Handbook of Experimental Pharmacology 184

Thomas C. Südhof
Klaus Starke
Editors

Pharmacology of Neurotransmitter Release

Springer

Handbook of Experimental Pharmacology

Volume 184

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Contributors

S. Boehm, J.-L. Boulland, D.A. Brown, M.K.L. Bredahl, F.A. Chaudhry, M.M. Dorostkar, R.H. Edwards, R. Feil, T.J. Feuerstein, R. Gilsbach, J. Gonçalves, L. Hein, R. Jahn, M. Jenstad, M. Kathmann, E.T. Kavalali, M. Khvotchev, A.E. Kisilevsky, T. Kleppisch, T. Lang, S.Z. Langer, D.M. Lovinger, C. Montecucco, G. Queiroz, M. Raiteri, A. Rohou, O. Rossetto, E. Schlicker, T.S. Sihra, T.C. Südhof, S. Sugita, Y.A. Ushkaryov, G.W. Zamponi

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Thomas C. Südhof MD
Howard Hughes Medical Institute
and Center for Basic Neuroscience
UT Southwestern Medical Center
6000 Harry Hines Blvd. NA4.118
Dallas TX 75390
USA
thomas.sudhof@utsouthwestern.edu

Prof. Dr. Klaus Starke
Institut für Experimentelle und
Klinische Pharmakologie und Toxikologie
Albert-Ludwigs-Universität Freiburg
Albertstrasse 25
D-79104 Freiburg i.Br.
Germany
klaus.starke@pharmakol.uni-freiburg.de

ISBN: 978-3-540-74804-5 e-ISBN: 978-3-540-74805-2

Handbook of Experimental Pharmacology ISSN 0171-2004

Library of Congress Control Number: 2007934514

Cover Design: WMXDesign GmbH, Heidelberg

Printed on acid-free paper

9 8 7 6 5 4 3 2 1

springer.com

Preface

This book is intended to provide an overview of the pharmacology of neurotransmitter release. Neurotransmitter release initiates synaptic transmission, the major mechanism by which neurons communicate with each other and with effector cells. Although a larger number of drugs act on the postsynaptic receptors that are the targets of the released neurotransmitters than on the release process itself, some of the oldest drug agents in medicine influence the release of subsets of neurotransmitters, for example, reserpine, which empties synaptic vesicles containing catecholamines and thereby blocks catecholamine release. Furthermore, some long-recognized compounds that act on neurotransmitter release are being increasingly used for new applications. For example, botulinum toxins are now among the most frequently administered cosmetic drugs employed to counteract the development of wrinkles; they act by inhibiting neurotransmitter release.

Dramatic progress has been made over the last decades in our understanding of neurotransmitter release. The principal mechanism that mediates release was elucidated by Bernhard Katz more some 50 years ago, but the molecular events remained obscure until the components and functions of nerve terminals were studied in recent years (reviewed in Südhof 2004). The basic mechanisms of release are discussed in the book's first part.

For a long time it was tacitly assumed that the amount of transmitter released per action potential was constant – at least at a given action potential frequency. However, this is not so – an almost baroque diversity of presynaptic plasticity mechanisms has emerged over the last two decades. Axon terminals are not only passively transmissive structures, but also represent actively computational elements. Synaptic neurotransmitter release changes as a function of use, often dramatically, in a manner that depends both on the release machinery and on extrinsic inputs. Indeed, nerve terminals are endowed with a large number of receptors for endogenous chemical signals – presynaptic receptors which, when activated, modulate the amount of transmitter being released.

Interestingly, the first experiment that retrospectively must be explained by presynaptic receptors was published in this handbook – in its second volume, in 1924, by the British pharmacologist Walter E. Dixon. Figure 1 shows that he injected

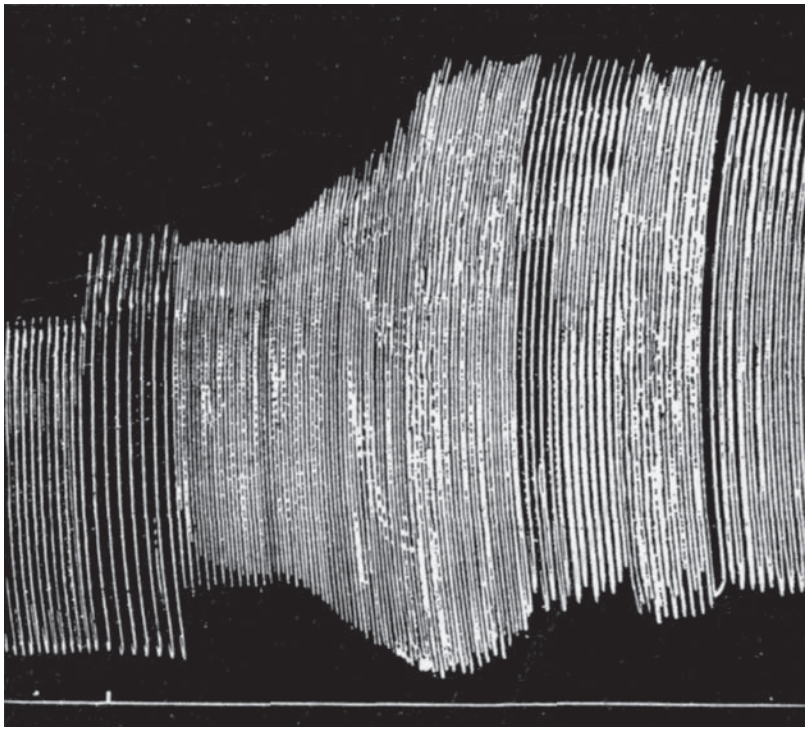

Fig. 1 Effect of nicotine on a rabbit isolated heart. From Dixon (1924).

nicotine into the isolated perfused heart of a rabbit. Immediately on injection, nicotine slowed the heart rate by stimulating intracardiac vagal ganglion cells. After a few seconds, however, bradycardia was replaced by marked tachycardia and an increase in contraction amplitude. Because the isolated heart does not contain sympathetic ganglion cells (and because an effect on the myocardium can be excluded), nicotine must have acted on the cardiac sympathetic axon terminals, on what we now call presynaptic nicotinic receptors.

