Ion Channels in Presynaptic Nerve Terminals and Control of Transmitter Release

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I. INTRODUCTION

One of the main ways of neuronal communication in the nervous system is by chemical synaptic transmission, where the presynaptic nerve terminal releases transmitters that interact with the postsynaptic target to produce the synaptic response. This review deals with one specific aspect of the function of the secreting presynaptic nerve terminal: the role of the ion channels in its activity.

The primary function of the presynaptic nerve terminal is to release transmitter and thus activate the postsynaptic target cell. In almost every step leading to the release of transmitter quanta, there is a substantial involvement of ion channels. In this review, the multitude of ion channels in the presynaptic terminal are surveyed. There are at least 12 different major categories of ion channels representing several tens of different ion channel types; the number of different ion channel molecules at presynaptic nerve terminals is many hundreds. We describe the different ion channel molecules at the surface membrane and inside the nerve terminal in the context of their possible role in the process of transmitter release. Frequently, a number of different ion channel molecules, with the same basic function, are present at the same nerve terminal. This is especially evident in the cases of calcium channels and potassium channels. This abundance of ion channels allows for a physiological and pharmacological fine tuning of the process of transmitter release and thus of synaptic transmission.

A. Steps in Presynaptic Activation

1) The first step in evoked presynaptic activation is the propagation of the action potential into the nerve terminals. This process involves the usual complement of ion channels that are found in the generation and propagation of action potentials in many cells. An excellent review by Jackson (380) summarizes the ion channels involved in presynaptic excitability.

2) The next step is the invasion of the action potential in the presynaptic nerve terminals causing the depolarization of the presynaptic membrane. In some cells, the action potential invades the presynaptic nerve terminal actively to its very end, whereas in other cells, the nerve terminals are depolarized by electrotonic spreading (380). Of special interest is the bifurcation point, which is frequently the site of failure of action potential propagation into the presynaptic nerve terminals (311, 635).

3) When the action potential invades the presynaptic nerve terminal it causes depolarization of the presynaptic nerve terminal membrane; this depolarization acts on a large number of voltage-regulated ion channels, such as sodium channels, calcium channels, potassium channels, nonselective channels, and chloride channels. Their probability of being in an open state is drastically changed (in most cases increased) by the depolarization.

4) Of special interest is the activation of the calcium channels in the presynaptic nerve terminal membrane that open upon depolarization. Their opening allows calcium to enter into the presynaptic nerve terminal and to increase the calcium concentration inside the nerve terminal. At rest, intracellular calcium concentration ([Ca$^{2+}$]$_i$) is very low ($\sim 10^{-7}$ M), but upon depolarization, this concentration increases substantially. In addition to calcium entry, there may also be a release of calcium from intracellular stores that affect the [Ca$^{2+}$]$_i$. (see Refs. 465, 611).

5) The increase of [Ca$^{2+}$]$_i$ causes a binding of calcium ions with special intracellular calcium sensors. Although the identity of all calcium sensors inside the presynaptic nerve terminal is not yet conclusive (107, 109,
149, 194, 290, 411, 477, 516, 624), there is a strong indication that synaptotagmin is one of the principal calcium sensors (91, 196). Synaptotagmin has C2 domains that have an important function in the calcium control of the exocytotic pathway (148, 777, 843, 848). The differential activation of transmitter release by calcium and strontium ions in peripheral (208, 537) and central synapses suggests the existence of two different sensors, which probably respond to different intracellular concentrations of calcium ions (294).

Activation of the calcium sensors leads to activation of the fusion machinery in the nerve terminal. It is well known that neurotransmitters are released from the presynaptic nerve terminal in two ways: quantal release (see Ref. 400) and molecular leakage (408). The main mode responsible for synaptic transmission is quantal release. Activation of the fusion machinery causes the fusion of the synaptic vesicle, which stores the neurotransmitter, with the surface membrane. In recent years, tremendous progress has been made in the identification and in the partial understanding of the role of fusion proteins, from yeast to vertebrates (43, 113, 118, 281, 385, 475, 477, 567, 809, 839, 883, 982). It is clear that many molecular steps are lumped together in the term fusion machinery (480).

Activation of the fusion machinery leads to fusion of the synaptic vesicle with the surface membrane and the quantal release of the neurotransmitter. Capacitance measurements have led to rapid advances in the evaluation and the interpretation of the fusion, mainly in other secreting cells rather than in presynaptic nerve terminals (471, 561).

It was recently suggested (683) that the release of transmitters can be controlled also after the fusion of the synaptic vesicle with the surface membrane. This postfusion control of transmitter release probably also involves the participation of ion channels in the vesicle membrane and in the nerve terminal membrane.

B. Overview of Channels in Presynaptic Nerve Terminals

Until June 1997, more than 40 different ion channels have been described in various nerve terminals. We present a brief overview of the main functions of the known ion channels, according to the ion that passes through the presynaptic nerve terminal membrane. We provide examples from “proper” presynaptic terminals that innervate the postsynaptic membrane and from secretory nerve terminals that secrete their products into the circulatory system.

Conceptually, ion channels are very simple molecular machines. They exist in two main states: open and closed (named also shut state) (see Ref. 743). When they are in an open state, they pass ions passively according their electrochemical gradient (the combined electrical and concentration driving forces). The transition between the open and the shut state is termed gating. In this review, we see predominantly two types of gating of ion channels in the presynaptic nerve terminals: voltage gating and ligand gating. The voltage-gated channels change their probability of transition, from shut to open state, according to the membrane potential of the nerve terminal. The ligand-gated channels respond to the concentration of a specific ligand inside the nerve terminal, in the extracellular space or in the membrane domain.

When ion channels open and ions flow through an open channel according to their electrochemical gradient, three main changes occur: 1) there are changes in the concentration of the relevant ions inside the nerve terminal and in the immediate extracellular space, 2) there is a change in the membrane potential, and 3) there is a change in the membrane resistance: the opening of channels causes an increase in conductance and hence the membrane resistance decreases. This in turn affects the space constant and the time constant of the membrane, and other ionic currents have an altered efficacy.

