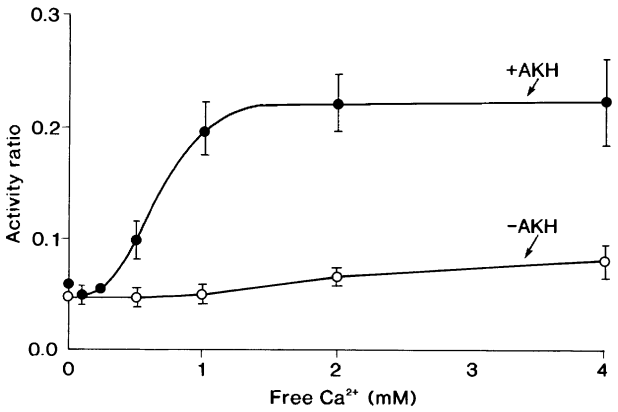

FIGURE 2 Dependence of AKH-induced activation of locust fat body phosphorylase on extracellular Ca^{2+}. Fat body was incubated for 15 min in the presence or absence of 40 nM AKH-I. Similar results were obtained with AKH-II and -III.

phosphorylase by cAMP in fat body was shown to be independent of extracellular Ca^{2+}, data suggest that at least part of the action of extracellular Ca^{2+} is at a site proximal to cAMP, e.g., the binding of the hormones to their receptor(s) or a Ca^{2+}-sensitive adenylyl cyclase.

All three locust AKHs are capable of stimulating the Ca^{2+} inflow into the fat body cells within 30 s with equal potency. This hormone-induced Ca^{2+} influx appeared to be mediated through depletion of intracellular Ca^{2+} stores, suggesting the functioning of a store-operated or capacitative Ca^{2+} entry mechanism. Simultaneously, the AKHs also enhance the efflux of Ca^{2+} from the fat body. At a physiological dose, AKH-III caused the strongest efflux and AKH-I the weakest; at a massive dose, their efficacy was equal. As the influx of Ca^{2+} exceeded the efflux, it is feasible that the intracellular Ca^{2+} concentration rises as a result of incubation of fat body with AKH.

C. Inositol Phosphates

For a maximal effect of AKH on glycogen phosphorylase activity in locust fat body, the release of Ca^{2+} from intracellular stores is required in addition to the availability of extracellular calcium. The same holds for the activation of glycogen phosphorylase and the stimulation of trehalose synthesis by HTH in *B. discoidalis* fat body. In the regulation of Ca^{2+} mobilization from intracellular stores, inositol phosphates ($InsP_n$) play a pivotal role, and the formation of these putative second messengers is induced by the locust AKHs. Each of the *Locusta* AKHs stimulates the synthesis of total $InsP_n$ within 1 min with different potencies: AKH-II barely induces any $InsP_n$ and AKH-III is more potent than AKH-I. The observation that the activation of glycogen phosphorylase by each of the AKHs is dampened by the phospholipase C (PLC) inhibitor U73122 suggests the involvement of $InsP_n$ (and/or diacylglycerol) in AKH signaling in the locust fat body.

All individual forms of $InsP_n$ are elevated by the AKHs, with $InsP_3$ and $InsP_4$ being the most interesting because of their presumed Ca^{2+} mobilizing actions (Fig. 3). With respect to $InsP_3$, AKH-III is again more potent than AKH-I, and the AKH-II-enhanced $InsP_3$ formation is quite small. The most prolonged effect on $InsP_3$ is caused by AKH-III. The high potency and prolonged effects of AKH-III with respect to the induction of various second-messenger systems apparently compensate (in part) for its low abundance relative to that of the other AKHs, and therefore, the effects of this hormone may be stronger than estimated from its relative amount in the circulation.

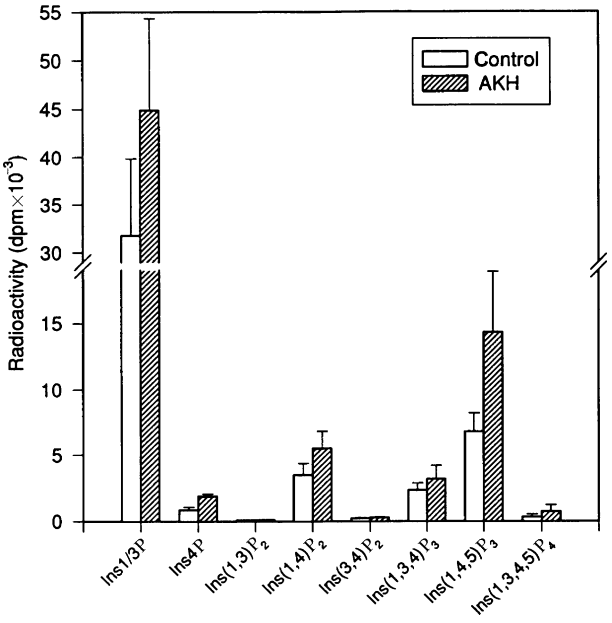

FIGURE 3 Effect of AKH on the formation of inositol phosphate isomers from locust fat body. Fat body tissue prelabeled with *myo*-[2-^{3}H]inositol was incubated for 1 min in the presence or absence (controls) of 40 nM AKH-I; then $InsP_n$ were isolated and separated by high-performance liquid chromatography and their radioactivity was measured. Results are expressed as disintegrations per minute per milligram of protein.

$Ins(1,4,5)P_3$ levels are greatly increased by HTH in the fat body of *B. discoidalis* in a time- and dose-dependent manner, which, along with the strong evidence for Ca^{2+} as component in the HTH second-messenger cascade, argues strongly for $InsP_3$ as a primary second messenger in response to HTH followed by the mobilization of intracellular Ca^{2+}.

D. Signaling Crosstalk

Crosstalk between signal transduction cascades provides the cell with a complex intracellular system for fine-tuning of hormone-induced signals. In locust fat body an elevation of cAMP levels does not influence the intracellular $InsP_n$ content, indicating that the basal PLC activity is not regulated by this cyclic nucleotide. Moreover, none of the signal transducing elements between the AKH receptor and PLC is affected by cAMP, since preincubation of fat body tissue with forskolin or dibutyryl-cAMP does not have an impact on AKH-induced $InsP_n$ production. Proof of a direct linkage between the AKH

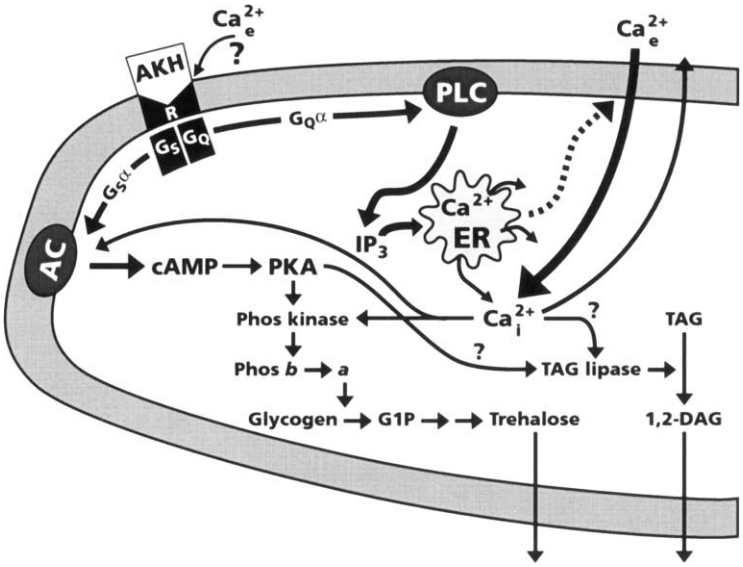

FIGURE 4 Tentative model for the coupling of AKH signaling pathways mediating the mobilization of energy substrates in the locust fat body cell. R, receptor; ER, endoplasmic reticulum; AC, adenylyl cyclase; PLC, phospholipase C; Phos, phosphorylase; G1P, glucose 1-phosphate; TAG, triacylglycerol; DAG, diacylglycerol.

receptor(s) and PLC (instead of a route via cAMP) came from the substantiation that the G-protein activator aluminum fluoride increases $InsP_n$ levels. Experiments using CTX, PTX, and GP antagonist-2A, a specific inhibitor of G_q, preclude the involvement of G_i and a G_s-sensitive isoform of PLC and evidence the involvement of G_q in the transduction of AKH signals toward fat body PLC (Fig. 4).

Glossary

adipokinetic hormone Peptide hormone in insects involved in the mobilization of substrates (predominantly lipids and carbohydrates) for energy generation needed by contracting flight muscles.

corpus cardiacum Neuroendocrine organ situated caudal to the brain to which it is connected by paired nerves. The organ combines the functions of neurohemal storage and adipokinetic/hypertrehalosemic hormone production.

fat body Organ whose functional role in insects is analogous to the combined functions of liver and adipose tissue in mammals. Fat body is the chief site of intermediary metabolism, detoxification, storage of nutrient reserves, and, in some species, deposition of nitrogenous waste products.

glycogenolysis Breakdown of glycogen; in insect fat body, the resulting glucose monomers are used for the biosynthesis of the disaccharide trehalose, the carbohydrate transport form in insects.

glycogen phosphorylase Enzyme that catalyzes the sequential removal of glycosyl residues from the nonreducing end (i.e., with a free 4′-OH group) of the glycogen molecule, using orthophosphate as a co-substrate and releasing glucose-1-phosphate.

hemolymph Blood of insects, which circulates in the body cavity between the various organs, bathing them directly. It consists of a fluid plasma in which the blood cells or hemocytes are suspended.

hypertrehalosemia Condition characterized by an increase in the level of trehalose in the hemolymph (insect blood) to values that significantly exceed the normal resting level.

hypertrehalosemic hormone Peptide hormone from the corpus cardiacum involved in the mobilization of fat body glycogen to fuel flight activity. For transport to the flight muscles, glycogen is converted into the disaccharide trehalose, the carbohydrate transport form in insects, whose level in hemolymph is raised during flight.

red pigment concentrating hormone Peptide hormone from the eyestalk of crustaceans; member of the adipokinetic hormone/red pigment-concentrating hormone family that comprises structurally related but functionally diverse peptides.

signaling cross-talk Occurrence of communication/interaction between different signaling pathways, resulting in an integrated response to a hormone-induced signal.

trehalase Enzyme that catalyzes the hydrolytic reaction by which trehalose is brought into the glycolytic mainstream: Trehalose + $H_2O \rightarrow$ 2D-glucose.

See Also the Following Articles

Adipokinetic Hormones and Lipid Mobilization

- **Adipokinetic Hormones: Structure and Biosynthesis**
- **Insect Endocrine System**

Further Reading

Becker, A., Liewald, J. F., and Wegener, G. (1998). Signal transduction in isolated fat body from the cockroach *Blaptica dubia* exposed to hypertrehalosaemic neuropeptide. *J. Comp. Physiol.* **168B**, 159–167.

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Adipokinetic Hormones and Lipid Mobilization

DICK J. VAN DER HORST AND ROB C. H. M. OUDEJANS
Utrecht University, The Netherlands

In the migratory locust, *Locusta migratoria*, the flight activity-induced release of adipokinetic hormones (AKHs; AKH-I, -II, and -III) from the neurosecretory cells of the corpus cardiacum, a neuroendocrine gland located caudal to the insect brain, has been studied extensively. The direct actions of these hormones on their fat body target cells trigger signal transduction processes that lead to the mobilization of lipid (diacylglycerol). This substrate fulfills a major role in energy metabolism of the contracting flight muscles during sustained flight activity. The molecular mechanism of diacylglycerol transport in insect blood involves a reversible conversion of the insect lipoprotein lipophorin, which has revealed a novel concept for lipid transport in the circulatory system.