Presynaptic nicotinic receptors are ligand-gated ion channels. Many other presynaptic receptors couple to G-proteins. Presynaptic receptors may be targets of bloodborne substances or substances secreted from neighboring cells, including neighboring axon terminals. In 1971 it was noticed with some surprise that many axon terminals even possess receptors for their own transmitter–presynaptic autoreceptors, the α_2-autoreceptors for noradrenaline being a prominent example (reviewed in Starke 2001). The various presynaptic ligand-gated ion channels and G-protein-coupled receptors are discussed in the second part of this volume. Questions regarding where the receptors' signal transduction pathways hit the exocytosis cascade and whether the receptors have therapeutic potential will be addressed in all chapters.

We attempt a synthesis of a large amount of information and cannot be expected to be totally successful. Nevertheless, we hope that the various contributions will be useful, and that the book will be of help to scientists in a wide number of fields.

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Dallas — *Thomas C. Südhof*
Freiburg i. Br. — *Klaus Starke*

Contents

Contributors

S. Boehm
Institute of Pharmacology, Center for Biomolecular Medicine and Pharmacology, Medical University of Vienna, Vienna, Austria, Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Universitätsplatz 4, 8010 Graz, Austria, stefan.boehm@meduni-graz.at

Jean-Luc Boulland
The Biotechnology Centre of Oslo, Centre for Molecular Biology and Neuroscience, University of Oslo, 0317 Oslo, Norway

David A. Brown
Department of Pharmacology, University College, Gower Street, London, WC1E 6BT, United Kingdom, d.a.brown@ucl.ac.uk

May K.L. Bredahl
The Biotechnology Centre of Oslo, University of Oslo, 0317 Oslo, Norway

Farrukh A. Chaudhry
The Biotechnology Centre of Oslo, Centre for Molecular Biology and Neuroscience, University of Oslo, P. O. Box 1125, Blindern, 0317 Oslo, Norway, f.a.chaudhry@biotek.uio.no

M.M. Dorostkar
Institute of Pharmacology, Center for Biomolecular Medicine and Pharmacology, Medical University of Vienna, Waehringer Straße 13a, 1090 Vienna, Austria

Robert H. Edwards
Departments of Neurology and Physiology, University of California, San Francisco School of Medicine, 600 16th Street, GH-N272B San Francisco, California 94143-2140, USA

Robert Feil
Interfakultäres Institut für Biochemie, Universität Tübingen, Hoppe-Seyler-Straße, 4, 72076 Tübingen, Germany

Thomas J. Feuerstein
Neurochirugische Universitätsklinik Breisacherstrasse 64 Freiburg, Germany, thomas.feuerstein@uniklinik-freiburg.de

Ralf Gilsbach
Institute of Experimental and Clinical Pharmacology and Toxicology, University of Freiburg, Albertstrasse 25, 79104 Freiburg, Germany

Jorge Gonalves
Department of Pharmacology, Faculty of Pharmacy, University of Porto, Porto, Portugal, jgonçalves@reit.up.pt

Lutz Hein
Institute of Experimental and Clinical Pharmacology, University of Freiburg, Albertstrasse 25, 79104 Freiburg, Germany, lutz.hein@pharmakol.uni-freiburg.de

Reinhard Jahn
Max Planck Institute for Biophysical Chemistry, Department of Neurobiology, Am Fassberg 11, 37077 Göttingen, Germany, rjahn@gwdg.de

Monica Jenstad
The Biotechnology Centre of Oslo, Centre for Molecular Biology and Neuroscience, University of Oslo, N-0317 Oslo, Norway

M. Kathmann
Institut für Pharmakologie und Toxikologie, Rheinische Friedrich-Wilhelms-Universität, Reuterstrasse 2b, 53113 Bonn, Germany

Ege T. Kavalali
Department of Neuroscience, Department of Physiology, The University of Texas, Southwestern Medical Center, 5323 Harry Hines Blvd. Dallas, Texas 75390-9111, United States

Mikhail Khvotchev
Department of Neuroscience, University of Texas, Southwestern Medical Center, Dallas, Texas 75390-9111, United States

Alexandra E. Kisilevsky
Hotchkiss Brain Institute and Department of Physiology and Biophysics, University of Calgary, Calgary, Canada

Thomas Kleppisch
Institut für Pharmakologie und Toxikologie, Technische Universität München, Biedersteiner Straße 29, D-80802 München, Germany, kleppisch@ipt.med.tu-muenchen.de

Thorsten Lang
Department of Neurobiology, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany, tlang@gwdg.de

S.Z. Langer
Alpha-2 Pharmaceutica AB, 8 H. Rosenblum Street, Apt. 4650, Tel Aviv 69379, Israel, langer.salomon@gmail.com

David M. Lovinger
Laboratory for Integrative Neuroscience, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, 5625 Fishers Lane, Bethesda, MD 20892-9411, USA, lovindav@mail.nih.gov

Cesare Montecucco
Departimento de Scienze Biomediche and Istituto CNR di Neuroscienze, Università di Padova, Viale G. Colombo 3, 35121 Padova, Italy, cesare.montecucco@unipd.it

Glória Queiroz
Department of Pharmacology, Faculty of Pharmacy, University of Porto, Porto, Portugal, gloria@ff.up.pt

M. Raiteri
Department of Experimental Medicine, Pharmacology and Toxicology Section, Center of Excellence for Biomedical Research, University of Genoa, 16132 Genoa, Italy, raiteri@pharmatox.unige.it

Alexis Rohou
Division of Cell and Molecular Biology, Imperial College London, London, SW7 2AY, United Kingdom, alexis.rohou@imperial.ac.uk

Ornella Rossetto
Departimento de Scienze Biomediche and Istituto CNR di Neuroscienze, Università di Padova, Viale G. Colombo 3, 35121 Padova, Italy, ornella.rossetto@unipd.it