We have seen in a previous section that the main “workhorse” in the process of quantal transmitter release is calcium. Therefore, we start the description of the ion channels in the presynaptic nerve terminal by discussing the properties of the calcium channels in some detail. Because most (but definitely not all) of the calcium channels in the nerve endings are voltage gated, we discuss thereafter the ion channels that control the membrane potential (and the membrane conductance) of the nerve terminal, namely, the potassium, the sodium, the nonselective, and the chloride channels (see Fig. 1). Finally, we briefly discuss the ligand-gated channels and the other channels present in the nerve terminal.

II. CALCIUM CHANNELS IN NERVE TERMINALS

A. Function of Calcium Channels

Calcium ions have many functions in neuronal cells. These include spike initiation (172, 226, 544, 582), rhythmic firing, neurite outgrowth, gene expression, and transmitter release (for review, see Ref. 161). The latter function is the central topic of this review article; hence, the other functions are mentioned only briefly.

B. A Primer on Classification of Calcium Channels

There are a large number of different calcium channels in the nervous system. In addition, confusion arises
from the use of different nomenclatures in referring to the same channels. We felt, therefore, that a brief introduction to the classification and the nomenclature of calcium channels would not be out of place. There are three different levels of classification, which roughly represent the historical development of the subject.

1. Voltage classification

Originally, it was assumed that there was only one type of calcium channel. However, the pioneering work of Hagiwara et al. (315) indicated that there may be more than one type of calcium current in the egg cell membrane of the starfish. Subsequently, it was found that such a distinction occurs in many other cells from different organisms (56, 58, 126, 595, 601, 702). The different currents have different voltage thresholds for activation; thus low voltage-activated channels (LVA) are those in which the activation is slightly above the resting potential, and high voltage-activated channels (HVA) are those in which the threshold for activation is substantially above the resting potential (towards 0 mV). In addition to the activation voltage, different calcium channels may be distinguished by their single-channel properties, activation kinetics, and inactivation kinetics. These properties of the channels in the nerve terminal are summarized in section II D.

2. Pharmacological classification

A) L, N, AND T CHANNELS. Once it was realized that there are at least two different types of calcium currents and channels, a search started to find specific pharmacological agents. A family of chemical substances, the dihydropyridines (DHP), was found to affect HVA channels (336) but not LVA channels (56). Some members of the family, such as nitredipine, inhibit the HVA channel activity, whereas other compounds, such as BAY K 8644, activate the HVA calcium channels (56, 336).

When calcium channels and currents in the dorsal root ganglion neurons of the chick were analyzed, it was found that not all HVA channel activity was affected by DHP (90, 125, 601). Hence, on the basis of the DHP sensitivity and kinetics, the HVA channels were further subdivided into DHP-sensitive channels (L-type calcium channels) and DHP-insensitive channels (N-type calcium channels) (see Fig. 2). The LVA channels were named T-type calcium channels (T for transient) (601). The search for an inhibitor of the N-type calcium channels yielded a toxin from the marine snail Conus geographus, namely, \( \omega \)-conotoxin GVIA (524).

B) MINDING THE P AND Q. The story does not end here. An additional type of HVA calcium channel, originally found in cerebellar Purkinje cells (156, 483, 910), is the P-type calcium channel, which is inhibited by funnel-web spider polypeptide and peptide toxins FTX and \( \omega \)-agatoxin IVA, respectively (551, 762). When the calcium channel sensitivity to agatoxin was tested on channels expressed in oocytes, it was found that these channels have a low sensitivity to agatoxin (~200 nM) (752), whereas the P-type sensitivity is much higher (~2 nM). This led to the proposal that there is yet another type of voltage-sensitive HVA calcium channel: the Q-type calcium channel (1003). The distinction between the P-type and Q-type calcium channels is not always obvious, and hence, they are frequently grouped together, as the P/Q type of calcium channels.
3. Molecular classification

A) THE SUBUNITS. Voltage-dependent calcium channels (VDCC), like many other channels, are composed of different subunits. There are five different subunits that are associated with the VDCC activity: \( \alpha_1 \), \( \alpha_2 \), \( \beta \), \( \gamma \), and \( \delta \). The \( \gamma \)-subunit has been found in skeletal muscle but not in brain and is not discussed here (211). The \( \alpha_\alpha \) _ and \( \delta \)-subunits are connected by a disulfide bridge and are referred to as a unitary complex \( \alpha_\alpha \delta \). Hence, in the mammalian nervous system, we are dealing with three distinct subunits \( \alpha_1 \), \( \alpha_2 \delta \), and \( \beta \) that together form a functional calcium channel (959, 1003).

The \( \alpha_1 \)-subunit is the pore-forming part of the molecule through which the calcium ions flow (see Ref. 806). The \( \beta \)-subunit interacts with \( \alpha_1 \) at the intracellular side and is postulated to alter the calcium channel inactivation (968). The function of the \( \alpha_\delta \delta \)-subunit is still unknown (for review, see Ref. 211).

The molecular diversity of the calcium channels in the nervous system and in other cells is achieved by different genes, alternative splicing, subunit assembly, and posttranslational modifications (79, 587, 649). It is not inconceivable that channel-associated proteins (773) may also alter the properties of the ion channels and thus increase their functional diversity.

B) \( \alpha_1 \)-SUBUNIT. Seven different genes were found to encode the \( \alpha_1 \)-subunit. They are named \( A \), \( B \), \( C \), \( D \), \( E \), \( G \), and \( S \). The \( S \) gene encodes the \( \alpha_1 \)-subunit in the skeletal muscle; the products of the other six genes were found in the brain. Each of the genes can produce more than one gene product by alternative splicing. Altogether, at least 18 different \( \alpha_1 \)-gene products have been identified in the nervous system (79, 648, 806).