I. INTRODUCTION

The flight activity of insects provides an exceptionally suitable yet relatively simple model system for studying the regulatory phenomena involved in energy expenditure during extreme physical activity. Similar to the processes used by vertebrates to generate energy for sustained locomotion, long-distance flying insects mobilize endogenous lipid (triacylglycerol) reserves to fuel oxidative metabolism during migratory flight. The fat body, which combines many of the properties and functions of vertebrate liver and adipose tissue, plays a fundamental role in lipid storage, as well as in the process of lipolysis controlled by adipokinetic hormones (AKHs). This article discusses the action of AKHs on lipid mobilization in fat body cells and the eventual AKH-induced transformation of the transport system of lipids in the insect blood, which will be exemplified using mainly the migratory locust (*Locusta migratoria*) as an insect model.

II. ADIPOKINETIC HORMONE SIGNAL TRANSDUCTION IN INSECT FAT BODY

Binding of the peptide adipokinetic hormones to their plasma membrane receptor(s) at the fat body cells results in the induction of a variety of signal transduction events that ultimately lead to the activation of target key enzymes. The first insect AKH receptors have recently been identified at the molecular level. The signal transduction mechanism of the three locust AKHs has been studied extensively and involves stimulation of cAMP production, which is dependent on the presence of extracellular Ca^{2+}. Additionally,

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the AKHs enhance the production of inositol phosphates including inositol 1,4,5-trisphosphate, which may mediate the mobilization of Ca^{2+} from intracellular stores. This depletion of Ca^{2+} from intracellular stores stimulates the influx of extracellular Ca^{2+}, indicative of the operation of a capacitative (store-operated) calcium entry mechanism. The interactions between the AKH signaling pathways ultimately result in the mobilization of stored reserves as fuels for flight. Although the carbohydrate (mainly trehalose) in the insect blood (hemolymph) provides the energy for the initial period of flight, additional trehalose is mobilized from fat body glycogen reserves by the AKH-induced activation of glycogen phosphorylase. At the same time, the concentration of lipid (diacylglycerol, DAG) in the hemolymph is increased progressively at the expense of stored triacylglycerol (TAG) reserves in the fat body and gradually constitutes the major substrate during prolonged flight.

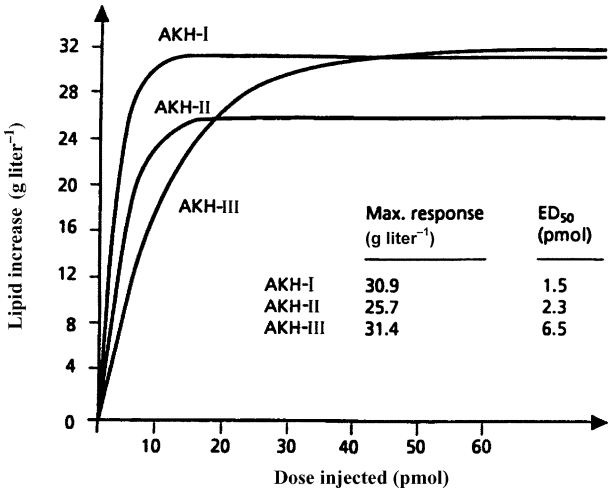

FIGURE 1 Dose–response curves for the lipid-mobilizing effects of AKH-I, -II, and -III in *L. migratoria*. Adult male locusts were injected with different doses of AKH or, in the controls, with saline, and after 120 min, the lipid content in hemolymph was determined. Responses represent increases in hemolymph lipid in AKH-injected locusts, expressed as grams per liter. For clarity, data points and standard error bars were omitted. ED_{50}, effective dose eliciting 50% of the maximum response.

III. EFFECT OF ADIPOKINETIC HORMONE ON LIPID MOBILIZATION

The namesake action of the AKHs on insect fat body cells results in the mobilization of lipid (TAG) stores. The increased production of DAG reflected by the increased concentration of DAG in the hemolymph indicates hormonal activation of the key enzyme, fat body TAG lipase. In a bioassay, all three *L. migratoria* AKHs (-I, -II, and -III) are able to stimulate lipid mobilization, although their relative potencies are different, as inferred from the dose–response curves shown in Fig. 1. This recalls the concept of a hormonally redundant system involving multiple regulatory molecules with overlapping actions. Results obtained with combinations of two or three AKHs, which are likely to occur together in locust hemolymph under physiological conditions *in vivo*, revealed that the maximal responses for the lipid-mobilizing effects were much lower than the theoretically calculated responses based on the dose–response curves for the individual hormones. In the lower (probably physiological) range, however, combinations of the AKHs were more effective than the theoretical values calculated from the responses elicited by the individual hormones.

The mechanism by which TAG lipase catalyzes AKH-controlled production of the DAG on which long-distance flight depends is only poorly understood, mainly due to technical problems in isolating or activating the lipase. In vertebrates, hormone-sensitive lipase (HSL) controls mobilization of TAG stores in adipose tissue, and although, in contrast to the situation in insects, free fatty acids (FFA) are released into the blood for uptake and oxidation in muscle, there is a clear functional similarity between vertebrate adipose tissue HSL and insect fat body TAG lipase. In vertebrate HSL, specific regulatory sites are phosphorylated *in vitro* by cAMP-dependent protein kinase (PKA), resulting in activation of HSL. The mechanism behind this activation of HSL upon phosphorylation by PKA is not well understood, but seems to involve translocation of HSL from the cytosol to the lipid droplet as well as conformational changes in the HSL molecule. Different phosphorylation sites in HSL may play different roles in the process of translocation and the increase in specific activity of the enzyme. Additionally, perilipins, a family of unique proteins intimately associated with the limiting surface of neutral lipid storage droplets, are acutely polyphosphorylated by PKA on stimulation of lipolysis, hinting at a role in this lipolytic process. Conformational changes of phosphorylated perilipins may expose the neutral lipid cores of the lipid droplets, facilitating the ensuing hydrolysis.

Even though a translocation of the insect fat body TAG lipase has not been established, the involvement

of AKHs in the process of lipolysis is beyond dispute. The effect of AKHs was demonstrated not only *in vivo* from enhanced levels of DAG in hemolymph of insects injected with the hormones as shown above, but also *in vitro* by the accumulation of DAG in isolated *L. migratoria* fat body tissue that was incubated in the presence of AKH. In later *in vitro* experiments, both cAMP and Ca^{2+} were shown to play an important role in the effect of AKH on lipolysis. The involvement of cAMP suggests a role for PKA in the phosphorylation and activation of fat body TAG lipase. In addition, an increase of fat body TAG lipase activity was measured in gregarious locusts *in vivo* when injected with AKH-I, whereas in solitary locusts, which do not store sufficient amounts of TAG in the fat body, AKH-I administration had no significant effect on lipase activity. The reason that the measured factorial increases of lipase activity after stimulation with lipolytic agents are relatively modest in these and other similar studies may reside in the applied experimental procedure, in which the enzyme is assayed in extracts of fat body or adipose tissue in the presence of optimally accessible TAG substrate. In this way, only the effects of (de)phosphorylation or other conformational changes of the enzyme on its lipolytic activity will be measured, and other potential effects such as enzyme translocation and the involvement of lipid droplet-associated proteins, factors that may be highly important for lipase activity as discussed above for vertebrate HSL, remain confounded. This may also explain why phosphorylation by PKA did not change the activity of TAG lipase that had been purified from fat body of the adult hawkmoth, *Manduca sexta*.

In two insect species that rely on lipid mobilization during sustained flight activity, *L. migratoria* and *M. sexta*, it has been shown that DAG, which is released from the fat body by the action of AKH, is stereospecific and has the *sn*-1,2-configuration. Although conclusive evidence on the pathway for the stereospecific synthesis of this *sn*-1,2-DAG is still lacking, the most probable route involves stereospecific hydrolysis of TAG into *sn*-1,2-DAG by a stereospecific lipase acting at the *sn*-3 position of the TAG.

IV. ADIPOKINETIC HORMONE-INDUCED LIPOPHORIN CONVERSIONS

For insect species that recruit fat body TAG depots to power their flight muscles during the vast distances covered nonstop by migratory flight, an efficient mechanism for lipid transfer is a premier issue. The action of AKH on lipid release has revealed a novel concept for lipid transport in the circulation of animal organisms. Insect hemolymph generally contains abundant amounts of a single multifunctional lipoprotein particle, high-density lipophorin (HDLp), that performs the tasks of transporting dietary and endogenously produced lipids to peripheral tissues during all developmental stages. A characteristic feature of HDLp is its ability to function as a reusable vehicle for a variety of lipids by the selective loading and unloading of lipid components at target tissues. In the flying insect, however, the increased transport of the released DAG in the hemolymph appears to require an AKH-stimulated transformation of the lipophorin particle, which is capable of alternating between the relatively lipid-poor HDLp form and a lipid-enriched (low-density lipophorin, LDLp) form. In this reversible conversion the exchangeable apolipoprotein apoLp-III, which exists in a lipid-free form and a lipid-bound form, plays an essential role. A schematic overview of the process is depicted in Fig. 2. Recent advances on the structural properties of HDLp and apoLp-III demonstrate a remarkable similarity to their counterparts in the mammalian system (the two nonexchangeable apolipoproteins of HDLp are similar to apoB; apoLp-III is similar to apoE). However, the functioning of the insect lipoprotein as a shuttle mechanism operating in energy transport during flight activity is intriguingly different, since in mammals lipoproteins do not play a role as carriers of mobilized lipids during exercise.

Both HDLp and apoLp-III are abundantly present in the hemolymph of resting insects. The loading of DAG onto HDLp particles at the fat body cell plasma membrane induces the association of multiple copies of apoLp-III with the expanding lipoprotein surface. The resulting LDLp, which is both larger and lipid-enriched, travels in the circulation to the flight muscles, where a lipophorin lipase specifically hydrolyzes the LDLp-carried DAG. The liberated FFA are imported into the muscle cells and oxidized to yield energy. Once the lipid content of the LDLp has diminished, apoLp-III dissociates from the particle, and finally, its HDLp constituent is recovered. Both HDLp and apoLp-III are recycled to the fat body and reutilized for DAG uptake (see Fig. 2). This concept of a reusable lipoprotein shuttle for lipid transport during flight activity was initially reported for the locust, but thereafter found to be present in many other long-distance flying insects.

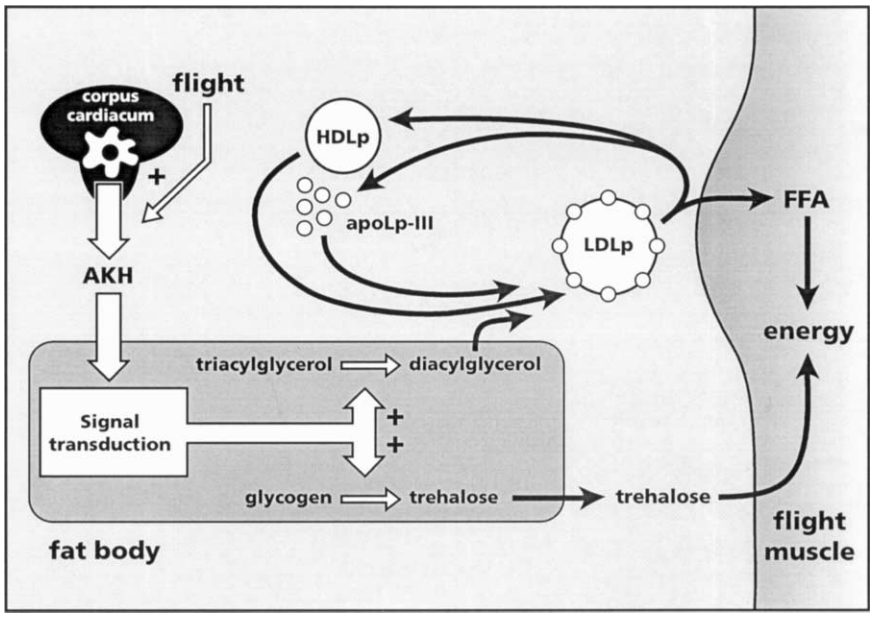

FIGURE 2 Schematic overview of AKH-controlled substrate mobilization from insect fat body during flight activity. AKH, adipokinetic hormones; HDLp, high-density lipophorin; LDLp, low-density lipophorin; apoLp-III, apolipophorin III; FFA, free fatty acids.