E. Schlicker
Institut für Pharmakologie und Toxikologie, Rheinische Friedrich-Wilhelms-Universität, Reuterstrasse 2b, 53113 Bonn, Germany, e.schlicker@uni-bonn.de

Talvinder S. Sihra
Department of Pharmacology, University College London, Gower Street, London, United Kingdom, t.sihra@ucl.ac.uk

Thomas C. Südhof
Departments of Neuroscience and Molecular Genetics, Howard Hughes Medical Institute, The University of Texas, Southwestern Medical Center, Dallas, Texas 75390-9111, USA, thomas.sudhof@utsouthwestern.edu

Shuzo Sugita
Division of Cellular and Molecular Biology, Toronto Western Research Institute, Toronto, Ontario M5T 2S8, Canada, ssugita@uhnres.utoronto.ca

Yuri A. Ushkaryov
Division of Cell and Molecular Biology, Imperial College London, London SW7 2AY, United Kingdom, y.ushkaryov@imperial.ac.uk

Gerald W. Zamponi
Department of Physiology and Biophysics, University of Calgary, 3330 Hospital Drive NW, Calgary T2N 4N1, Canada, Zamponi@ucalgary.ca

Neurotransmitter Release

Thomas C. Südhof

Abstract Neurons send out a multitude of chemical signals, called neurotransmitters, to communicate between neurons in brain, and between neurons and target cells in the periphery. The most important of these communication processes is synaptic transmission, which accounts for the ability of the brain to rapidly process information, and which is characterized by the fast and localized transfer of a signal from a presynaptic neuron to a postsynaptic cell. Other communication processes, such as the modulation of the neuronal state in entire brain regions by neuromodulators, provide an essential component of this information processing capacity. A large number of diverse neurotransmitters are used by neurons, ranging from classical fast transmitters such as glycine and glutamate over neuropeptides to lipophilic compounds

Thomas C. Südhof
Departments of Neuroscience and Molecular Genetics, and Howard Hughes Medical Institute, The University of Texas Southwestern Medical Center, Dallas, TX 75390-9111, USA
thomas.sudhof@utsouthwestern.edu

T.C. Südhof, K. Starke (eds.), *Pharmacology of Neurotransmitter Release.*
Handbook of Experimental Pharmacology 184.

and gases such as endocannabinoids and nitric oxide. Most of these transmitters are released by exocytosis, the i.e. the fusion of secretory vesicles with the plasma membrane, which exhibits distinct properties for different types of neurotransmitters. The present chapter will provide an overview of the process of neurotransmitter release and its historical context, and give a reference point for the other chapters in this book.

1 Principles of Neurotransmitter Release

Neurons communicate with each other and their target cells via two principal mechanisms: the secretion and reception of chemical messengers called neurotransmitters, and the direct transfer of intercellular signals via gap junctions. Communication via neurotransmitters occurs in several forms that range from classical synaptic transmission at synapses to diffuse secretion of neuromodulators which mediate volume transmission. Communication via gap junctions occurs at so-called electrical synapses. Almost all of the neuronal communication is mediated by neurotransmitters, and electrical synapses are exceedingly rare in vertebrate brain. Both types of communication are not unique to neurons. Secretion of neuromodulators and neuropeptides is also mediated by endocrine cells and even some highly differentiated cells such as adipocytes, and diffusible neurotransmitters such as nitric oxide are released by many non-neuronal cells. Only the presynaptic secretion of classical neurotransmitters in the context of a synapse is specific to neurons, although the postsynaptic cell can be either a neuron (most of the time) or an effector cell (e.g., a muscle cell). The present book will only deal with communication by neurotransmitters, and only with the release of such transmitters and the pharmacology of this release.

What is a neurotransmitter, and how many different "types" of neurotransmitter release exist? At least five types of neurotransmitter release can be defined.

1. Synaptic neurotransmitter release occurs in a classical, electron microscopically observable synapse, and is mediated by synaptic vesicle exocytosis from nerve terminals (Figure 1; Katz, 1969; Südhof, 2004; note that a "nerve terminal" is not necessarily the end of an axon, but generally is formed by axons en passant as they arborize throughout the brain). Synaptic neurotransmitter release, the first step in synaptic transmission, transfers information extremely rapidly (in milliseconds) in a highly localized manner (restricted to an area of less than a square micrometer; reviewed in Südhof, 2004). Synaptic release secretes "classical" neurotransmitters: GABA, glycine, glutamate, acetylcholine, and ATP. It has been suggested that in addition to neurons, astrocytes also secrete classical neurotransmitters by a similar mechanism (**?**), but this type of secretion has not been directly demonstrated.
2. Monoaminergic neurotransmitters (dopamine, noradrenaline, adrenaline, histamine, and serotonin) are released by exocytosis of small dense-core vesicles from