C) \( \beta \)-SUBUNIT. Four different genes were found to encode the \( \beta \)-subunit and are named \( 1 \), \( 2 \), \( 3 \), and \( 4 \). Each of the genes can produce more than one gene product by alternative splicing. At least eight different \( \beta \)-gene products have been identified in the brain (79, 133, 374, 647).

D) \( \alpha_\delta \)-SUBUNIT. The \( \delta \)-subunit seems to have an identical structure to that of the COOH-terminal part of the \( \alpha_\delta \)-subunit and hence may be the product of the same gene, altered by posttranslational modification. A splice variant was found in the rat brain (199). This subunit may be involved in the regulation of secretion (967).

4. Who is who?

There are three different classifications of the voltage-sensitive calcium channels. Clearly, it is of great interest to find which channel corresponds to what molecular structure; however, only a partial achievement of this aim is possible at present. The DHP-sensitive L-type calcium channels in the nervous system have the \( \alpha_\alpha \delta \)-subunit and hence may be the product of the same gene, altered by posttranslational modification. A splice variant was found in the rat brain (199). This subunit may be involved in the regulation of secretion (967).

5. Diversity and multitude of calcium routes into the neuron

Calcium ions can enter the neuron interior by different routes. In section \( \mu \)A we discussed voltage-activated calcium channels. In addition, calcium ions can enter the
nerve through nonselective ion channels, through ligand-gated cation channels, and in the reverse mode of the sodium/calcium exchanger.

To appreciate the diversity of the calcium routes into the neuron and the nerve terminal, let us make a rough estimate of only one of the components of the calcium entry systems into the nerve: the voltage-gated calcium-selective ion channels. Such a calculation shows that the number of possible molecular species is really astounding. There are at least 18 different \( \alpha_1 \)-subunit genes including splice variants and at least 8 different \( \beta \)-subunits, without taking into consideration posttranslational modifications. If there are no forbidden assemblies, at least 144 different types of calcium channels can be generated with these 2 subunits only. If one takes into account also the splice variants of the \( \alpha_2 \delta \)-subunit, one reaches at least 288 different calcium channel molecules. Of course, not all the permutations are possible, since not all the genes are expressed in the same cell, but the possibility for diversity is extensive. The diversity is even larger envisaging the molecular interactions of calcium channels with other proteins (see, for example, Ref. 698), transmitters, and second messengers. If one also takes into consideration the other possible routes for calcium entry, then there is no doubt that the nerve cell possesses many hundreds (if not thousands) of different ways to alter the [\( \text{Ca}^{2+} \)]. Even more surprising is that many of the various routes are present in the same nerve terminal and may be responsible for transmitter release. Why does the nerve terminal need such a wide variety of calcium entry routes? We speculate that such multitude of control of the same basic function allows a great plasticity in transmitter release and thus in synaptic transmission in the nervous system (see below). The presence of different calcium channels in the same nerve terminal may also have an importance in the partial protection against animal toxins. If a toxin is “administered” in the body, it will block only part of the calcium channels; this may have an obvious evolutionary advantage.

It is no wonder then that such molecular diversity leads to a large number of pharmacological actions. In section II.C.8 and Table 2 we present the pharmacological modification of the presynaptic nerve terminal function, using agents acting on one or more molecular targets.

C. Methods for Studying Calcium Channels in Nerve Terminals

The most desirable methods to study the properties of the calcium channels in the presynaptic nerve terminals are direct electrophysiological methods and specific molecular probes for calcium channels. However, there is a great gap between what is desirable and what is available. The calcium channels in the nerve terminals can be studied directly, at the single-channel level using the patch-clamp technique, only in a very limited number of preparations, namely, the chick calyx synapse (820, 845), the rat calyx of Held (52, 95, 264, 372, 861), and the peptidergic nerve terminals of the rat neurohypophysis which release their content into the bloodstream (292, 417, 766, 945). (Calcium currents can be studied in a larger number of preparations.) Thus some extrapolation from these preparations, where direct methods are feasible, to other interesting structures is necessary. Most of our knowledge, regarding calcium channel properties in nerve terminals, comes from indirect methods. In section I, we outlined the various steps in presynaptic function. They may also serve as an indication of how far the method is from the actual activity of the calcium channels in the presynaptic nerve terminal. We briefly summarize here these indirect methods and their inherent difficulties, to assess the strength of various arguments regarding the role of calcium channels in the regulation of presynaptic function.

1. Quantal transmitter release

The primary function of the nerve terminal is to release quanta of transmitter (200, 247). Because the release of transmitter quanta is a function of [\( \text{Ca}^{2+} \)], and because [\( \text{Ca}^{2+} \)] is a function of the activity of the calcium channels at the surface membrane, it was assumed that measurements of evoked quantal transmitter release represent mainly the activity of the calcium channels. The drawback of this method, however, is the nonlinearity between calcium concentration and transmitter release (209). Because this nonlinear relation has a sigmoidal shape (45, 209, 360) and because [\( \text{Ca}^{2+} \)] can be affected by additional pathways and not only by the surface membrane calcium channels, the conclusions from such studies are indirect.

2. Amplitude of the postsynaptic response

The previous method requires knowledge of the unitary quantal event. In many cases, however, this quantity cannot be estimated accurately because of its small size or multiple inputs. In such cases, even a more indirect measure was used to evaluate the activity of the calcium channels in the presynaptic nerve terminals: the amplitude of the postsynaptic potential or current. Despite the
inherent difficulties of these methods, many valuable results were obtained by this method for excitatory and inhibitory responses in peripheral and central synapses.

3. Release of transmitters from synaptosomes

The release of transmitter can be measured not only by physiological means, but also by biochemical methods using pinched-off nerve terminals (591) (for review, see Ref. 962), as a function of calcium concentration (256, 323, 367, 384, 489, 623, 696, 705, 736, 895, 899, 900). The difficulty with this method is that the synaptosome is usually not amenable to physiological stimulation, and the depolarization needed for the opening of the calcium channels to evoke transmitter release is achieved by elevating the extracellular potassium concentration. Because changes in extracellular potassium concentration ([K\textsuperscript{+}]\textsubscript{o}) produce usually a much slower and more prolonged effect on the membrane potential, channel inactivation probably occurs. An additional difficulty is that the release of transmitter to the extracellular medium reflects not only evoked transmitter release from the presynaptic nerve terminal, but also the molecular leakage found to occur in a number of synapses (see Ref. 262, 408, 433).