This shuttle mechanism is specific to the adult stage of the insect. During all stages of locust development, high circulating levels of HDLp are present to transport lipid components between tissues. However, the apoLp-III gene is expressed only after the first week of adult life and is apparently related to the adult-specific capacity of the insect to fly. The molecular basis of the interaction of apoLp-III with the lipoprotein surface is of great interest and value since the locust apoLp-III represents the only full-length apolipoprotein for which a three-dimensional structure has been disclosed. In the absence of lipid, apoLp-III reveals a globular bundle of five amphipathic α-helices. These helices are oriented such that the hydrophobic amino acid side chains are buried in the bundle interior and the hydrophilic residues face outward. A plausible model postulates that lipid binding induces a significant reorganization of the helical segments, allowing interaction of the hydrophobic face of the helices with the lipoprotein lipid surface. A critical event in lipid surface recognition seems to be the disturbance of the phospholipid monolayer by the appearance of patches of DAG molecules in the lipoprotein surface. Mechanistic details underlying the opening of the amphipathic helix bundle and the lipid-binding activity of apoLp-III, however, remain to be defined.

Glossary

adipokinetic hormone Peptide hormone in insects involved in the mobilization of substrates (predominantly lipids and carbohydrates) for energy generation needed by contracting flight muscles.

apolipophorin III (apoLp-III) Exchangeable apolipoprotein (mol wt 17–20 kDa) in the hemolymph of insects engaging in long-distance flights for which lipids are used as an energy source. In the resting blood, apolipophorin III forms a stable particle consisting of a bundle of five amphipathic α-helices; during lipid loading of lipophorin the conformation of apoLp-III changes and allows the peptide to unfold and reversibly bind to the expanding particle.

fat body Organ whose functional role in insects is analogous to the combined functions of liver and adipose tissue in mammals. Fat body is the chief site of intermediary metabolism, detoxification, storage of nutrient reserves, and, in some species, deposition of nitrogenous waste products.

HDLp High-density lipophorin (density approximately 1.12 g/ml), the abundant and generally single lipoprotein particle in insect hemolymph, transporting several classes of lipids between organs and tissues in the resting situation.

LDLp Low-density lipophorin (density approximately 1.04 g/ml), the adipokinetic hormone-induced form of lipoprotein in the hemolymph of insects engaging in long-distance flights for which lipids are used as an

energy source. The particle consists of lipid (diacylglycerol)-loaded and apoLp-III-associated HDLp.

lipophorin The abundant and generally single lipoprotein particle in insect hemolymph. The density of lipophorin (lipid-bearing protein) is similar to that of human high-density lipoprotein (HDL); for the insect lipophorin, HDLp is used to distinguish it from HDL.

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Adipokinetic Hormones: Structure and Biosynthesis

ROB C. H. M. OUDEJANS AND
DICK J. VAN DER HORST
Utrecht University, The Netherlands

The structure and biosynthesis of the adipokinetic hormones (AKHs) are reviewed and their storage, release, and inactivation in insect blood (hemolymph) are discussed. Since most of our knowledge on the biosynthesis of the AKHs has been obtained from locust species, these insects will serve as a general model system.

I. INTRODUCTION

Insect long-distance flight activity involves extremely high metabolic rates, and insect flight muscles are among the most energy-demanding tissues known. The energy substrates needed to fuel the flight muscles are stored in the fat body and their mobilization is under the control of adipokinetic hormones (AKHs). Additional functions of the AKHs are revealed during a variety of other metabolic "stress" situations (energy-consuming processes), such as starvation, diapause, and molting. The AKHs are peptide hormones that (with a few exceptions) are synthesized and stored in the so-called adipokinetic cells of the glandular lobes of the corpus cardiacum, a neuroendocrine organ situated in the insect head and connected to the central nervous system.

II. PRIMARY STRUCTURE OF ADIPOKINETIC HORMONES

Since the elucidation of the structure of the first AKH in 1976 from 3000 locust corpora cardiaca, analytical techniques have been revolutionized and today it is possible to determine the structure of a new AKH from just a few picomoles of the hormone. To date, the structures of some 37 different AKHs are known from representatives of most insect orders (Table I).

energy source. The particle consists of lipid (diacylglycerol)-loaded and apoLp-III-associated HDLp.

lipophorin The abundant and generally single lipoprotein particle in insect hemolymph. The density of lipophorin (lipid-bearing protein) is similar to that of human high-density lipoprotein (HDL); for the insect lipophorin, HDLp is used to distinguish it from HDL.

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Adipokinetic Hormones: Structure and Biosynthesis

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The structure and biosynthesis of the adipokinetic hormones (AKHs) are reviewed and their storage, release, and inactivation in insect blood (hemolymph) are discussed. Since most of our knowledge on the biosynthesis of the AKHs has been obtained from locust species, these insects will serve as a general model system.

I. INTRODUCTION

Insect long-distance flight activity involves extremely high metabolic rates, and insect flight muscles are among the most energy-demanding tissues known. The energy substrates needed to fuel the flight muscles are stored in the fat body and their mobilization is under the control of adipokinetic hormones (AKHs). Additional functions of the AKHs are revealed during a variety of other metabolic "stress" situations (energy-consuming processes), such as starvation, diapause, and molting. The AKHs are peptide hormones that (with a few exceptions) are synthesized and stored in the so-called adipokinetic cells of the glandular lobes of the corpus cardiacum, a neuroendocrine organ situated in the insect head and connected to the central nervous system.

II. PRIMARY STRUCTURE OF ADIPOKINETIC HORMONES

Since the elucidation of the structure of the first AKH in 1976 from 3000 locust corpora cardiaca, analytical techniques have been revolutionized and today it is possible to determine the structure of a new AKH from just a few picomoles of the hormone. To date, the structures of some 37 different AKHs are known from representatives of most insect orders (Table I).

TABLE I Amino Acid Sequences of Peptide Hormones of the AKH/RPCH Family

Hormone (by acronym)	Amino acid sequence
Miv-CC	pGlu-Ile-Asn-Phe-Thr-Pro-Asn-Trp-NH_2
Phm-AKH-III	pGlu-Ile-Asn-Phe-Thr-Pro-Trp-Trp-NH_2
Poa-HrTH	pGlu-Ile-Thr-Phe-Thr-Pro-Asn-Trp-NH_2
Lom-AKH-II	pGlu-Leu-Asn-Phe-Ser-Ala-Gly-Trp-NH_2
Tem-HrTH	pGlu-Leu-Asn-Phe-Ser-Pro-Asn-Trp-NH_2
Pab-RPCH	pGlu-Leu-Asn-Phe-Ser-Pro-Gly-Trp-NH_2
Scg-AKH-II	pGlu-Leu-Asn-Phe-Ser-Thr-Gly-Trp-NH_2
Pya-AKH	pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-NH_2
Ers-AKH	pGlu-Leu-Asn-Phe-Thr-Pro-Ser-Trp-NH_2
Lom-AKH-III	pGlu-Leu-Asn-Phe-Thr-Pro-Trp-Trp-NH_2
Mem-CC	pGlu-Leu-Asn-Tyr-Ser-Pro-Asp-Trp-NH_2
Pht-HrTH	pGlu-Leu-Thr-Phe-Ser-Pro-Asp-Trp-NH_2
Pea-CAH-II	pGlu-Leu-Thr-Phe-Thr-Pro-Asn-Trp-NH_2
Taa-AKH	PGlu-Leu-Thr-Phe-Thr-Pro-Gly-Trp-NH_2
Ona-CC-II	PGlu-Phe-Asn-Tyr-Ser-Phe-Asp-Trp-NH_2
Ona-CC-I	PGlu-Tyr-Asn-Phe-Ser-Thr-Gly-Trp-NH_2
Pea-CAH-I	pGlu-Val-Asn-Phe-Ser-Pro-Asn-Trp-NH_2
Ani-AKH	pGlu-Val-Asn-Phe-Ser-Pro-Ser-Trp-NH_2
Grb-AKH	pGlu-Val-Asn-Phe-Ser-Thr-Gly-Trp-NH_2
Emp-AKH	pGlu-Val-Asn-Phe-Thr-Pro-Asn-Trp-NH_2
Psi-AKH	pGlu-Val-Asn-Phe-Thr-Pro-Gly-Trp-NH_2
Lia-AKH	pGlu-Val-Asn-Phe-Thr-Pro-Ser-Trp-NH_2
Mas-AKH	pGlu-Leu-Thr-Phe-Thr-Ser-Ser-Trp-Gly-NH_2
Del-CC	pGlu-Leu-Asn-Phe-Ser-Pro-Asn-Trp-Gly-Asn-NH_2
Tea-HrTH	pGlu-Leu-Asn-Phe-Ser-Thr-Gly-Trp-Gly-Gly-NH_2
Phm-AKH-I	pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Ser-NH_2
Lom-AKH-I	pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2
Hez-HrTH	pGlu-Leu-Thr-Phe-Ser-Ser-Gly-Trp-Gly-Asn-NH_2
Phl-CC	pGlu-Leu-Thr-Phe-Thr-Pro-Asn-Trp-Gly-Ser-NH_2
Cam-HrTH-II	pGlu-Leu-Thr-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2
Cam-HrTH-I	pGlu-Leu-Thr-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2 | Hexose
Taa-HoTH	pGlu-Leu-Thr-Phe-Thr-Pro-Gly-Trp-Gly-Tyr-NH_2
Bld-HrTH	pGlu-Val-Asn-Phe-Ser-Pro-Gly-Trp-Gly-Thr-NH_2
Plc-HrTH-II	pGlu-Val-Asn-Phe-Ser-Pro-Ser-Trp-Gly-Asn-NH_2
Rom-CC-I	pGlu-Val-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2
Lom-HrTH	pGlu-Val-Thr-Phe-Ser-Arg-Asp-Trp-Ser-Pro-NH_2
Vac-AKH	pGlu-Leu-Thr-Phe-Thr-Ser-Ser-Trp-Gly-Gly-Lys

	Variation per residue position										
Position:	1	2	3	4	5	6	7	8	9	10	11
	pGlu	Ile Leu Phe Tyr Val	Asn Thr	Phe Tyr	Ser Thr	Ala Arg Phe Pro Ser Thr	Asn Asp Gly Ser Trp	Trp	Gly Ser	Asn Gly Pro Ser Thr Tyr	Lys

They are short peptides consisting of 8–11 amino acid residues and constitute the so-called AKH/RPCH (red pigment-concentrating hormone) family. The nomenclature of the members of this family (by acronym) is based on the use of the first two characters of the genus name and the first character of the species name of the animal from which the hormone was first isolated, supplemented by an abbreviation of its function.

The name is followed by a roman numeral in case there is more than one hormone per function. So Lom-AKH-I, -II, and -III are the three AKHs isolated from *Locusta migratoria*, and Lom-AKH-I and Scg-AKH-II are the two AKHs from *Schistocerca gregaria*.