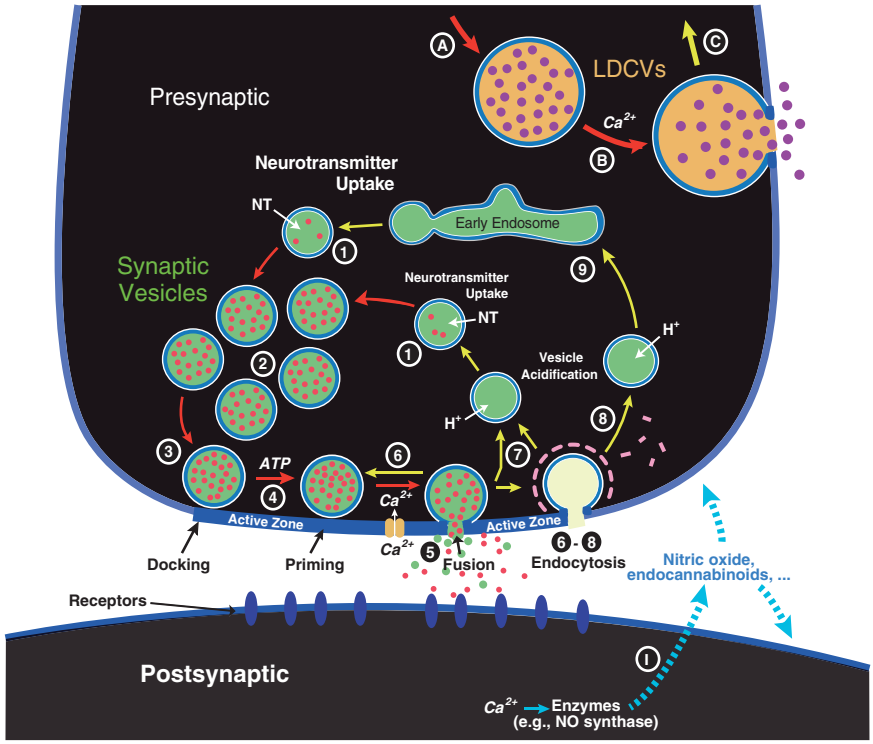

Fig. 1 Secretory pathways in neurons. The drawing schematically illustrates the three major neurotransmitter release pathways. (**a**) Release of classical neurotransmitters by synaptic vesicle exocytosis (center; steps 1–9). Classical neurotransmitter release depends on an underlying synaptic vesicle cycle that starts when synaptic vesicles are filled with neurotransmitters by active transport (step 1), and form the vesicle cluster (step 2). Filled vesicles dock at the active zone (step 3), where they undergo a priming reaction (step 4) that makes them competent for Ca^{2+}-triggered fusion-pore opening (step 5). After fusion-pore opening, synaptic vesicles undergo endocytosis and recycle via three alternative pathways: local reuse (step 6; also called kiss-and-stay), fast recycling without an endosomal intermediate (step 7; also called kiss-and-run), or clathrin-mediated endocytosis (step 8) with recycling via endosomes (step 9). Steps in exocytosis are indicated by red arrows, and steps in endocytosis and recycling by yellow arrows. (**b**) Release of neuropeptides and biogenic amines by LDCV exocytosis. LDCVs are generated in the cell body by budding from the Golgi complex filled with neuropeptides (not shown). LDCVs are then transported from the cell body to the axons or dendrites (step A, as shown for nerve terminals). A Ca^{2+}-signal triggers the translocation and fusion of LDCVs with the plasma membrane outside of the active zone (step B). After exocytosis, empty LDCVs recycle and refill by transport to the cell body and recycling via the Golgi complex (step C). (**c**) Release of gaseous or lipidic neurotransmitters, which are synthesized in either the pre- or the postsynaptic neuron (only the postsynaptic synthesis is shown), and secreted by diffusion across the plasma membrane (step I) to act on local extracellular receptors (e.g., CB1 receptors for endocannabinoids) or intracellular targets (e.g., guanylate cyclase for nitric oxide). (Modified from Südhof, 2004).

axonal varicosities that are largely not associated with a specialized postsynaptic structure (i.e., are outside of synapses; Brock and Cunnane, 1987; Stjärne, 2000). However, at least in the case of dopamine, postsynaptic specializations can occur with presynaptic small dense-core vesicles.

3. Neuropeptides are secreted by exocytosis of large dense-core vesicles (LDCVs) outside of synapses (Figure 1; Salio et al., 2006). LDCVs undergo exocytosis in all parts of a neuron, most often in axon terminals and dendrites. Monoamines are often co-stored with neuropeptides in LDCVs and co-secreted with them upon exocytosis. For all intents and purposes, LDCV-mediated secretion resembles hormone secretion in endocrine cells.
4. Classical neurotransmitters and monoamines may rarely be secreted by neurons, not by exocytosis, but by transporter reversal. This mechanism involves the transport of neurotransmitters from the cytosol to the extracellular fluid via transporters that normally remove neurotransmitters from the extracellular fluid. This mechanism appears to account for the burst of dopamine released by amphetamines (Fleckenstein et al., 2007), but its physiological occurrence remains unclear.
5. A fifth pathway, finally, is the well-established secretion of small membrane-permeable mediators by diffusion. This mechanism is used for the secretion of nitric oxide, endocannabinoids, and other important lipidic or gaseous neurotransmitters. The major point of regulation of release here is the synthesis of the respective compounds, not their actual secretion.

Only the first type of neurotransmitter release mediates the fast point-to-point synaptic transmission process at classical synapses (sometimes referred to as wiring transmission). All of the other types of neurotransmitter release effect one or another form of "volume transmission" whereby the neurotransmitter signal acts diffusely over more prolonged time periods (Agnati et al., 1995). Of these volume transmitter pathways, the time constants and volumes involved differ considerably. For example, diffusible neurotransmitters such as nitric oxide act relatively briefly in a localized manner, whereas at least some neuropeptides act on the whole brain, and can additionally act outside of it (i.e., function as hormones). There is an overlap between wiring and volume neurotransmission in that all classical neurotransmitters act as wiring transmitters via ionotropic receptors, and also act as "volume transmitters" via G-protein-coupled receptors. Moreover, neuromodulators in turn feed back onto classical synaptic transmission.

Quantitatively, synaptic transmission is the dominant form of communication between neurons. A single look at an electron micrograph reveals that synapses with their appendant organelles, especially synaptic vesicles, are abundant in brain, whereas LDCVs are only observed occasionally (Figure 2). However, this does not mean that synaptic transmission is more important than the volume transmission pathways. The two principally different signaling pathways play distinct roles in information processing by the brain, and both are essential for brain function.