4. Radioactive calcium fluxes into synaptosomes

Using radioactively labeled calcium, one can measure calcium influx into the nerve terminal (mainly synaptosomes but also in brain slices) (287, 486, 580, 703) and the resulting transmitter release. The main disadvantage of this technique for both influx and transmitter release is the time resolution, which is usually too slow for detailed characterization of the presynaptic processes involved.

5. Fluorescence measurements of [Ca\textsuperscript{2+}]\textsubscript{i}

By using fluorescent compounds, which change their emission in response to the calcium concentration in their vicinity, one can measure the changes in [Ca\textsuperscript{2+}]\textsubscript{i} (and intraterminal calcium concentration). This method is also widely used for studying calcium channels in presynaptic terminal membranes (105, 119, 189, 235, 332, 445, 461, 538, 550, 685, 805, 832, 833, 880, 977). In passing, we want to mention that fluorescence probes have also been used to monitor the membrane potential and the shape of the nerve terminals (539).

6. Capacitance measurements in nerve terminals and secretory cells

In most studies, the release of a quantum of transmitter was measured by its postsynaptic action. However, this information can also be obtained from the properties of the releasing cell, using variants of the patch-clamp technique (see Ref. 743). The whole cell patch-clamp technique enables one to monitor the changes in membrane surface area via changes in capacitance, and therefore to measure the exocytosis process directly. Combining this method with pharmacological studies reveals the contribution of the calcium channels to the transmitter release process (418, 469, 766, 767, 927).

7. Presynaptic calcium currents and calcium channels

In the calyx synapse of the chick, it was possible to record both “whole terminal” and single calcium channel activity (817, 824, 989, 990). This presynaptic terminal is exposed after enzymatic treatment. Almost all the methods described above were used in the terminals of the rat neurohypophysis. The role and contribution of calcium channels to secretion could be compared by different methods such as single-channel recordings (458), intraterminal recordings (99), whole terminal recordings (264, 943), calcium imaging, neurotransmitter release (833), and exocytosis measurements (766).

Single-channel recording has obvious advantages for monitoring the channel activity directly, enabling detailed electrophysiological and pharmacological characterization. The disadvantage is that the channel is usually studied in isolation, and the interpretation of its contribution to a physiological process is not direct. On the other hand, whole terminal recording and intraterminal recording supply the characteristics of the macroscopic current that compensates for this disadvantage in the single-channel recording. Single-channel recordings were made also in altered preparations of presynaptic terminal such as fused synaptosomes (535, 536) or presynaptic channels expressed in model systems (904).

Extracellular recording was used in many preparations to overcome the difficulty of recording calcium channels in the small presynaptic terminal. By blocking the potassium and sodium currents using pharmacological tools, many laboratories were able to record extracellular currents defined as carried by calcium ions either by calcium depletion or by agents known to block calcium currents. It was used mainly in neuromuscular junction preparations (see, for example, Refs. 23, 508). One of the main drawbacks of this method is that it is impossible to define in detail the biophysical characteristics of the current measured.

8. Pharmacology

Usually the various methods are combined with specific agonists or antagonists that determine the existence and define the type of calcium channel. It should be mentioned that the basic pharmacology was usually investigated not in nerve terminal preparations; hence, some extrapolation was often necessary to reach the conclusions regarding the nerve terminal. Because the number of pharmacological studies of transmitter release
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<td>SNX-260</td>
<td>297, 470, 703, 900, 917</td>
</tr>
<tr>
<td>Cd&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>844</td>
</tr>
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</table>

**Q type**

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>o-&lt;em&gt;Conotoxin MVIIC&lt;/em&gt;</td>
<td>299, 705, 960</td>
</tr>
<tr>
<td>o-&lt;em&gt;Agatoxin IVA&lt;/em&gt;</td>
<td>960</td>
</tr>
<tr>
<td>Cd&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>705</td>
</tr>
</tbody>
</table>
D. Functional Properties of Identified Calcium Channels in Nerve Terminals

The methods described in the previous section were used to determine the functional properties of calcium channels in the nerve terminals (see Table 3). Valuable information is available from nerve terminals where direct patch-clamp recordings were made, and calcium channels were characterized on the single-channel or on the whole terminal level. This was achieved in a very limited number of nerve terminals, among them the peptidergic terminals of the rat neurohypophysis (943), the calyx synapse of the chick (818), and at the synapse of Held (52, 264, 372, 861).

Before starting the description of the various channels, we want to point out two generalizations. First, the voltage-activated calcium channels characterized until now at the nerve terminals are of the high voltage-activated (HVA channels) variety and, therefore, are activated by an action potential invading the nerve terminal. Second, most of the channels with all their characteristics do not fit into the conventional nomenclature of voltage-activated calcium channels. In most cases, we use the names proposed in the original articles.

1. L type

An L-type calcium channel has been described in rat neurohypophysial terminals; it displays a single-channel conductance of 25 pS (with 110 mM Ba\(^{2+}\)). The threshold for activation is around \(-20\) mV, and it shows no voltage-dependent inactivation. The decay time kinetics of the ensemble currents are \(<500\) ms at the single-channel level (943) and \(1,250\) ms for the whole terminal current (943). The mean open time during voltage pulses from a holding potential of \(-50\) mV to test potential of \(-10\) mV is \(0.49\) ms, and the closed time distribution has two components with means of \(2.02\) and \(79.91\) ms. The activity (\(N_{Po}\)) of the channel increases with voltage. There is no information about the activation and deactivation kinetics.