In addition to the AKHs, the family contains hypertrehalosemic (HrTH) and hypotrehalosemic hormones (HoTH), corpus cardiacum factors (CC), etc. All members are found in insects, except for RPCH, which is restricted to the X-organ-sinus gland of crustaceans. Curiously, the variation in the structure of AKHs of insects is extremely large, whereas in all crustaceans investigated to date, just one RPCH structure has been found. All members (except Vac-AKH) are fully blocked peptides: They are N-terminally blocked by a pyroglutamate (pGlu) residue and C-terminally blocked by an amide group. Although the variation in structure has increased by an increasing number of new hormones, characteristic features are still present. At the bottom of Table I the variation per amino acid residue position is indicated. At position 1 a pGlu is always found, at position 8 a Trp is always found, and at position 4 an aromatic amino acid residue is always found. At position 2 most members contain a branched-chain amino acid residue, and at position 5 either a Ser or a Thr is present. Except for Lom-HrTH, all longer members possess a Gly at position 9. Most variation occurs at positions 6, 7, and 10. In only one member (Cam-HrTH-I), a sugar moiety with an as yet unknown structure has been found as a posttranslational modification. Except for a few members (Lom-HrTH, Mem-CC, Ona-CC-II, and Pht-HrTH), all peptides are uncharged.

Adipokinetic activity of a substance can be shown in the locust (*L. migratoria*) by using a simple bioassay of injecting the substance into resting animals and measuring the increase in lipid content in the hemolymph. Potential hypertrehalosemic activity can be measured using a bioassay in the cockroach (*Periplaneta americana*). Interestingly, all members of the AKH/RPCH family (including RPCH itself) exert an adipokinetic effect in the locust assay, suggesting that the AKH receptor(s) of *L. migratoria* is (are) less specialized or that all AKHs share structural similarities. In contrast to locusts and cockroaches, some insect species have a low response to their own AKH and a higher response to the hormone from other species. There are even members of the family with an as yet unknown function (for instance, Lom-HrTH, which stimulates in a heterologous assay the mobilization of carbohydrates only in the cockroach). In many insect species a particular physiological function seems to be supported by two, or in some locust and grasshopper species even three, hormones, suggesting sophisticated hormonal control. Whether there are quantitative or qualitative differences that result in the fine-tuning of apparently identical or just pleiotropic functions is still under investigation.

III. BIOSYNTHESIS OF ADIPOKINETIC HORMONES

All AKHs studied thus far are translated from separate mRNAs. They code for preprohormones with a simple structure: a signal sequence, a monocopy AKH sequence (starting with a Gln to form pGlu), a Gly residue (to form the terminal AKH amide group), a dibasic processing site, and an AKH-associated peptide sequence. In particular, in *L. migratoria* the signal sequences of the three preprohormones are cotranslationally cleaved, and the resulting prohormones of AKH-I and -II dimerize at random by oxidation of their Cys residues and give rise to two homodimeric precursor molecules (AKH-I/I and AKH-II/II) and one heterodimeric precursor molecule (AKH-I/II). This dimerization is a rather unique phenomenon, first established for another locust species (*S. gregaria*). Further proteolytic processing of the dimeric products in the secretory granules of the AKH cells of the corpus cardiacum results in the bioactive hormones and one heterodimeric and two homodimeric AKH-precursor-related peptides (APRPs) with as yet unknown functions (see Fig. 1). The biosynthesis of AKH-III is not yet fully understood. Recently, dimerization of the AKH-III prohormone with itself (it contains two Cys residues itself) has been established. Dimerization with the prohormones of AKH-I and -II has not been observed, and whether a cyclic prohormone of AKH-III can arise by formation of an internal disulfide bond is unknown.

In vitro studies on the biosynthesis of AKHs in locusts have revealed that the total time required for their biosynthesis from the starting point when radiolabeled amino acids are made available to the moment that radiolabeled bioactive hormones appear is 30–60 min. In *L. migratoria* the time for biosynthesis of AKH-III is shorter than that for AKH-I and -II, suggesting that there are two different pathways or different processing procedures for AKH-I/II, on the one hand, and for AKH-III, on the other.

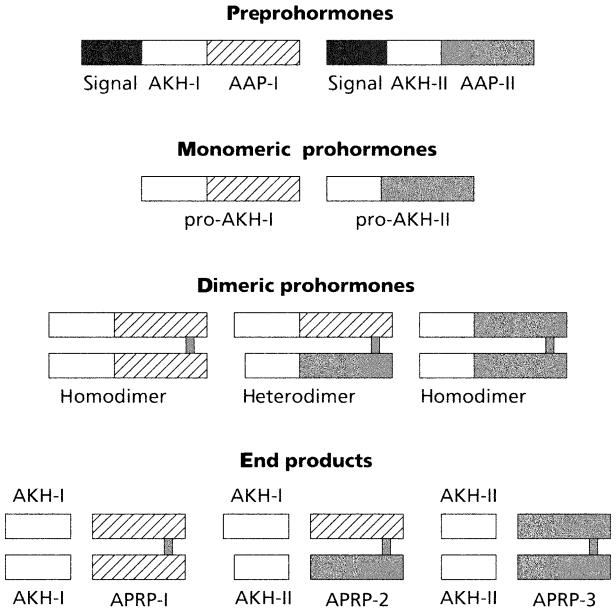

FIGURE 1 The biosynthesis of the locust adipokinetic hormones AKH-I and -II and the adipokinetic precursor-related peptides APRP-1, -2, and -3. After cleavage of the signal peptide from the preprohormones, the resulting monomeric prohormones, which consist of the AKH sequence and the AKH-associated peptide (AAP), form three dimeric prohormones, which are then processed to the AKHs and the APRPs.

IV. STORAGE, RELEASE, AND INACTIVATION OF ADIPOKINETIC HORMONES

AKHs are stored in the secretory granules of the adipokinetic cells of the corpus cardiacum. In *L. migratoria* the total content of the AKHs increases continuously during the larval stages and throughout adult life. In aging locusts an increasing number of intracisternal granules that contain stores of AKH prohormones/precursors are also found. The three AKHs, colocalized and stored in the same secretory granules, are released during flight. Since the membrane of the pertinent secretory granule fuses with the plasma membrane, the total contents of the granule are released into the hemolymph: the bioactive AKHs, the APRPs, and possibly other end products.

The release of the AKHs in *L. migratoria* is subjected to many regulatory substances, which are of either a stimulatory or an inhibitory nature and can be of both neural and humoral origin. A detailed description falls beyond the scope of this article. The only natural stimulus of release of the AKHs is flight and the relative contributions of all known substances effective in the release process remain to be established *in vivo*.

The situation in *L. migratoria* is even more complex, since secretory granules of only a particular age can be released. Newly formed granules containing the AKHs must mature before they can release their contents (or before they can fuse with the plasma membrane). Granules more than 8 h old are believed to enter a nonreleasable pool. Determination of the total hormone content of a neuroendocrine structure has therefore a limited physiological value, because only the releasable amount of hormone is relevant.

Finally, the balance between released hormones (a mixture of many compounds present in the secretory granule) and the rate of their inactivation prior to reaching the target tissue will be of eminent importance for their ultimate effect. The three AKHs of *L. migratoria* appear to be inactivated differentially after their release, with half-lives during flight of 35, 37, and 3 min obtained for AKH-I, -II, and -III, respectively.

Glossary

adipokinetic hormone Peptide hormone in insects involved in the mobilization of substrates (predominantly lipids and carbohydrates) for energy generation needed by contracting flight muscles.

corpus cardiacum Neuroendocrine organ situated caudal to the brain to which it is connected by paired nerves. The organ combines the functions of neurohemal storage and adipokinetic/hypertrehalosemic hormone production.

fat body Organ whose functional role in insects is analogous to the combined functions of liver and adipose tissue in mammals. Fat body is the chief site of intermediary metabolism, detoxification, storage of nutrient reserves, and, in some species, deposition of nitrogenous waste products.

hemolymph Blood of insects, which circulates in the body cavity between the various organs, bathing them directly. It consists of a fluid plasma in which the blood cells or hemocytes are suspended.

hypertrehalosemic hormone Peptide hormone from the corpus cardiacum involved in the mobilization of fat body glycogen to fuel flight activity. For transport to the flight muscles, glycogen is converted into the disaccharide trehalose, the carbohydrate transport form in insects, whose level in hemolymph is raised during flight.

red pigment concentrating hormone Peptide hormone from the eyestalk of crustaceans, a member of the adipokinetic hormone/red pigment-concentrating hormone family that comprises structurally related but functionally diverse peptides.

See Also the Following Articles

Adipokinetic Hormones and Carbohydrate Metabolism • Adipokinetic Hormones and Lipid Mobilization • Insect Endocrine System

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Adrenal Cortex Role in Medullary Synthesis of PNMT

I. C. McMillen and M. B. Adams
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During development, two anatomically and functionally distinct structures merge to form the adrenal gland. These structures comprise the central adrenal medulla, which synthesizes and secretes the catecholamines noradrenaline and adrenaline, and the peripheral adrenal cortex, which synthesizes and secretes steroid hormones, most notably the mineralocorticoids (e.g., aldosterone) and the glucocorticoids (e.g., corticosterone or cortisol).

I. INTRODUCTION

Although the adrenal medulla and cortex have different embryological origins and are regulated predominantly by separate reflex neurogenic and endocrine mechanisms, respectively, there is substantial functional cross talk between these two separate components of the adrenal gland, which is important in the maintenance of an adequate physiological stress response to a range of metabolic and other physiological stressors. One important aspect of the functional interaction between the adrenal cortex and the medulla is the role of the adrenal cortex in the control of the synthesis of the adrenaline-synthesizing hormone, phenylethanolamine *N*-methyltransferase (PNMT). This article reviews the separate developmental origins of the adrenal medulla and adrenal cortex, the anatomical relationship between these two structures, and the role of the glucocorticoid hormones in the induction and maintenance of PNMT synthesis during different physiological conditions.

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I. INTRODUCTION

Although the adrenal medulla and cortex have different embryological origins and are regulated predominantly by separate reflex neurogenic and endocrine mechanisms, respectively, there is substantial functional cross talk between these two separate components of the adrenal gland, which is important in the maintenance of an adequate physiological stress response to a range of metabolic and other physiological stressors. One important aspect of the functional interaction between the adrenal cortex and the medulla is the role of the adrenal cortex in the control of the synthesis of the adrenaline-synthesizing hormone, phenylethanolamine *N*-methyltransferase (PNMT). This article reviews the separate developmental origins of the adrenal medulla and adrenal cortex, the anatomical relationship between these two structures, and the role of the glucocorticoid hormones in the induction and maintenance of PNMT synthesis during different physiological conditions.

II. THE ADRENAL MEDULLA AND ADRENAL CORTEX—ANATOMY AND DEVELOPMENT

In early development, ectodermal neural crest cells migrate ventrally from the apex of the neural tube to the dorsal aorta, where they aggregate and differentiate to form sympathetic neurons, or to the adrenal gland primordia, where they differentiate to form chromaffin cells. The migratory primitive chromaffin cells invade the medial side of the developing adrenal cortical anlage and pass between the cortical cells and as the medulla occupy the center of the gland. In the developing mammalian adrenal medulla, three types of cells can be observed depending on the stage of development. The first type includes the primitive sympathetic migratory neurons, which are known as sympathogonia and are totipotent, being capable of differentiating into either sympathetic neurons or chromaffin cells depending on migratory route and the levels of environmental factors, such as the presence of glucocorticoids, nerve growth factor (NGF), and fibroblast growth factors. These cells are characterized by a small rounded nucleus with only a very thin peripheral rim of cytoplasm. The second type of cell is the phaeochromoblast, which has large elongated nuclei and a cytoplasm that is devoid of catecholamine secretory granules. The third and final cell type is the mature "chromaffin" cell or phaeochromocyte, which possesses a smaller ovoid nucleus and contains catecholamine-storing granules, with the cytoplasm giving a positive staining reaction to chromic acid due to the oxidation of the catecholamines to melanin. The mammalian adrenal cortex has its embryological origins in the mesoderm arising from mesenchymal tissue adjacent to the coelomic epithelium lying close to the urogenital ridge. Mesothelial cells between the root of the mesentery and the developing gonad undergo proliferation and subsequently penetrate the underlying mesenchyme. These cells then differentiate to form the adrenocortical masses, which are invaded during development by migrating sympathochromaffin cells. An important feature of the anatomical juxtaposition of the adrenal medulla and cortex is the fact that the continuous networks of blood vessels that supply the adrenal cortex converge and empty into the larger sinusoids present within the corticomedullary and medullary regions, so that the medulla is directly exposed to blood that has passed through the adrenal cortex. The medullary sinusoids then drain into medullary veins.