With the multitude of different types of transmitters, the question arises whether a single neuron can release more than one transmitter. Dale's principle stated that

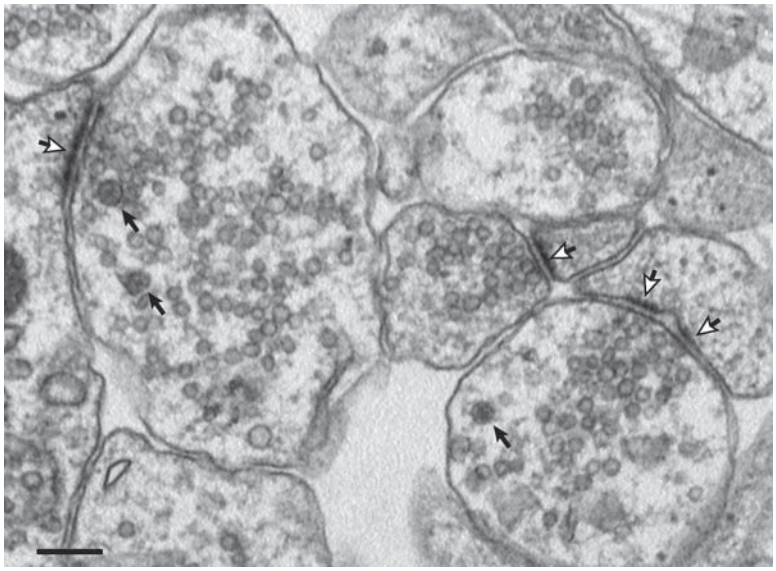

Fig. 2 Electron micrograph of synapses. The image shows synapses formed by cultured cortical neurons from mouse. Note abundant synaptic vesicles in nerve terminals adjacent to synaptic junctions that are composed of presynaptic active zones and postsynaptic densities (open arrows point to postsynaptic densities of synaptic junctions; synapse on the right contains two junctions). In addition to synaptic vesicles, two of the nerve terminals contain LDCVs (closed arrows). Calibration bar = 500 nm. (Image courtesy of Dr. Xinran Liu, UT Southwestern).

this is not the case, but seems to be incorrect given the fact that virtually all neurons secrete neuropeptides and either classical neurotransmitters or monoamines (Salio et al., 2006). Moreover, many neurons additionally secrete diffusible neurotransmitters. Thus, a neuron usually operates by multiple neurotransmitter pathways simultaneously. To add to the complexity of these parallel signaling pathways, the relatively small number of neurons that secrete monoamines from axonal varicosities may also secrete classical neurotransmitters in separate classical synapses (Trudeau, 2004). Despite this complexity, however, Dale has to be given credit for his principle because the multiple transmitters secreted by a given neuron generally operate in distinct secretory and effector pathways. A given neuron usually releases only one type of classical neurotransmitter (with a few exceptions), suggesting that a modified Dale principle is still correct cotransmission.

2 Very Short History of the Analysis of Neurotransmitter Release

Our current concept of synaptic transmission, as mediated by intercellular junctions formed by one neuron with another neuron or target cell, is fairly recent. This concept was proposed in the second half of the 19th century, and proven

only in the 20th century. It was embedded in a larger debate of whether neurons form a "reticular" network of connected cells, or a network of cells whose connections are discontinuous (the so-called neuron theory). Like with everything else in neuroscience, Ramón y Cajal is usually credited with the major discoveries in this field, but the actual concept predates him, and the development of the current view of synaptic transmission is due to a team effort. When Ramón y Cajal followed in the footsteps of scientists like Kühne, Koelliker, and His, who had formulated the first concept of synapses, even though the actual term was coined much later, Cajal's elegant prose and the fortunate opposition of Emilio Golgi to the neuron theory enhanced the influence of his writings and somewhat obscured the fact that the actual concepts that Cajal was presenting were already well established in the literature.

The term synapse was coined in 1897 by the physiologist Charles Sherrington in M. Foster's *Textbook of Physiology*, but the idea of the chemical synapse was developed almost half a century earlier in studies on the neuromuscular junction. As always in science, technical advance spawned conceptual breakthroughs. The three technical advances that fueled the progress in neuroscience in the second half of the 19th century were the improvements in light microscopy, chiefly due to Lister's invention of apochromatic lenses, the continuous development of staining methods culminating in Golgi's epynomous stain, and the application of more precise electrical recordings, allowing the emergence of electrophysiology to complement anatomy. Each historical stage in the discovery process is coupled to a particular preparation and technical approach, and major progress was usually achieved when a new technique was applied to a new preparation. This pattern also applies to the discovery of the synapse which was first described, without naming it, at the neuromuscular junction.

In the middle of the 19th century, it was known from the work of Volta, Galvani, and others that the nerve stimulates muscle contractions at the neuromuscular junction, and that electrical signals were somehow involved. Using the tools of cellular neuroanatomists, Kühne (1862) and Krause (1863) first demonstrated that the neuromuscular junction is not composed of a direct cellular connection between nerve and muscle as had been believed, but is discontinuous. Fifteen years later, the electrophysiologist Emil du Bois-Reymond (1877) proposed that the transmission of a synaptic signal is chemical. Subsequent work by Koelliker, Cajal, and Sherrington generalized this concept of a discontinuous synaptic connection that mediates intercellular signaling to the interneuronal synapses. Although the concept of the synapse continued to be disputed until well into the 20th century (e.g., see Golgi's Nobel lecture), the very existence of these disputes should not prevent us from recognizing that the actual description of synaptic transmission, and at least its proof for one particular synapse, the neuromuscular junction, had been established 50 years earlier.