In goldfish retinal bipolar synaptic terminals, the only calcium current is of the L type. Its threshold for activation is \(-50\) mV, and its maximal amplitude is at \(-15\) mV (332). This current shows slow calcium-dependent inactivation with a minimal time constant of \(4.6\) s. The whole cell calcium current inactivation is due not to change in the voltage dependence of the channel, but to the closure of channels (928).

2. N\(_{i}\) type

In neurohypophysial terminals, the N\(_{i}\) type has a single-channel conductance of \(11\) pS (with \(110\) mM Ba\(^{2+}\)).

### Table 2. Continued

<table>
<thead>
<tr>
<th>Antagonist</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Mg(^{2+})</td>
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</tr>
<tr>
<td>Mn(^{2+})</td>
<td>50, 138, 150, 302, 518, 568, 665, 858</td>
</tr>
<tr>
<td>Co(^{2+})</td>
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<tr>
<td>Pb(^{2+})</td>
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<tr>
<td>Cd(^{2+})</td>
<td>138, 174, 175, 215, 221, 470, 550, 568, 605, 623, 646, 651, 665, 693, 829, 846, 899, 906</td>
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<tr>
<td>Ni(^{2+})</td>
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<tr>
<td>Sn(^{2+})</td>
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<td>Gd(^{3+})</td>
<td>138</td>
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<tr>
<td>La(^{3+})</td>
<td>123</td>
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<td>897</td>
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<tr>
<td>ω-Conotoxin MVIIA</td>
<td>917, 992</td>
</tr>
<tr>
<td>ω-Grammotoxin SIA</td>
<td>900</td>
</tr>
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<td>Aminoglycoside antibiotics</td>
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<td>Hololena toxin</td>
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<tr>
<td>Ethanol</td>
<td>467, 852</td>
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<td>(−)-Daurosline</td>
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<tr>
<td>Wagler in-I</td>
<td>891</td>
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<tr>
<td>CNS 1237</td>
<td>297</td>
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<tr>
<td>Leptinotarsin*</td>
<td>546</td>
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<tr>
<td>CGP 28.392*</td>
<td>654</td>
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<tr>
<td>Phentolamine*</td>
<td>141</td>
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<tr>
<td>Extract of the marine algal flagellate</td>
<td>541</td>
</tr>
<tr>
<td>Prymnesium patelli ferum*</td>
<td>541</td>
</tr>
<tr>
<td>l-Triiodothyronine*</td>
<td>323</td>
</tr>
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</table>

* Agonist.
TABLE 3. Functional properties of calcium channels in nerve terminals

<table>
<thead>
<tr>
<th>Type</th>
<th>γ, pS</th>
<th>( V_{th}, \text{mV} )</th>
<th>Activation</th>
<th>Inactivation</th>
<th>Animal and Terminal</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>25</td>
<td>−20</td>
<td>Time to peak −21 ms</td>
<td>( \tau \sim 500–1,250 \text{ ms} )</td>
<td>Rat neurohypophysis</td>
<td>943</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−50</td>
<td></td>
<td>( \tau \sim 4,600 \text{ ms Ca}^{2+} ) dependent</td>
<td>Goldfish retinal bipolar</td>
<td>332, 928</td>
</tr>
<tr>
<td>( N_t )</td>
<td>11</td>
<td>−10</td>
<td></td>
<td>( \tau \sim 50–100 \text{ ms} ), ( V_{1/2} \sim ) −68 mV</td>
<td>Rat neurohypophysis</td>
<td>943</td>
</tr>
<tr>
<td>NPT</td>
<td>11–14</td>
<td>−30</td>
<td>( \tau \sim 1.5 \text{ ms} )</td>
<td></td>
<td>Chick ciliary ganglion</td>
<td>990</td>
</tr>
<tr>
<td>P</td>
<td>9–19*</td>
<td>−40</td>
<td>Voltage dependent, maximal at +20 mV, ( \tau \sim 0.5 \text{ ms} )</td>
<td></td>
<td>Squid giant synapse</td>
<td>46</td>
</tr>
<tr>
<td>Q</td>
<td>16</td>
<td>−10</td>
<td>Time to peak −5 ms</td>
<td>( \tau \sim 116 \text{ ms} )</td>
<td>Rat hippocampus expression in Xenopus</td>
<td>960</td>
</tr>
</tbody>
</table>

For L and Q type, channel and current properties were not characterized in presynaptic terminal channel (752). \( N_t \)-type channel is probably a combination of N- and Q-type channels. *Single-channel properties were not characterized in presynaptic terminal channel (910). \( \tau \), time constant.

The threshold for activation is around −10 mV, and it shows voltage-dependent inactivation (half-maximal voltage is around −68.5 mV). The decay time kinetics of the ensemble currents are <50 ms at the single-channel level (943) and 100 ms for the whole terminal current (943). The mean open time during voltage pulses from a holding potential of −90 mV to test potential of −10 mV is 0.34 ms, and the closed time has two components with means of 1.78 and 86.6 ms. The activity (\( NPO \)) of the channel increases with voltage. In some of the patches, the channel did not inactivate and continued to be open throughout the test pulse.

3. NPT type

In the presynaptic nerve terminals of the chick ciliary ganglion, one type of channel was characterized on a single-channel level, and two components were dissected on pharmacological and inactivation bases (990). The single-channel conductance ranges between 11 and 14 pS (with 110 mM Ba\(^{2+} \)) (818) and is reduced to 10 pS with 6 mM Ca\(^{2+} \) and 6 mM Ba\(^{2+} \) (819). The threshold for activation is around −30 mV, and it has little voltage-dependent inactivation. The activation rate constant in whole terminal current is around 1.5 ms at positive potentials, and the deactivation rate at −80 mV is 0.77 ms (824). Although no inactivation was observed in the “whole cell” current when barium was the charge carrier, clear inactivation was detected when calcium ions replaced it, and the rate of inactivation increased with external calcium concentration (990). This calcium-dependent inactivation may be applicable to other VDCC in nerve terminals that were examined with barium.