Aside from the cortical effluent, which drains into the medullary sinusoids, the medulla also receives blood directly from arteries known as arteriae medullae. The presence of arteriae medullae within the adrenal gland is species specific, with relatively few being observed in the rat adrenal gland, whereas substantially greater numbers are found in cat and bovine adrenals. The arteriae medullae derive from branches of the large capsular arteries that pass directly through the adrenal cortex before entering the medulla and branching into arterioles and capillaries. These vessels then empty into the medullary veins, which in turn empty into the central adrenal vein.

From a range of studies in different species, it appears that the adrenal medullary cells are exposed to high concentrations of glucocorticoids delivered either through the vasculature or through paracrine interactions within the gland. In the human adrenal, it has been shown, for instance, that there are variously protrusions, clusters, islets, and single cortical cells that occur diffusely within the adrenal medulla, providing the opportunity for paracrine interactions. Similarly, specific immunostaining for the neuroendocrine protein chromogranin-A has identified the occurrence of chromaffin cells within all three zones of the human adrenal cortex, again supporting a close functional relationship between these cell types. In species such as the sheep or cow, adrenomedullary cells directly adjacent to the zona reticularis and zona fasiculata of the adrenal cortex characteristically synthesize large amounts of adrenaline and the area of the adrenaline-synthesizing zone varies directly with the activity of the adrenal steroidogenic cells. Before discussing the range of mechanisms by which adrenal glucocorticoids may regulate the synthesis of adrenaline, it is necessary to review the synthetic pathway for both of the major adrenal catecholamines, noradrenaline and adrenaline.

Cholinesterase staining and nerve degeneration studies confirm that the majority of the nerve fibers that project to the chromaffin cells of the mammalian adrenal medulla are preganglionic cholinergic sympathetic fibers arising from the splanchnic nerve. Retrograde tracer studies reveal that the preganglionic sympathetic fibers that innervate the adrenal gland arise ipsilaterally from the intermediolateral horn of the spinal cord between thoracic level 3 (T3) and lumbar level 2, with the majority arising from T8–T11. There is a body of evidence to suggest that splanchnic nerve stimulation can enhance ACTH-induced glucocorticoid output from the adrenal cortex. Postganglionic sympathetic fibers containing catecholamines have been found using fluorescence

histochemistry to run in close association with vascular supply of the gland. The adrenal gland also appears to have an intrinsic innervation with ganglion cells having been identified within the gland with their numbers being species specific. In the rat, two populations of ganglion cells have been identified. One population of cells, termed the type I ganglion cells, are relatively large and exhibit properties consistent with a postganglionic, noradrenergic phenotype. These cells are probably derived from neural crest cells that invaded the cortical anlage but unlike the chromaffin cells were exposed to a different set of environmental factors that led to their differentiation into neurons. Type II ganglion cells are smaller, have a nonclassical peptidergic transmitter phenotype, and express nitric oxide synthase. Intra-adrenal ganglion cells have fibers that project to cortical, medullary, and capsular regions of the gland, with a number appearing in close proximity to blood vessels and chromaffin cells. Little is known about the physiological function of these intrinsic ganglion cells but they may act to regulate adrenal blood flow and also secretory output from the adrenal medullar and adrenal cortex.

III. SYNTHESIS OF ADRENOMEDULLARY CATECHOLAMINES

Catecholamines are synthesized in the chromaffin cells of the adrenal gland from the dietary amino acid tyrosine, as illustrated in Fig. 1. The initial step in the synthesis of adrenomedullary catecholamines is the oxidation of tyrosine to dihydroxyphenylalanine (DOPA) by the enzyme tyrosine hydroxylase [TH; tyrosine 3-monooxygenase; tyrosine, tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating), EC 1.14.16.2]. TH is a mixed-function oxidase, which requires molecular oxygen and utilizes a tetrahydrobiopterine as a co-substrate. The tetrahydropteridine co-substrate is oxidized to dihydrobiopteridine in the conversion of tyrosine to DOPA; dihydropteredine reductase (EC 1.6.99.7) and nicotinamide adenine dinucleotide phosphate are required to regenerate the pool of the reduced form of the biopterine. Tyrosine hydroxylase is the rate-limiting enzyme in the catecholamine synthetic pathway, as TH has a substantially lower specific activity than any of the other catecholamine synthetic enzymes and the pool of tyrosine is greater than that of any of the other catecholamine synthetic enzyme substrates. Tyrosine hydroxylase is located in the soluble fractions of adrenal medullary homogenates, indicating its presence in the cytoplasm of the chromaffin cells. DOPA is subsequently decarboxylated by DOPA decarboxylase, also known as aromatic L-amino acid decarboxylase (AADC; EC 4.1.1.28), to form dopamine. AADC is not specific for catecholamine synthesis as it is involved in the decarboxylation of a number of aromatic L-amino acids and appears to have a wide distribution in a number of tissues. Like TH, AADC activity in the chromaffin cell is found in the water-soluble fraction of adrenal homogenates, indicating that it is located in the cytosol of chromaffin cells. Dopamine is actively transported into the chromaffin granules and converted to noradrenaline by dopamine β-hydroxylase [DBH; 3,4-dihydroxyphenylethylamine, ascorbate:oxygen oxidoreductase (β-hydroxylating), EC 1.14.17.1]. DBH is the only enzyme in the catecholamine biosynthetic pathway located within the chromaffin granules; all of the other enzymes are situated in the cytoplasm of the chromaffin cells. DBH is present in the chromaffin granules in one of two similar forms, either bound to

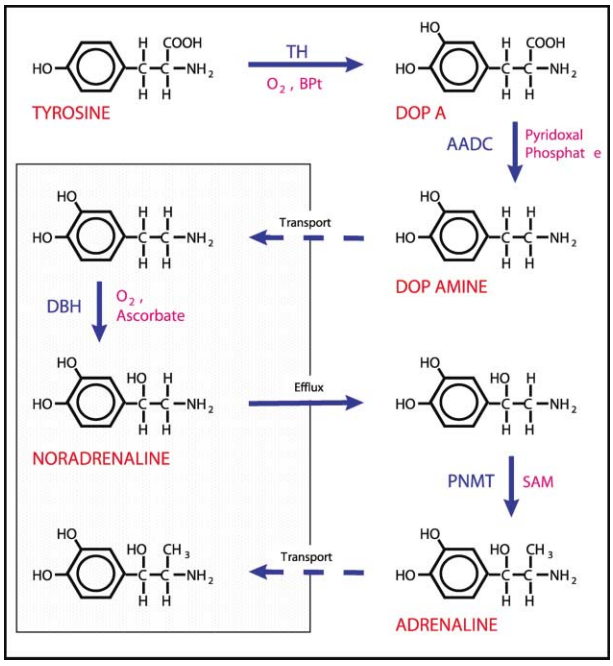

FIGURE 1 Biosynthetic pathway of adrenomedullary catecholamines. Schematic diagram of the synthesis of catecholamines within the chromaffin cells of the adrenal medulla, with substrates and products, enzymes, and co-factors indicated. Shaded area represents chromaffin granule; the unshaded area depicts the cytosol of the chromaffin cell. DOPA, dihydroxyphenylalanine; TH, tyrosine hydroxylase; AADC, aromatic amino acid decarboxylase; DBH, dopamine β-hydroxylase; PNMT, phenylethanolamine *N*-methyltransferase; BPt, tetrahydrobiopterine; SAM, *S*-adenosylmethionine.

the internal membrane or free in the internal matrix of the chromaffin granule. The relative proportions of the enzyme that are free versus membrane-bound vary between species, with the free form probably being derived from a membrane-bound precursor that undergoes proteolysis. Like TH, DBH is a mixed-function oxidase and it catalyzes the oxidation of dopamine to noradrenaline, requires molecular oxygen, and utilizes ascorbate as a co-factor. The final step in the catecholamine biosynthetic pathway is the N-methylation of noradrenaline, which passively permeates into the cytosol from chromaffin granules, to adrenaline. This is achieved by transfer of the *S*-methyl group from *S*-adenosylmethionine to the primary nitrogen group of noradrenaline by the enzyme PNMT.

IV. GLUCOCORTICOIDS AND PNMT SYNTHESIS

Early endocrine ablation and replacement experiments clearly demonstrated that glucocorticoids play a role in the maintenance of the activity of TH and PNMT. Hypophysectomy of the adult rat, which abolishes pituitary ACTH secretion and reduces adrenal corticosterone output, dramatically decreases both adrenaline content and PNMT activity within the adrenal gland. Administration of corticosterone or the potent synthetic glucocorticoid dexamethasone reverses the fall in PNMT activity after hypophysectomy. Glucocorticoids are also found to inhibit the enhanced degradation of the PNMT enzyme that occurs as a consequence of hypophysectomy, by stabilization of the enzyme co-substrate, *S*-adenosylmethionine. A number of studies have found that glucocorticoid administration to hypophysectomized rats also increases PNMT mRNA expression due to increased transcription of the PNMT gene. Analysis of the PNMT gene has revealed the presence of consensus sequences for the glucocorticoid-response element in the 5′ upstream regulatory/promoter sequence. In primary cultures of isolated bovine chromaffin cells that have been removed from any cortical influence, dexamethasone is a powerful inducer of PNMT activity with an EC_{50} for PNMT mRNA and activity induction of 1–10 nM. Bovine chromaffin cells have been found to possess glucocorticoid-binding receptors with a K_d of approximately 1 nM, and binding of glucocorticoids to the type II glucocorticoid receptors (GRs) present in chromaffin cells results in dimerization and transport into the nucleus, where the complex interacts with glucocorticoid-response elements on the PNMT gene to stimulate transcription. Furthermore, substantially higher levels of GR immunoreactivity have been identified in the adrenaline-containing cells than in the noradrenaline-containing cells of the adrenal medulla of the rat. There is evidence from a range of studies that glucocorticoids appear to play a crucial role in the induction of adrenal PNMT activity and mRNA expression that occurs in response to a range of stressors, thus demonstrating the importance of the anatomical juxtaposition of the adrenal cortex and adrenal medulla in the generation of the adaptive stress response.

V. GLUCOCORTICOIDS AND PNMT SYNTHESIS DURING DEVELOPMENT

Previous studies have shown that when dissociated chromaffin cells from immature adrenals are cultured in the absence of glucocorticoids they exhibit poor long-term survival in culture. The addition of NGF to these cultures is able to rescue many of the chromaffin cells; however, they subsequently undergo a phenotypical change exhibiting extensive neurite outgrowth. Glucocorticoid administration is able to block or delay NGF-induced neurite outgrowth. Long-term culture of rat adrenal chromaffin cells in the presence of NGF and the absence of glucocorticoids results in a complete transition to a sympathetic neuron phenotype as assessed by a number of morphological and biochemical criteria. Hence, glucocorticoids appear to act as a survival factor for chromaffin cells and repress the neuronal transdifferentiation of these cells. The levels of corticosterone present in the fetal rat adrenal gland at the time of invasion by the SA progenitor cells would be more than sufficient to suppress neural transdifferentiation by these cells. It has therefore been considered that the sympathetic progenitor cells that migrate from the sympathetic ganglion primordium through the developing adrenal anlage develop into chromaffin cells in the adrenal medulla under the influence of the glucocorticoid-rich environment. Recent studies, however, have challenged this view as an analysis of mice carrying targeted mutations of the GR gene found that the mice lacking a GR gene product had normal numbers of adrenal chromaffin cells. The GR mutant mice did, however, lack adrenomedullary PNMT. It therefore appears that the role of glucocorticoids in development is to modulate directly or indirectly the expression of PNMT, but that

the expression of PNMT itself does not determine the chromaffin phenotype.