The next major step forward in deciphering the mechanisms of synaptic transmission occurred in the neuropharmacological studies of Henry Dale, Otto Loewi, Wilhelm Feldberg, and their colleagues. Although, as in the discovery of the synapse as an intercellular noncontinuous junction, many individuals contributed, Loewi is generally credited with the single decisive experiment. This is probably fair, since

Loewi demonstrated directly that a chemical mediator (acetylcholine) is responsible for the transmission of the signal from the vagus nerve to the heart (Loewi, 1921). Despite Loewi's, Dale's, and Feldberg's advances, however, doubts lingered as to whether a chemical signal could be fast enough to account for the speed of synaptic transmission. Many scientists, with John Eccles (one of Sherrington's last pupils) as the most vocal protagonist, continued to espouse the view that fast synaptic transmission is essentially electrical, whereas chemical signaling serves only as a slow modulatory event. In other words, these views proposed a clean division of transmission into fast synaptic wiring transmission that is electrical, and slow volume transmission that is chemical. The doubts about the speed of chemical neurotransmission, and its general validity, were only definitively laid to rest by Bernhard Katz's seminal experiments on the frog neuromuscular junction, demonstrating that synaptic transmission operates as a quantal chemical event (Katz, 1969). It is remarkable that from Kühne's to Katz's studies, the major contributions to establishing synaptic transmission as the major mechanism by which neurons communicate came from the neuromuscular junction. The concept of the synapse was first postulated at the neuromuscular junction, the first genuine neurotransmitter was identified with acetylcholine as the neuromuscular junction neurotransmitter, and the chemical quantal nature of synaptic transmission was revealed at the neuromuscular junction.

The findings of Katz and colleagues raised two major questions: what are the mechanisms that allow the fast secretion of neurotransmitters from presynaptic terminals in response to an action potential? What molecules mediate the fast recognition of these neurotransmitters by the postsynaptic cell? The elucidation of the basic mechanisms of release again started with the cholinergic system in the description and isolation of synaptic vesicles as the central organelle, chiefly by Victor Whittaker (Whittaker and Sheridan, 1965). The progress in the field, however, then shifted to central synapses, with the identification of the major molecules involved in release of neurotransmitters, and the description of the mechanism by which Ca^{2+}-influx into nerve terminals achieves the fast triggering of release via binding to synaptotagmins (reviewed in Südhof, 2004). The discovery of neurotransmitter receptors and their properties was initiated by classical pharmacological approaches dating back to the British school founded by Langley (1921), but the definitive description of these receptors was enabled by the simultaneous development of patch clamping by Neher and Sakmann (1976) and of molecular cloning of these receptors by S. Numa (Noda et al., 1982).

3 Basic Mechanisms of Release by Exocytosis

Most neurotransmitter release occurs by exocytosis of secretory vesicles, which involves the fusion of the secretory vesicles (synaptic vesicles and LDCVs) with the plasma membrane. All intracellular membrane fusion (except for mitochondrial fusion) is thought to operate by the same fundamental mechanism that involves a core machinery composed of four classes of proteins: SNARE-proteins, SM-proteins (for

Sec1/Munc18-like proteins), Rab-proteins, and Rab-effectors (Jahn et al., 2003). The specific isoforms of these proteins that are being used vary tremendously between fusion reactions, but the general principle by which these proteins act seems to be always similar: Rab and Rab-effector proteins appear to proofread the docking and fusion reaction between the two target membranes and may even mediate the docking at least in part, whereas SNARE- and SM-proteins catalyze the actual fusion reaction.

3.1 Rab-Proteins and Rab-Effectors

Rab-proteins are GTP-binding proteins that interact with effectors in a GTP-dependent manner. Rab3A, 3B, 3C, and 3D represent a family of Rab-proteins that are highly enriched on synaptic vesicles and other secretory organelles throughout the body. In addition, Rab27A and 27B are also generally found on secretory vesicles, although it is unclear whether they are present on synaptic vesicles (Südhof, 2004). Rab3/27 proteins together function in exocytosis, and mediate vesicle docking at least in part. Two classes of Rab3/27 effectors were described: rabphilins and RIMs. Both effector classes include multiple members encoded by distinct genes. Rabphilins are cytosolic proteins that are recruited to secretory vesicles by Rab3/27, but their function has remained largely obscure. RIMs are components of the detergent-insoluble protein complex that makes up the active zone, the part of the presynaptic plasma membrane where synaptic vesicles dock and fuse (Figure 3). The active zone is composed of the RIM-containing protein complex that includes several other large proteins, in particular Munc13s, piccolo/bassoon, ELKS, and α-liprins, all of which are crucial for normal synaptic vesicle exocytosis. It is noticeable that in most intracellular fusion reactions, Rab-effectors are composed of large complexes that do more than just bind the Rab-protein, but perform several functions in the fusion process, with the Rab-protein often being involved in the docking of the membranes for fusion and in the regulation of the other activities of the complex during the fusion reaction. The same appears to be true for Rab3/27 binding to the RIM-containing active zone protein complex. The whole active zone complex could be considered as a single large Rab-effector complex (Figure 3), and is likely involved not only in the docking of synaptic vesicles, but also in organizing the actual fusion reaction and in synaptic plasticity (see below).

3.2 SNARE Proteins

Membrane fusion consists of merging two negatively charged phospholipid bilayers, and thus requires overcoming a major energy barrier (Jahn et al., 2003). SNARE proteins represent a family of membrane proteins that are present on opposing membranes destined to fuse. As first proposed by Jahn, Heuser, Rothman and colleagues