4. P like

In the squid giant presynaptic terminal, the calcium current is probably carried via P channels (150). The threshold for activation is around −40 mV, and it shows no voltage-dependent inactivation during 25-ms voltage pulse. The activation is half-maximal at −13 mV and maximal at −20 mV. Activation half times were reduced from 1.5 ms at −20 mV to 0.5 ms at −20 mV (46).

5. Q type

This type of channel was not characterized directly in a nerve terminal preparation but was suggested to be involved in the transmitter release process and therefore to be located at the presynaptic nerve terminal (960). Characterization of this channel by expressing class A \( \alpha_1 \)-subunit in Xenopus oocytes revealed that this subunit is the Q type (752). The threshold for activation is around −10 mV, and it shows steep voltage-dependent inactivation. Its single-channel conductance is 16 pS, and the time to peak current amplitude is ∼5 ms, time for half decay (inactivation) is 116 ms, and the single-channel open time distribution has two components of 0.5 and 2.6 ms.

6. R type

Recently, it was shown by Wu et al. (976) that R-type calcium channels may be involved in the regulation of transmitter release at the calyx-type synapse of the rat medial nucleus of the trapezoid body. After abolishing the L, N, and the P/Q type of calcium channels by pharmacological agents, they recorded the remaining calcium current that constituted 20% of the total calcium current. It fitted the classification of R-type calcium current. This current was large enough to generate release of transmitter sufficient for a suprathreshold postsynaptic response. There are three very interesting properties of this R-type calcium channel: the activity of this channel is inhibited by metabotropic glutamate receptors, the activity is inhibited by GABA\(_B\) receptors, and the calcium sensitivity of release induced by the activation of these channels is substantially lower than in other cases (see sect. nF).
E. “Demography” and “Geography” of Calcium Channels

1. Calcium channels are localized at presynaptic terminals

The existence of calcium-conducting pathways into the nerve terminal has been shown by a variety of methods. Some of the methods are rather indirect and show that the $[\text{Ca}^{2+}]_i$ increases in the nerve terminal; other methods are more direct in nature, enabling the visualization of the channel protein.

The initial proposal that calcium channels exist in the nerve terminals came from electrophysiological experimentation at the squid giant synapse (45, 46, 151, 406) and at the neuromuscular junction of a number of species (see Refs. 23, 106, 458, 508). In this section we present some recent articles showing an increase in $[\text{Ca}^{2+}]_i$ in the nerve terminal, or the existence of presumed specific channel proteins, in different vertebrate and invertebrate species.

A) SQUID. With the use of fura 2 measurements, it was found at the squid giant presynaptic terminals that calcium concentration was highest in the compartment closest to the postsynaptic neuron. The colocalization of calcium transients and active zones strongly suggests that neurons cluster calcium channels selectively at active zones, and this localization enhances the magnitude of calcium signals in the vicinity of the active zones (484, 805).

B) BARNACLE. Presynaptic terminal region, and individual photoreceptor terminals of the barnacle, Balanus nubilus, were studied using the calcium indicator dyes arsenazo III and fura 2. It was found that calcium entry occurs in a restricted region $<50 \mu$m in length, which corresponded closely to the region of synaptic contact with second-order cells (830), and that depolarizing pulses produced voltage-dependent calcium entry, that was confined to the tips of the arborization (119).

C) TORPEDO. With the use of a combination of colloidal gold labeling and freeze-fracture techniques, it was found in nerve terminals isolated from the electric organ of Torpedo marmorata, that antagonist specific for voltage-activated calcium channels binds to intramembrane particles in presynaptic membranes. Biotinylated derivative of $\omega$-conotoxin exerts an inhibitory action on the high potassium-evoked release of ATP (243).

D) GOLDFISH. In goldfish cultured retinal ganglion cell growth cones, with the use of a circular vibrating microprobe, it was found that cell growth cones generate steady inward currents at their tips. The major part of this current is carried by calcium ions, which are suggested to flow through a population of voltage-sensitive calcium channels located on the filopodial tips (271).

E) FROG. The initial proposal of the localization of calcium ions at the active zones came from early morphological studies (339, 340). More recently, fluorescent probes were used; at the frog motor nerve terminals, tetramethylrhodamine-conjugated $\omega$-conotoxin fluorescent stain consisted of a series of narrow bands (in face views) or dots (in side views) $\sim 1 \mu$m apart on the synaptic rather than the nonsynaptic side of the nerve terminal. The bands and dots of stain were in spatial register with the postsynaptic junctional folds as revealed by combined staining of ACh receptors (166). In frog hair cells from sacculus, the calcium indicator fluo 3 was imaged by fluorescence confocal microscopy; when a cell was depolarized, on its basolateral surface, several foci of transiently enhanced fluorescence due to local calcium influx occurred. After protracted recording, each cell displayed on average 18 brightly and permanently fluorescent spots at the same positions. Measurement of currents through membrane patches at fluorescently labeled active zones demonstrated the presence of both voltage-activated calcium channels and calcium-activated potassium channels (376).

F) LIZARD. In lizard axon terminals on twitch and tonic muscle fibers in intercostal muscles, the freeze-fracture technique was used. Differences in quantal output are related to the observed differences in the number of active zone particles flanking synaptic vesicles at the active zone (which are probably the calcium channels; Ref. 935). Evidence for the presence of calcium channels in these nerve terminals was obtained by electrophysiological and optical methods (23, 473, 474, 538, 568).

G) CHICK. In chick calyx-type nerve terminal of the ciliary ganglion, atomic force microscopy revealed low (1/µm²) and high (55/µm²) calcium channel density. Prominent interchannel spacing of 20 nm indicated an intermolecular linkage. Particles were observed in clusters and short linear or parallel linear arrays (328).

H) RAT AND MOUSE. In rat neuromuscular junctions, using electron cytochemical analysis, it was found that “A” sites are located at the openings of junctional folds; these triangular elements are identical to presynaptic protrusions of the active zone and probably comprise calcium channels of the presynaptic membrane (184).