There are also important interactions between the adrenal cortex and the adrenal medulla in those species, such as sheep and human, that have a prolonged gestation period and in which there are intact neuroendocrine responses to intrauterine stressors present before birth. Classical studies in the sheep fetus have shown that adrenaline content in the adrenal gland increased markedly during the 10–15 days preceding parturition coincident with the prepartum surge in adrenal cortisol output. Intrafetal administration of adrenaline to the sheep halts lung liquid secretion and stimulates lung liquid absorption by a β-adrenergic-dependent mechanism. Stimulation of β_2-adrenoreceptors in the fetal sheep and fetal rabbit lung with adrenaline and specific β_2-adrenoreceptor agonists also elicits an increase in pulmonary phospholipid synthesis and also the levels of phospholipids in lung lavage fluid. Catecholamines, in particular adrenaline, via β_2-adrenoreceptor stimulation are able to stimulate an increase in pulmonary surfactant secretion and synthesis. The well-established actions of glucocorticoids on surfactant synthesis in the lungs are therefore enhanced by the concomitant actions of the catecholamines. Newborns that are delivered by caesarean section do not experience the substantial catecholamine surge that occurs in babies that are vaginally delivered and have a lower lung compliance after delivery.

VI. SUMMARY

The functional interactions between the neighboring cells of the adrenal medulla and adrenal cortex are initiated early in development and are maintained by the paracrine and endocrine actions of the adrenocortical glucocorticoids on PNMT and hence adrenaline synthesis in the adrenomedullary chromaffin cells. The array of strategies that exist across a range of species to ensure that adrenomedullary cells that synthesize adrenaline are in close contact with the adrenocortical cells highlight the importance of a coordinated adrenocortical–medullary response to a range of acute and chronic stressors from before birth and into adult life. The chromaffin cells are therefore well placed to integrate both neurogenic and hormonal stimuli to ensure that adrenaline and the glucocorticoids act in a tightly regulated partnership to enhance the metabolic, cardiovascular, and respiratory responses to physiological challenges that threaten homeostasis.

Glossary

chromaffin cell The functional cell of the adrenal medulla; it contains catecholamine-storing granules, with the cytoplasm giving a positive staining reaction with chromic acid due to the oxidation of the catecholamines.

dopamine β-hydroxylase The only enzyme in the catecholamine biosynthetic pathway located within the catecholamine-containing granules of the chromaffin cell, where it catalyzes the conversion of dopamine to noradrenaline.

glucocorticoid receptors Proteins that are present in chromaffin cells and which, after binding with the glucocorticoids, dimerize and are transported to the nucleus of the cell, where the complex interacts with the glucocorticoid-response elements on the phenylethanolamine *N*-methyltransferase gene to stimulate transcription.

phenylethanolamine N-methyltransferase An enzyme that catalyzes the final step in the catecholamine biosynthetic pathway, i.e., the N-methylation of noradrenaline to result in the synthesis of adrenaline.

tyrosine hydroxylase The enzyme that catalyzes the oxidation of tyrosine to dihydroxyphenylalanine; it is the rate-limiting enzyme in the catecholamine synthetic pathway in the adrenal medulla.

See Also the Following Articles

Adrenocorticosteroids and Cancer • Adrenocorticotropic Hormone (ACTH) and Other Proopiomelanocortin Peptides • Glucocorticoid Effects on Physiology and Gene Expression • Mineralocorticoid Biosynthesis • Mineralocorticoid Effects on Physiology and Gene Expression

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Adrenocorticosteroids and Cancer

THOMAS J. DILLING AND W. GILLIES MCKENNA
University of Pennsylvania

Adrenocorticosteroids play several distinct roles for the clinical oncologist. Rarely, patients may have a primary tumor of the adrenal gland itself, leading to a clinical excess (or dearth) of adrenocortical hormones. Sometimes, too, tumors outside the adrenal axis can produce these hormones, causing well-described clinical syndromes. Expansile tumors in the brain or spinal column are an oncologic emergency, managed in part with adrenocorticosteroids. Sometimes steroids are also used as a primary chemotherapeutic agent in the treatment of the cancer itself. Finally, despite the oncologist's best efforts, patients sometimes experience side effects from chemotherapy and/or radiation, particularly in the lung; these side effects can effectively be managed by adrenocorticosteroids in many cases.

I. ADRENOCORTICAL TUMORS

Adrenal cortical carcinoma is a very rare tumor, comprising 0.05 to 0.20% of all cancers. It may be either functional or nonfunctional in terms of hormone production. When functional, the carcinomas may secrete excessive amounts of either adrenocorticotropic hormone (ACTH, or corticotropin) or sex hormones. Children less than 6 years of age can present with evidence of virilization, precocious puberty, or Cushing's syndrome. Adults (typically between the ages of 40 and 50) can also present with these tumors, as evidenced by hormonal syndromes (feminization, virilization, hypercortisolism, or hyperaldosteronism). Surgery is curative if total resection is possible, though 70% of patients present with positive lymph nodes or distant metastasis and therefore require chemotherapy.

II. PARANEOPLASTIC SYNDROMES

It should be noted that tumors outside the adrenal axis sometimes secrete adrenal corticosteroids. The classic tumors with ectopic endocrine production are some small-cell cancers of the lung and pituitary adenomas, though other less frequent tumors appear on this list as well. Pituitary ACTH overproduction (also known as Cushing's disease) is the most common syndrome, occurring in 55 to 82% of patients, and adrenal dysfunction occurs in 5 to 32% of patients, ectopic ACTH production in 11 to 25% of patients, and CRH overproduction in approximately 1% of patients. The resultant paraneoplastic syndromes are numerous. Cushing's initial description of the peripheral effects of hyperfunctioning pituitary adenoma included truncal obesity, purple striae, hypertension, fatigue, moon facies, buffalo hump, weakness, depression, amenorrhea, hirsutism, and edema. Complete treatment of this vast topic is beyond the scope of this article, though the reader is encouraged to consult the citations under Further Reading for additional information.

III. MANAGEMENT OF TUMOR SEQUELAE

Two dreaded complications of malignancy include spinal cord compression and life-threatening brain edema. In the former condition, a metastatic clone of malignant cells grows and expands within the spinal column, eventually compressing the spinal cord to the point that permanent paralysis can quickly ensue if not treated urgently. Likewise, primary brain tumors (or lesions metastatic to the brain) can expand within the fixed volume of the skull and cause life-threatening edema, leading to gross alteration of personality, seizures, or death.

Adrenocorticosteroids have a potent anti-inflammatory activity that plays an important role in the management of these conditions. Dexamethasone is commonly prescribed in doses ranging from 16 to

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Adrenocorticosteroids have a potent anti-inflammatory activity that plays an important role in the management of these conditions. Dexamethasone is commonly prescribed in doses ranging from 16 to

100 mg/day, tapering over time. Studies on spinal cord compression have failed to demonstrate a distinct advantage for the higher doses, though patients do appear to experience diminished pain after 24 h on the higher doses. Lower doses are usually used to treat brain edema. In any case, radiation therapy or surgical decompression is then performed urgently to debulk the tumor.

IV. TREATMENT OF CANCER

In 1943, a corticosteroid (the so-called Compound E) was reported to cause atrophy of lymphatic tissue in mice. Cortisone acetate and adrenocorticotropic hormone became clinically available in 1948. In November 1949, a group of researchers published a report in which they noted cancer regression in a pair of patients with different types of lymphoma. They stated that enlargement of lymph nodes occurred approximately 6 weeks after ACTH was discontinued in the patient with Hodgkin's disease, but found that there was no recurrence in the patient with lymphosarcoma 10 weeks after discontinuation of therapy. A number of studies followed in the 1950s and 1960s, confirming the earlier results regarding the importance of prednisone in treating lymphoma.

Although individual compounds were shown to be helpful, they did not always completely cure patients of these lymphomas or produce lasting remission. In 1968, Vincent DeVita and co-workers reported their landmark study, first published in 1970, in which they combined four different chemotherapeutics that had previously demonstrated individual activity against Hodgkin's disease. They combined cyclophosphamide, vincristine, procarbazine, and prednisone to create the MOPP chemotherapy regime. When tested on 43 patients, it induced complete remission in 35, 17 of whom had lasting remission—a vast improvement over any previously published results. In the years since, prednisone has also been shown to have activity against chronic leukemia and non-Hodgkin's lymphoma. In addition, different combinations (most notably CHOP, which also contains prednisone) have been tested and have become standards in the treatment of some lymphatic/leukemic cancers.

V. MANAGEMENT OF SEQUELAE FROM CANCER TREATMENT

In the years since chemotherapy and radiation therapy were introduced, physicians and scientists have improved the efficacy of these treatments while simultaneously diminishing their side effects on the patient. Occasionally, however, despite physicians' great determination, patients do experience problems related to the cancer therapy itself. While the specifics of all these potential problems are far beyond the scope of this article, one requires mention here. The lung is a relatively sensitive organ, and the tumors that occupy it can be quite resistant to treatment, requiring high doses of radiation or chemotherapy in an attempt to achieve patient cure. Clinicians, therefore, must balance these opposing problems by examining the therapeutic ratio—the ability to sterilize the tumor while sparing normal tissues.

Chemotherapy and radiation sometimes cause a hypersensitivity-type reaction called radiation pneumonitis. Chemotherapy has been shown to be a radiation sensitizer in the lung, which is important to remember in this era of combination chemotherapy and radiation. Approximately 43% of patients demonstrate radiologic changes within the lung from chemoradiotherapy treatment. Interestingly, symptomatic radiation pneumonitis occurs in only approximately 7% of patients. Patient variability seems to play a role. The volume of lung actually being irradiated, which can vary greatly depending upon the exact disease process, also determines whether a patient will experience this problem. It has also been shown that prior irradiation or chemotherapy can predispose a patient to radiation pneumonitis. Preexisting lung disease has also been implicated. Likewise, it has been shown that subclinical radiation pneumonitis can become symptomatic after steroid withdrawal.

Clinically, the syndrome generally manifests itself within about 2 to 3 months after the completion of therapy. Occasionally it presents as quickly as 1 month or as late as 6 months after the completion of treatment. Patients present with shortness of breath, which can vary from mild to severe. Cough is also typically prominent in about half of these patients. Fever is also sometimes seen. The symptoms can be relatively minor if the area of lung treated is small. On the other hand, the clinical course can be fulminant, leading to respiratory insufficiency and cyanosis, progressing to cor pulmonale (right heart failure) in a matter of days.

The histopathology and cellular biology of this disease process are fairly well elucidated. At 0 to 2 months after radiation, injury to small vessels and capillaries can be seen. This leads to vascular congestion and increased capillary permeability. As a result, proteinaceous material is deposited within

the alveoli of the lungs. In the range of 2 to 9 months postradiation, one can note microscopic evidence of obstruction of pulmonary capillaries. In addition, one sees hyperplasia of the vascular endothelium, and the walls of the alveoli become infiltrated with fibroblasts. If the radiation injury is mild, these changes may subside. However, if the injury is severe enough, it can progress to a chronic stage.

Corticosteroid administration dramatically improves the physiologic changes in mice and decreases their mortality. When given prophylactically, the steroids failed to prevent radiation pneumonitis, but when given at the first sign of clinical symptoms, they produced a clinical response. Other reports, however, failed to show amelioration of severe radiation pneumonitis with steroid administration. No controlled clinical trials have been conducted, however, to prove their efficacy in humans. Despite this fact, they are commonly given when the diagnosis is reasonably ascertained, at a dose of 1 mg/kg. This dose is maintained for several weeks and then slowly weaned.