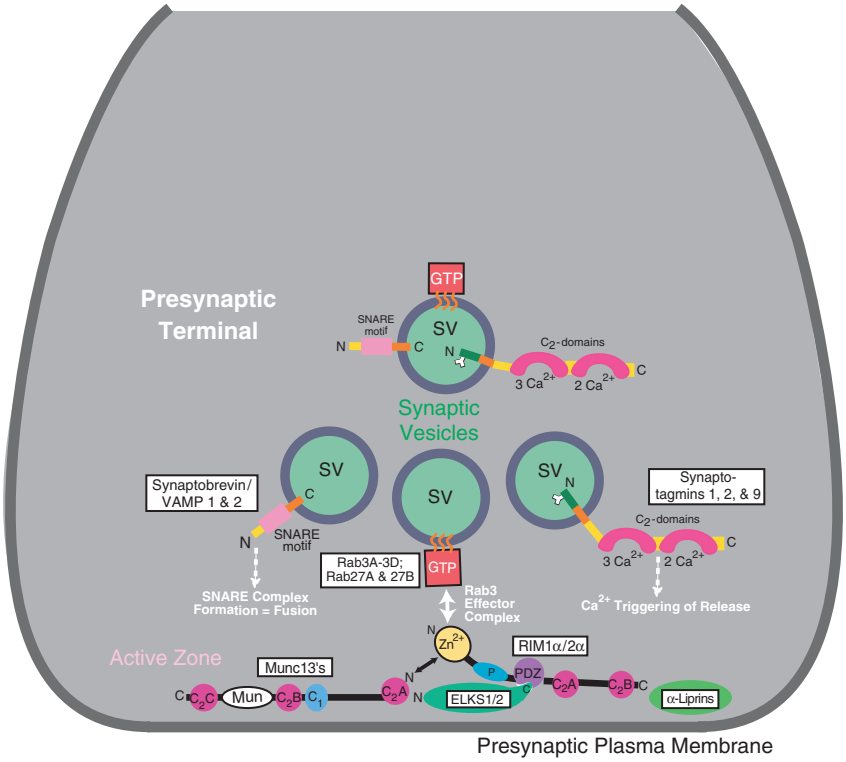

Fig. 3 Interaction of Rab3 and Rab27 on synaptic vesicles with the active zone protein complex containing Munc13s, RIMs, ELKS, and liprins. The schematic drawing depicts a nerve terminal with a few synaptic vesicles containing the three vesicle proteins that mediate exocytosis: the SNARE protein synaptobrevin/VAMP that participates in fusion (see Figure 4), the Rab-proteins Rab3 and Rab27 that attach synaptic vesicles to the active zone protein complex as shown, and the Ca^{2+}-sensor protein synaptotagmin that translates the Ca^{2+}-signal into release (Figure 5). The active zone protein complex is composed of Munc13, RIM, ELKS, and liprins, so that RIM binds to all of the three other active zone proteins, and additionally interacts with Rab3/27 via its N-terminal domain. The active zone protein complex likely contains other protein components that are not shown, in particular piccolo/bassoon. (Modified from Südhof, 2004).

(Hanson et al., 1997; Weber et al., 1998), formation of a "trans-complex" by SNARE proteins on opposing membranes forces these membranes together, thereby overcoming the energy barrier (Figure 4). SNARE proteins contain a characteristic 60-residue sequence, the so-called SNARE motif. SNARE complexes are assembled from four types of SNARE motifs (called R, Qa, Qb, and Qc, classified based on sequence homologies and the central residue) that fold into a tight four-helical bundle which always contains one copy for each type of SNARE motif. The close approximation of two membranes by SNARE-complex assembly destabilizes their negatively charged surfaces, thereby initiating the intermixing of their hydrophobic lipid interiors. This is thought to provide the energy for membrane fusion.

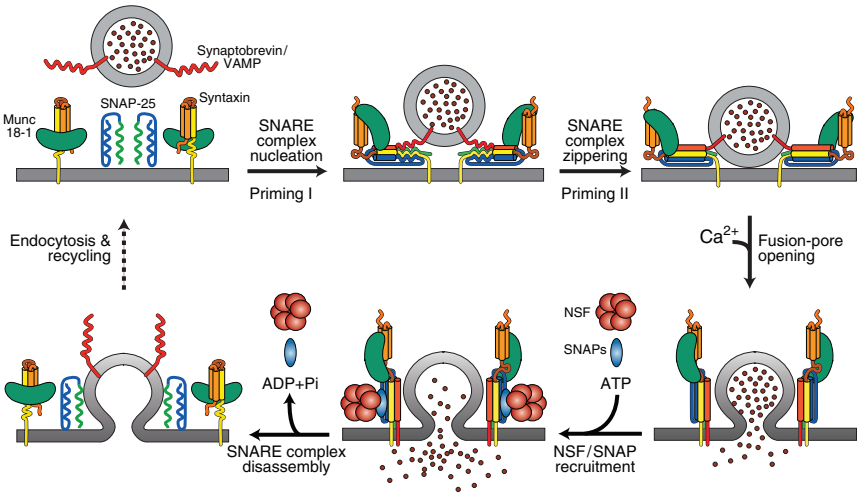

Fig. 4 Schematic diagram of the SNARE protein/Munc18 cycle. Docked synaptic vesicles (top left) may be attached to the active zone via the Rab/RIM interaction (see Figure 3) but contain SNARE proteins that have not yet formed a complex with each other (synaptobrevin/VAMP on synaptic vesicles and SNAP-25 and syntaxin-1 on the plasma membrane; note that syntaxin-1 is thought to be complexed to the SM-protein Munc18-1). Priming is envisioned to occur in two steps that involve the successive assembly of SNARE-complexes (priming I and II). During priming, Munc18-1 is thought to be continuously associated with syntaxin-1, shifting from a heterodimeric binding mode in which it was attached to syntaxin-1 alone to a heteromultimeric binding mode in which it is attached to the entire SNARE complex (top right). After priming, Ca^{2+} triggers fusion-pore opening to release the neurotransmitters by binding to synaptotagmin (see Figure 5). After fusion-pore opening, SNAPs (no relation to SNAP-25) and NSF (an ATPase) bind to the assembled SNARE complexes, disassemble them with ATP-hydrolysis, thereby allowing synaptic vesicles to undergo re-endocytosis and to recycle with synaptobrevin on the vesicle, while leaving SNAP-25 and syntaxin-1/Munc18-1 on the plasma membrane. Note that the overall effect is that SNARE/Munc18-proteins undergo a cycle of association/dissociation that fuels the membrane fusion reaction which underlies release. (Modified from Rizo and Südhof, 2002).

Synaptic exocytosis involves three SNARE proteins: the R-SNARE synaptobrevin/VAMP (isoforms 1 and 2) on the vesicle, and the Q-SNAREs syntaxin (isoforms 1 and 2) and SNAP-25 on the plasma membrane (Figure 4). Since SNAP-25 has two SNARE-motifs, synaptobrevin, syntaxin, and SNAP-25 together have four SNARE-motifs. Synaptobrevins and SNAP-25 are relatively simple SNARE proteins that are composed of little else besides SNARE motifs and membrane-attachment sequences (a transmembrane region for synaptobrevin, and a cysteine-rich palmitoylated sequence for SNAP-25). Syntaxins, in contrast, are complex proteins. The N-terminal two-thirds of syntaxins include a separate, autonomously folded domain (the so-called H_{abc}-domain), while the C-terminal third is composed of a SNARE motif and transmembrane region just like synaptobrevin.