In mouse neuromuscular junction, miniature endplate potential frequency analysis and mathematical models reveal that release is normally governed by intracellular calcium close to points of calcium entry through channels; stochastic factors give rise to more release than if calcium was homogeneously distributed. If calcium channels are uniformly close to release sites, the average number of channels opened per site per action potential may be as low as 4 (674).

I) GUINEA PIG. In guinea pig transverse slices of the hippocampus, histochemical methods showed that electrical stimulation combined with application of aminopyridine compounds led to electron-dense deposits of 60–400 nm diameter, mainly restricted to the activated input.
layers. Deposits were predominantly found at presynaptic sites (435) and may represent calcium channels.

2. Localization of calcium channels in mammalian tissues

Section II illustrated the possible existence of calcium channels in nerve terminals of different species. In this section, we present the distribution of the various calcium channels in terminals from different tissues in the same animal, namely, the rat.

In the rat retina (and in some endocrine cells) (89, 485), L-type channels control secretion (628). On the other hand, on motor nerve terminals innervating both skeletal and smooth muscle, it seems at the moment that only N-type calcium channels have been found to control neurotransmission (37, 272, 318, 576, 707). In the rat central nervous system (CNS), it seems that the picture is even more complex; in spinal cord, brain stem, neurohypophysis, cerebellum, midbrain, hippocampus, and cortex, it was suggested that more than one type of VDCC exists in the nerve terminals (see also sect. II). Spinal cord sensory neurons possess mainly N-type calcium channels, but also L- and P-type calcium channels have been described. (543). However, in dorsal horn and superior cervical ganglion, there is P-type dominance with smaller N-type contribution (300, 862); the same picture emerges in brain stem interneurons (905). In the neurohypophysis, it was established that there are L, N or N-like, and P/Q channels (832, 944, 945), but it was also suggested that only N-type calcium channels contribute to secretion (930). In the cerebellum, again the picture is of P-type dominance with smaller N-type contribution and no L-type calcium channels (695, 862), although L-type was suggested to be involved in the modulation of calcium currents and glutamate release by GABA (367). In the midbrain, the picture is even more complex, with different types of neurons releasing different types of neurotransmitters (898). In GABA release, there is N-type dominance and small L-type contribution (421). In dopamine release, the contribution is either almost equal for N, L, and P/Q (131) or mainly P with slight N (898, 981) while only P in glutamate release (898). In the modulation of the calcium signal by adenosine and ATP (655), both N and L types are involved. In cultures of hippocampal neurons, it is mainly N type that mediates exocytosis, with small contribution of P/Q or both P/Q and L (700). In hippocampal slice preparations, P/Q-type channels dominate transmission, and N-type channels contribute much less (489, 615, 862); in other studies, N-type channels were also suggested as the main route for calcium entry (379, 487, 666, 736, 876). In the different areas of the rat cortex, there are mainly P type in cerebrocortical synaptosomes (872, 880) (790) in frontal cortex synaptosomes (609), and in neocortical mini-slices (287), whereas L and N were found (736) in other cortical synaptosomes. The data on the types of channels in rat nerve terminals are summarized in Table 4.

3. Localization of calcium channels in motor nerve terminals

The types of VDCC differ in different species and anatomical localization. In motor nerve terminals (innervating skeletal muscle), all the types of nerve terminal calcium channels (L, N, P, and Q) were found in different species. Usually a combination of more than one type of channel in each terminal was suggested (see sect. II). In insect (grasshopper and housefly) motor nerve terminals, it is P and/or Q (78). In lobster, it is the N-type channel that underlies transmitter release (310). In the lizard, it is L type (473); in the frog and *Xenopus*, it is L and N (30, 254, 273, 324, 414, 718, 720); and in electromotor neurons of electric fish, it is N (244) and Q (705). In mammals, L type was found in the mouse (19, 356) together with N (357, 579), P (669, 901), and P/Q (844), whereas in rat only N type (318) and in humans only P type (668) were found.

It should be noted that similar pharmacology does not constitute a proof of channel identity. In this context, we want to quote the experiments of Fisher and Bourque (259) on the somata and nerve terminals of rat magnocellular neurons of the supraoptic nucleus (see also Ref. 796). Both structures possess calcium currents sensitive to ω-agatoxin IVA. However, the ω-agatoxin IVA-sensitive currents at the nerve terminals have a rapid inactivation, whereas those of the cell body have a much slower inactivation. It will be of interest to see in the future whether this biophysical difference between the ω-agatoxin IVA-sensitive currents is due to subunit composition or post-translational modification and how the targeting is achieved. For these types of questions, one needs “dissecting” toxins and antibodies for a relevant answer.

4. Colocalization of calcium channels in the same nerve terminal

Numerous studies have been conducted in which different specific pharmacological agents were used to inhibit one type of calcium channels, and the effect was examined either on the calcium currents or on the release of transmitter (directly or on the amplitude of the postsynaptic response). The distinction between P and Q channels is frequently based on the concentration of ω-agatoxin IVA used. If only high concentrations of ω-agatoxin IVA were used, then the channels were classified as P/Q-type calcium channels.

The results of the colocalization experiments are interesting in two aspects. First, it is quite clear that many nerve terminals possess more than one type of calcium channel involved in transmitter release. Second, the dif-
ferent types of calcium channels cooperate in a very complex way.