Glossary

paraneoplastic syndromes Clinical syndromes experienced by some cancer patients due to the production of hormones or other substances by a tumor. The symptoms of the syndrome depend upon the exact substance(s) produced.

radiation pneumonitis Pathologically demonstrable series of changes seen in the lung tissue of some patients after irradiation. The syndrome can range from subclinical to highly toxic in severity and may be either self-limited or chronic.

spinal cord compression Oncologic emergency in which a clone of tumor cells expands within the spinal column, leading to permanent paralysis if not treated urgently.

See Also the Following Articles

Adrenal Cortex Role in Medulla Synthesis of PNMT • Adrenocorticotropic Hormone (ACTH) and Other Proopiomelanocortin Peptides • Androgen Receptors and Prostate Cancer • Apoptosis, Glucocorticoid-Induced • Cancer Cells and Progrowth/Prosurvival Signaling • Estrogen and Progesterone Receptors in Breast Cancer • Glucocorticoid Effects on Physiology and Gene Expression

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Adrenocorticotropic Hormone (ACTH) and Other Proopiomelanocortin (POMC) Peptides

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Adrenocorticotropin (ACTH) is a 39-amino-acid peptide derived from the prohormone proopiomelanocortin (POMC). ACTH stimulates adrenocortical steroid synthesis in response to stress and circadian fluctuations. ACTH is synthesized in corticotropes in the anterior lobe of the pituitary gland and, to a much lesser extent, in melantropes of the intermediate pituitary of many species with the exception of humans. It was first thought that ACTH was exclusively an endocrine hormone; however,

the alveoli of the lungs. In the range of 2 to 9 months postradiation, one can note microscopic evidence of obstruction of pulmonary capillaries. In addition, one sees hyperplasia of the vascular endothelium, and the walls of the alveoli become infiltrated with fibroblasts. If the radiation injury is mild, these changes may subside. However, if the injury is severe enough, it can progress to a chronic stage.

Corticosteroid administration dramatically improves the physiologic changes in mice and decreases their mortality. When given prophylactically, the steroids failed to prevent radiation pneumonitis, but when given at the first sign of clinical symptoms, they produced a clinical response. Other reports, however, failed to show amelioration of severe radiation pneumonitis with steroid administration. No controlled clinical trials have been conducted, however, to prove their efficacy in humans. Despite this fact, they are commonly given when the diagnosis is reasonably ascertained, at a dose of 1 mg/kg. This dose is maintained for several weeks and then slowly weaned.

Glossary

paraneoplastic syndromes Clinical syndromes experienced by some cancer patients due to the production of hormones or other substances by a tumor. The symptoms of the syndrome depend upon the exact substance(s) produced.

radiation pneumonitis Pathologically demonstrable series of changes seen in the lung tissue of some patients after irradiation. The syndrome can range from subclinical to highly toxic in severity and may be either self-limited or chronic.

spinal cord compression Oncologic emergency in which a clone of tumor cells expands within the spinal column, leading to permanent paralysis if not treated urgently.

See Also the Following Articles

Adrenal Cortex Role in Medulla Synthesis of PNMT • Adrenocorticotropic Hormone (ACTH) and Other Proopiomelanocortin Peptides • Androgen Receptors and Prostate Cancer • Apoptosis, Glucocorticoid-Induced • Cancer Cells and Progrowth/Prosurvival Signaling • Estrogen and Progesterone Receptors in Breast Cancer • Glucocorticoid Effects on Physiology and Gene Expression

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Adrenocorticotropic Hormone (ACTH) and Other Proopiomelanocortin (POMC) Peptides

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Adrenocorticotropin (ACTH) is a 39-amino-acid peptide derived from the prohormone proopiomelanocortin (POMC). ACTH stimulates adrenocortical steroid synthesis in response to stress and circadian fluctuations. ACTH is synthesized in corticotropes in the anterior lobe of the pituitary gland and, to a much lesser extent, in melantropes of the intermediate pituitary of many species with the exception of humans. It was first thought that ACTH was exclusively an endocrine hormone; however,

ACTH and its POMC relatives are expressed abundantly in the nervous system. POMC is a complex prohormone cleaved by specific prohormone convertases. In addition to ACTH, POMC contains the amino acid sequences of β-lipotropic hormone (β-LPH), β-endorphin, the melanocortins, corticotropin intermediate lobe peptide, and joining peptide. POMC is also highly expressed in neurons of the arcuate nucleus of the hypothalamus and the nuclear trachis solitacius. Even though the structure of POMC is the same wherever it is expressed, posttranslational cleavage of POMC can produce a different variety of biologically active peptides in each cell.

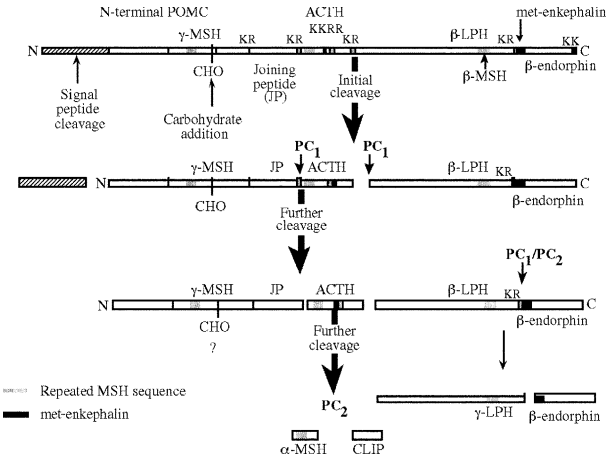

FIGURE 1 Processing of human proopiomelanocortin (POMC). The processing proceeds in stages, yielding a variety of forms of secreted peptides; N-terminal peptide, adrenocorticotropic hormone (ACTH), and β-lipotropin are the principal circulating forms produced by the anterior pituitary corticotroph. In the rodent, approximately 40% of ACTH has a posttranslational addition of a phosphate moiety. γ-MSH, γ-melanocyte-stimulating hormone; α-MSH, α-melanocyte-stimulating hormone; β-LPH, β-lipotropin, CHO, carbohydrate. In the CNS, ACTH is converted to α-MSH and CLIP.

I. ACTH AND POMC PEPTIDES

The biologically active peptides derived from POMC are the result of proteolytic processing by the prohormone convertases PC1/3 and PC2. Several such enzymes have been characterized and include the PCs, as well as furan and PACE 4. These enzymes are members of the family of kexin/subtilisin-like serine proteases. PCs cleave at single or multiple basic amino acid residues; however, as is the case with PC2, an accessory protein may be required for activity. The basic amino acid sequence lysine, arginine (KR) is the most often observed cleavage motif, but KK, RR, and RK will subserve this function as well. The PCs have different affinities for these basic cleavage motifs and thus generate different bioactivities from a common prohormone in a given cell type.

Peptide processing studies established that the principal peptide derivatives of POMC in the anterior lobe cells of humans are the N-terminal peptide, ACTH, and β-lipotropin (Fig. 1). The intermediate lobe POMC cells, called melanotropes, are present in other mammals and in the human fetus, but largely are vestigial in the human adult. The melanotropes produce additional products such as α-melanocyte-stimulating hormone (α-MSH) and corticotropin-like intermediate lobe peptide (CLIP) from ACTH, and β-endorphin from γ-lipotropin. The smaller POMC peptides are also produced in the human nervous system, with ACTH serving as a biosynthetic intermediate for the production of α-MSH. In rodents, the biological activity of α-MSH is enhanced by α-N-acetylation; however, the acetylation state of human α-MSHs is unclear at present. Human β-MSH probably is not secreted as a distinct hormone but is a by-product of postsecretory proteolysis. It is also interesting to note that the nonhuman primate exhibits the same lobe-specific POMC processing found in rodents and not humans.

In humans, each of the three principal products of POMC contains a common tetrapeptide core (His-Phe-Arg-Trp), which in turn is contained in the three melanocortin peptides, α-MSH, β-MSH, and γ-MSH. In addition, ACTH itself contains the tetrapeptide core and has melanotropic (pigment-producing) activity. This tetrapeptide core is the recognition site for the ACTH and melanocortin receptors. Previously, it was suggested that the hyperpigmentation found in disorders of excess ACTH may be related to ACTH itself or to the other melanotropic derivatives of the POMC molecule. The melanotropic activities of these peptides, particularly γ-MSH, have not been fully elucidated, and the circulating concentrations of these melanotropin peptides have not yet been determined simultaneously. Nonetheless, the absence of significant amounts of γ-MSH, and of β-MSH in humans, support ACTH as the likely candidate.

The cloning of five different melanocortin receptors (MC1–5) began a new era in studying POMC peptides. All of the receptors for POMC peptides that have been cloned belong to the seven-transmembrane G-protein-coupled receptor superfamily. ACTH binds to the adrenal ACTH receptor (MC2) with

nanomolar affinity. It then activates adenylyl cyclase via Gαs, increasing intracellular cAMP, activating protein kinase A, and thus resulting in the phosphorylation of proteins. Activation of this pathway induces cortisol synthesis and secretion.

The melanocortins (the MSHs) bind to MC1 and MC3–5 with nanomolar affinity to mediate a variety of physiological actions described below. All of the MC receptors stimulate cAMP production when activated by their respective ligands. In contrast, β-endorphin binds the μ class of opioid receptors and inhibits cAMP production through the Gαi/o class of G-protein-coupled receptors. The receptor for β-LPH has not been cloned.

II. PHYSIOLOGY OF ACTH AND POMC-DERIVED PEPTIDES

ACTH, released from the anterior pituitary corticotroph, is the major physiologic regulator of the synthesis and secretion of glucocorticoids by the adrenal glands. Glucocorticoids act on many processes mainly by altering gene transcription and protein synthesis in the target cells. In, addition, glucocorticoids permit metabolic adaptation during fasting to prevent low blood sugar. They also play an important role in the body response to physical and emotional stress. Other actions include their inhibitory effect on inflammation and regulation of vascular responsiveness to norepinephrine.

ACTH rapidly stimulates steroidogenesis, which can result in a great rise in blood glucocorticoids within seconds or minutes. To support this function, ACTH stimulates adrenal blood flow and the rate-limiting step of glucocorticoid synthesis: the conversion of cholesterol to pregnenolone. It also exerts several long-term tropic effects on adrenal cells that maintain the cellular machinery necessary to carry out steroidogenesis.

In addition to ACTH, β-lipotropin is cleaved from POMC in anterior pituitary corticotrophs. β-Lipotropin has effects on lipid metabolism, but its physiologic function in humans has not yet been established. When stimulated, corticotrophs secrete ACTH and β-lipotropin in a 1:1 ratio into the bloodstream.

The circulating levels of ACTH and glucocorticoids are greatly influenced by stress. When an individual is exposed to a stressful situation, levels of ACTH and glucocorticoids rise rapidly in the blood. Stress stimulates the hypothalamic–pituitary–adrenal axis regardless of the existing concentrations of circulation glucocorticoids. This occurs because stress increases neural activity in the CNS, stimulating the hypothalamic parvicellular neurons in the paraventricular nucleus to secrete corticotropin-releasing hormone (CRH) at a greater rate. If stress persists, the hypothalamic–pituitary–adrenal axis will function at a higher setpoint, maintaining higher concentrations of ACTH and glucocorticoids, which may eventually have detrimental effects on the individual. The role of the intermediate lobe melanotrope in stress response is not clear. For instance, in all nonhuman species studied, the majority of the endorphins produced by the intermediate lobe are α-N-acetylated and thus do not bind the opioid receptors. In lower organisms, circulating melanocortins may be involved in pigmentation responses to stress.