3.3 SM Proteins

Genes for SM-proteins were discovered in genetic screens in *C. elegans* (unc18) and yeast (sec1), and their connection to membrane fusion was identified when the SM-protein Munc18-1 was found to directly bind to syntaxin-1 (Brenner, 1974; Novick et al., 1980; Hata et al., 1993). SM-proteins are composed of a conserved ∼600 amino acid sequence that folds into an arch-shaped structure. With seven members in mammals and four in yeast, SM-proteins constitute a small family of highly homologous proteins. SM proteins have essential roles in all fusion reactions tested. Three SM proteins (Munc18-1, −2, and −3) are involved in exocytosis, where they are at least as essential as SNARE proteins. For example, deletion of Munc18-1 in mice has more severe consequences for synaptic vesicle exocytosis than deletion of synaptobrevin or SNAP-25 (Verhage et al., 2000).

Initially, Munc18-1 was found to bind only to monomeric syntaxin-1 in a manner that is incompatible with SNARE-complex formation. Puzzlingly, however, other SM proteins were subsequently found to bind to assembled SNARE complexes. This puzzle was resolved with the discovery that Munc18-1 (and presumably −2) participates in two distinct modes of SNARE interactions: the originally defined binding to monomeric syntaxins, and a novel mode of direct binding to assembled SNARE complexes (Dulubova et al., 2007; Shen et al., 2007). These results suggested that all SM-proteins directly or indirectly interact with assembled SNARE complexes in fusion. The additional binding of Munc18-1 to the closed conformation of syntaxin prior to SNARE complex formation renders Ca^{2+}-triggered exocytosis unique among fusion reactions, possibly in order to achieve a tighter control of the fusion reaction.

3.4 Mechanism of SNARE and SM Protein Catalyzed Fusion

Both SNARE and SM proteins are required as components of the minimal fusion machinery. At the synapse, for example, deletion of Munc18-1 leads to a loss of all synaptic vesicle fusion, revealing Munc18-1 as an essential component of the fusion machine (Verhage et al., 2000). It is likely that SNARE proteins first force membranes together by forming trans-complexes, thereby creating a fusion intermediate that at least for synaptic vesicles appears to consist of a hemifusion stalk (Figure 4). Since the unifying property of SM proteins is to bind to assembled SNARE complexes, they likely act after such a fusion intermediate has formed, but their exact role remains unknown.

Each intracellular fusion reaction exhibits characteristic properties, and involves a different combination of SM and SNARE proteins. The specificity of fusion reactions appears to be independent of SNARE proteins because SNARE complex formation is nonspecific as long as the Q/R-rule is not violated (i.e., the fact that SNARE complexes need to be formed by SNARE proteins containing R-, Qa-, Qb-, and Qc-SNARE motifs), and of SM proteins because SM proteins often function in

multiple fusion reactions. Fusion specificity must be determined by other mechanisms, possibly GTP-binding proteins of the rab family.

4 Mechanism of Ca^{2+}-Triggering: Ca^{2+}-Channels, Ca^{2+}-Buffering, and Synaptotagmin

Neurotransmitter release is triggered by Ca^{2+} when an action potential invades the nerve terminal and gates the opening of voltage-sensitive Ca^{2+}-channels. Thus, there are two determinants of neurotransmitter release: (1) The Ca^{2+}-dynamics in the nerve terminal that are dictated by the properties and location of the Ca^{2+}-channels; the concentration, affinities, and kinetics of local Ca^{2+}-buffers; and the Ca^{2+}-extrusion mechanisms and (2) the action of the Ca^{2+}-receptors that translate the Ca^{2+}-signal into release, with most release being mediated by Ca^{2+}-binding to synaptotagmins (see below).

4.1 Ca^{2+}*-Dynamics*

The Ca^{2+}-concentration in a nerve terminal depends on the number and temporal pattern of action potentials, the effectiveness of these action potentials to open Ca^{2+}-channels, and the properties and concentrations of Ca^{2+}-buffers. Not only the time course of changes in Ca^{2+}-concentrations, but also the spatial distribution of Ca^{2+}, is important because Ca^{2+} is not uniformly distributed in a nerve terminal. Moreover, the Ca^{2+}-dynamics of a nerve terminal differ between nerve terminals, and play a central role in synaptic plasticity (e.g., see Rozov et al., 2001; Zucker and Regehr, 2002).

Ca^{2+}-channels are well investigated, have proven to be great drug targets, and will be discussed at length in the chapter by Kisilevsky and Zamponi. Two types of Ca^{2+}-channels, the so-called P/Q- and N-type channels (referred to as Cav2.1 and 2.2) account for the vast majority of releases. These Ca^{2+}-channels are located in the active zone of the presynaptic terminal (Llinas et al., 1992), although their precise location is unknown. Ca^{2+}-channels are – not surprisingly – tightly regulated by several signaling systems. As a result of their non-uniform localization and their stringent regulation, the Ca^{2+}-signal produced by the opening of Ca^{2+}-channels by a given action potential cannot be predicted, but varies greatly between synapses in amplitude, space and time (Rozov et al., 2001). This variation is increased by differences in Ca^{2+}-buffering between synapses. Ca^{2+}-buffers are much less understood than Ca^{2+}-channels because of the large number of different types of buffers, the difficulty in manipulating them pharmacologically or genetically, and the problems in measuring them. The most important nerve terminal Ca^{2+}-buffer likely is ATP, which has a relatively low Ca^{2+}-affinity but a high concentration and is highly mobile, rendering it an effective buffer at peak Ca^{2+}-concentrations (Meinrenken et al.,