Whereas the first two decades of POMC research were heavily focused on endorphins, their expression in the pituitary, and the expression of these peptides in the CNS, recently there has been intense interest in POMC peptides and feeding behavior. The cloning of the melanocortin and ACTH receptors contributed greatly to this interest as α-MSH had been implicated in food intake regulation for many years. The melanocortins also have wide variety of physiological effects including alterations in motor activity and sexual behavior, analgesia, memory, nerve regeneration, antipyretic actions, pigmentation, and grooming behavior.

Though many studies have been performed in rodent models examining POMC peptides and obesity, only in the past 5 years has firm evidence for POMC's involvement in obesity emerged. Much of this information comes from genetic studies. A current hypothesis is that mutations in regulatory elements of the POMC gene decrease the levels of POMC expression in the brain, thus causing alterations in energy metabolism.

There have been a small number of children with null mutations in the POMC gene resulting in undetectable levels of ACTH. The common phenotype presented was red hair, adrenal insufficiency, and severe, early onset obesity. Furthermore, recessive and dominant mutations in the MC4 receptor (MC4-R) gene have been seen in humans and these mutations are proposed to cause 5% of childhood obesity. These and other data suggest that obesity may be a genetic disease of the hypothalamus and that POMC may be a major player.

The POMC and neuropeptide Y systems are the most extensively studied regarding energy intake. The POMC system receives input detailing

the nutritional status and energy storage via insulin and leptin signaling mediated by receptors on POMC neurons in the arcuate nucleus of the hypothalamus. There is a complex interaction of food intake inhibition by α-MSH and agouti-related protein at the level MC4-R. CRH is the mediator of food intake and environmental conditions. It inhibits food intake and weight gain, activates the hypothalamic–pituitary axis, and modulates energy storage in adipose tissue. The link between the CRH system and POMC neurons in the hypothalamus and other brain areas remains to be completely defined.

III. REGULATION OF ACTH AND POMC-DERIVED PEPTIDES

Pituitary ACTH secretion is under two primary regulatory influences. There is a positive peptidergic influence from the hypothalamus, mediated by CRH, which varies diurnally and is subject to extreme perturbation by stress. There also is a negative feedback influence of glucocorticoids, acting both at the pituitary level to inhibit the corticotrope and at the hypothalamic level to inhibit CRH neurons. Cortisol is the major physiologic glucocorticoid inhibitor of ACTH secretion; there is no other important endogenous ACTH inhibitor in humans. Figure 2 shows a scheme that depicts a variety of neurotransmitter inputs to the hypothalamus that have been postulated to regulate CRH release and ultimately, the secretion of ACTH. Further, vasopressin (AVP) and melatonin have been implicated in the regulation of ACTH secretion. In particular, AVP and melatonin released from the suprachiasmatic nucleus appear to be intimately involved in the circadian rhythm of glucocorticoids. In addition, AVP released from the neural lobe of the pituitary has long been thought to act synergistically with CRH to enhance ACTH secretion from the anterior lobe.

In humans, ACTH has a diurnal rhythm, exhibiting the highest plasma levels at approximately 6–8 AM and a nadir at approximately midnight. In rats, this pattern is reversed, and both lighting and activity may alter this cycle. In humans, there is also a free-running cycle of plasma cortisol that varies between 25 and 33 h. All of the other peptides derived from pituitary POMC demonstrate these cycles; however, due to differences in the half-lives of the peptides, the plasma concentrations are not superimposable.

The negative feedback effect of glucocorticoids on ACTH secretion results from actions on both the hypothalamus and the corticotroph. Glucocorticoids act directly on the corticotrophs to decrease POMC

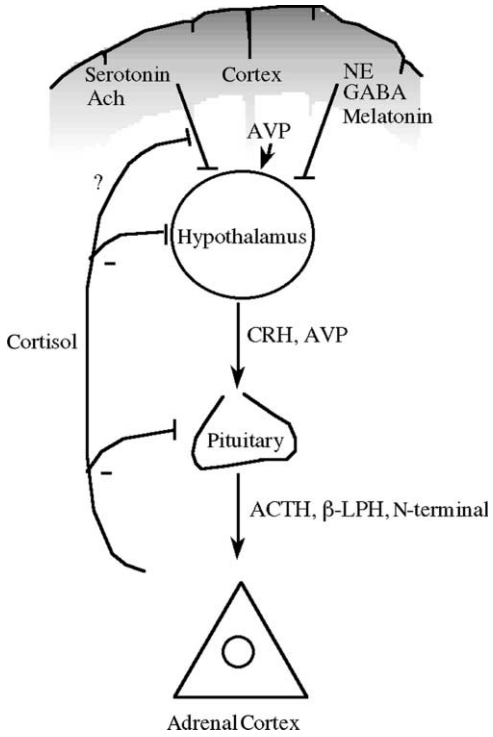

FIGURE 2 Scheme for control of ACTH secretion. Ach, acetylcholine; NE, norepinephrine; GABA, γ-aminobutyric acid; CRH, corticotropin-releasing hormone; AVP, arginine vasopressin.

synthesis and secretion. Glucocorticoids inhibit the release of CRH from the hypothalamus, which also decreases ACTH release. In addition, glucocorticoids inhibit ACTH secretion by acting directly on the corticotroph to inhibit the action of CRH. As a result, CRH becomes less effective at stimulating ACTH release. In species other than humans, the synthesis and secretion of POMC peptides are tonically inhibited by hypothalamic neurons releasing dopamine. Intermediate lobe melanotropes do not express the glucocorticoid receptor and therefore are not regulated by glucocorticoids.

Cortisol and its synthetic analogues (e.g., prednisone and dexamethasone) suppress ACTH secretion. After the administration of a dose of a glucocorticoid, ACTH secretion diminishes within minutes ("fast feedback") and, in a second phase ("delayed feedback"), is suppressed for hours or days, depending on the glucocorticoid used. It is unclear whether the fast and delayed feedback actions are associated with different anatomic sites. It is likely, however, that delayed feedback is an intracellular event operating through changes in nuclear mechanisms, whereas fast feedback may be dependent on

effects on the cell membrane mediated through other classes of receptors.

Although pituitary POMC regulation by circulating glucocorticoids is fairly well understood, much less is known about POMC peptide regulation in the brain. In the rodent, a large cell group of POMC neurons is bilaterally distributed in the arcuate nucleus of the hypothalamus and projects extensively to other regions of the brain. These projections pass through limbic structures, the diencephalon, and amygdala, as well as the thalamus and periaqueductal gray, areas associated with nociception and sensory integration. In addition, there are projections to the brainstem, which are thought to be involved in the regulation of cardiovascular and respiratory systems. In the nucleus tractus solitarius, POMC neurons send projections to the spinal cord and probably interact with local POMC circuits in the spinal cord itself. It appears that human central nervous system POMC expression resembles that found in the rodent, thus serving as a good model for future POMC peptide research.

IV. SUMMARY

POMC is a prohormone that gives rise to several biologically active peptides that are expressed primarily in the pituitary and brain. ACTH, the melanotropins, and endorphins are liberated by specific proteolytic enzymes called PCs. By expressing the PCs in a cell-type-specific fashion, a variety of POMC-derived products can be differentially expressed. When combined with the tissue-specific expression of POMC peptides and their cognate receptors, an incredibly diverse array of physiological responses can be produced. Since all of the peptides derived from POMC are co-released from large secretory granules, a myriad of biological activities are modulated simultaneously. In the case of POMC, these are glucocorticoid production, food intake, stress responses, and modulation of pain perception. There is much to learn about how the modulation of these specific POMC-derived bioactivities evolved to be regulated in a coordinated manner.

Glossary

adrenocorticotropic hormone A peptide that elicits glucocorticoid secretion from the adrenal cortex.

β-endorphin A peptide possessing analgesic properties mediated through the opioid receptors.

β-LPH A peptide derived from proopioimelanocortin containing γ-MSH and β-endorphin.

α-MSH, β-MSH, γ-MSH α-, β-, and γ-Melanocyte-stimulating hormones, collectively called the melanocortins. These peptides are thought to stimulate pigmentation and may be involved in appetite and feeding behavior.

prohormone convertase A class of proteolytic enzymes responsible for cleaving proopioimelanocortin into biologically active peptides.

prohormone A protein that is converted to biologically active molecules via posttranslational processing by specific proteolytic enzymes or other modifications.

proopioimelanocortin A prohormone expressed in the neuroendocrine system.

See Also the Following Articles

Appetite Regulation, Neuronal Control • Corticotropin-Releasing Hormone, Stress, and the Immune System • Endocrine Rhythms: Generation, Regulation, and Integration • Glucocorticoid Effects on Physiology and Gene Expression • Melatonin in Humans • Neuropeptides and Control of Anterior Pituitary • Stress • Vasopressin (AVP)

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Amino Acid and Nitric Oxide Control of the Anterior Pituitary

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Hypothalamic neurosecretory neurons regulate the secretion of the six anterior pituitary tropic hormones. In turn, these first-order, final common pathway neurons of the neuroendocrine system are immediately controlled by second-order afferent neurons. Most of these hypothalamic afferent neurons utilize either the inhibitory neurotransmitter γ-aminobutyric acid (GABA) or the excitatory neurotransmitter glutamate (Glu). Second-order neurons also regulate the neurosecretory neurons by elaborating the neuroactive gas nitric oxide (NO). Second-order GABA neurons uniformly decrease the secretion of the anterior pituitary tropic hormones, whereas Glu neurons increase their secretion. Second-order NO neurons mostly increase, but in one case decrease, anterior pituitary tropic hormone secretion.

I. HYPOTHALAMIC CONTROL OF PITUITARY GLAND FUNCTION

A. Hypothalamic Releasing and Inhibiting Hormones

Neurons situated in the hypothalamus control the synthesis and secretion of the hormones of the anterior and posterior pituitary gland (Fig. 1). The posterior pituitary hormones, oxytocin and vasopressin, are synthesized in the cell bodies of neurons located in the hypothalamic supraoptic and paraventricular nuclei. Axons of these neurons project to terminals in the posterior lobe (PL), from which oxytocin and vasopressin are released on depolarization. In contrast, the anterior pituitary hormones are controlled by hypothalamic neurons, whose cell bodies are located in arcuate nuclei (AN) and other

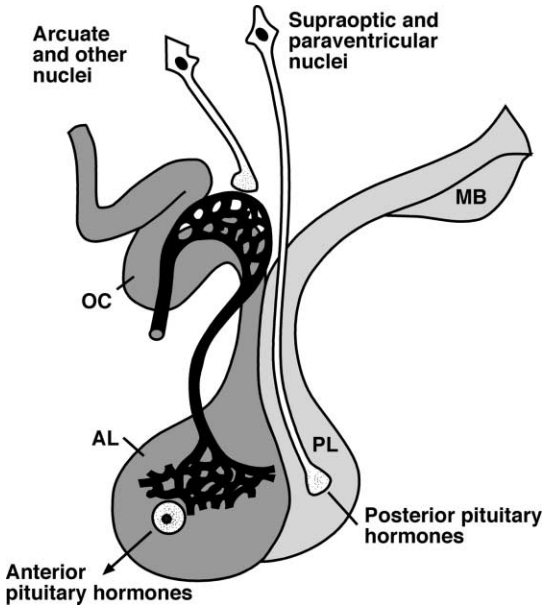

FIGURE 1 Secretion of hypothalamic hormones. The hormones of the posterior lobe (PL) are released into the general circulation from the endings of supraoptic and paraventricular neurons, while hypophysiotropic hormones are secreted into the hypophysial portal circulation from the endings of arcuate and other hypothalamic neurons. The hormones circulate in the portal vessels to the anterior lobe (AL), where they control the synthesis and secretion of the anterior pituitary tropic hormones. MB, Mamillary bodies; OC, optic chiasm. Modified from Ganong (2001), with permission.