The contribution of L-type calcium channels to transmitter release or presynaptic calcium currents is small (see Table 5). Single L-type channels were recorded in the rat neurohypophysis (943), and their contribution to the whole terminal calcium current was estimated to be ~20% (938, 943). The L-type DHP channel blockers partly inhibited the release or the presynaptic calcium current in Aplysia neuronal synapse in buccal ganglion (266), in lizard motor nerve ending (473), and in mouse neuromuscular junction (355, 356). In some other terminals, the L-type contribution was estimated as 11–20% of the total: rat central terminals of visceral sensory neurons (543), rat striatal midbrain synaptosomes (131, 421), and guinea pig hippocampus (134). In rat cultured hippocampal neurons, the L-type contribution was found to occur in only a percentage of the boutons and was estimated to be 23% (700). On the other hand, there was no contribution for L type: in the rat tail arteries (272), in the rat brain stem inhibitory interneurons (905), in the rat cerebellum (550, 695, 862), in the rat spinal slice (862), in the rat (215, 237, 615, 862, 961), and in the rat frontal cortex slice preparation (987). In conclusion, it seems that only a small fraction of the terminal calcium channels is of the L type, and their contribution to transmission is usually smaller than that of other channels.

Most of the studies on the colocalization of different types of calcium channels in nerve terminals indicate that the majority of channels are either N- or P/Q-type channels. The fraction of these types varies in different preparations. In rat tail artery, 70–80% of the calcium currents are of the N type (272); there is no evidence yet for the identity of the other types responsible for the unaccounted fraction. In rat central terminals of visceral sensory neurons, 57% of the calcium current is carried by N type and only 12% by P/Q type (543). In rat striatal synaptosomes, GABA release is dominated by N type (421) (48%), with no indication of P/Q while dopamine and glutamate release are dominated by P/Q type (898) (50–70%). In the rat brain stem inhibitory interneuron terminals, 50% of the calcium channels are of the P type and

### Table 4. Type of channels in rat nerve terminals and secretory cells

<table>
<thead>
<tr>
<th>Location</th>
<th>Preparation</th>
<th>Types</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autonomic</td>
<td>Postganglionic nerves in tail artery</td>
<td>N</td>
<td>37, 272</td>
</tr>
<tr>
<td></td>
<td>Cultured sympathetic neurons</td>
<td>N</td>
<td>886</td>
</tr>
<tr>
<td></td>
<td>Anococcygeus muscle</td>
<td>N</td>
<td>576</td>
</tr>
<tr>
<td></td>
<td>Iris, sympathetic nerve terminals</td>
<td>N</td>
<td>707</td>
</tr>
<tr>
<td>Motor</td>
<td>Motor nerve terminals</td>
<td>N</td>
<td>318</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Pancreatic β-cells</td>
<td>L</td>
<td>89</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>Ad gland</td>
<td>L</td>
<td>485</td>
</tr>
<tr>
<td>Retina</td>
<td>Rat retinal bipolar cell terminals</td>
<td>L</td>
<td>628</td>
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<tr>
<td>Spinal cord</td>
<td>Central terminals of visceral sensory neurons</td>
<td>L, N, P</td>
<td>543</td>
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<td></td>
<td>Superior cervical ganglion</td>
<td>N, P</td>
<td>300</td>
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<tr>
<td></td>
<td>Thin spinal slices</td>
<td>N, P</td>
<td>862</td>
</tr>
<tr>
<td>Brain stem</td>
<td>Presynaptic interneurons in brain stem</td>
<td>N, P</td>
<td>905</td>
</tr>
<tr>
<td>Neurohypophysis</td>
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<td></td>
<td>Isolated neurohypophysis</td>
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<td>930</td>
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<td>Cerebellum</td>
<td>Cerebellar granule neurons</td>
<td>L, N, P/Q</td>
<td>367</td>
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<tr>
<td></td>
<td>Cerebellar slices</td>
<td>N, P</td>
<td>695, 862</td>
</tr>
<tr>
<td>Midbrain</td>
<td>Striatal nerve terminals</td>
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<td>421</td>
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<td>Striatal slices</td>
<td>N, P/Q</td>
<td>981</td>
</tr>
<tr>
<td></td>
<td>Midbrain synaptic terminals</td>
<td>N, L</td>
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<td></td>
<td>Striatal synaptosomes</td>
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<td>Hippocampal mossy fiber synaptosomes</td>
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<td>CA1 neurons in hippocampus slices</td>
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<td>Hippocampal cultures</td>
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<td>Thin slices of central nervous system</td>
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<td>862</td>
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<td></td>
<td>Hippocampal slices, CA1 pyramidal neurons</td>
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<td>489</td>
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<tr>
<td>Cortex</td>
<td>Cortical synaptosomes</td>
<td>L, N</td>
<td>736</td>
</tr>
<tr>
<td></td>
<td>Cerebrocortical synaptosomes</td>
<td>P</td>
<td>870, 880</td>
</tr>
<tr>
<td></td>
<td>Frontal cortex synaptosomes</td>
<td>P</td>
<td>699</td>
</tr>
<tr>
<td></td>
<td>Neocortical mini-slices</td>
<td>P</td>
<td>287</td>
</tr>
<tr>
<td></td>
<td>Cerebrocortical synaptosomes</td>
<td>P/Q</td>
<td>105</td>
</tr>
<tr>
<td>Brain</td>
<td>Rat brain N-type channels</td>
<td>N</td>
<td>65, 66, 233</td>
</tr>
<tr>
<td></td>
<td>Synaptosomes</td>
<td>N</td>
<td>232, 461, 463, 493, 703, 778, 958, 993</td>
</tr>
<tr>
<td></td>
<td>Rat brain cannabinoid receptor</td>
<td>Q</td>
<td>499</td>
</tr>
</tbody>
</table>
In three types of synapses, both excitatory and inhibitory in rat cerebellum, there is a clear dominance of P/Q type (550, 695, 862). In rat hippocampal slice preparations, most of the channels are of the P/Q type (215, 237, 489, 862, 961). On the other hand, it should be noted that in cultured hippocampal neurons, the picture is quite the opposite, with the majority of the channels of the N type (615, 700). In guinea pig hippocampal slices, most of the channels are of the P/Q type, colocalized with the N type (134, 983); the same pattern is seen in rat frontal cortex synaptosomes (897). The N-like channel (\(I_{Nt}\)) described at the rat neurohypophysis (943) was thought to be responsible for the transient component of the calcium current. Later, it was found that a large part of this transient current is sensitive to FTX and \(\omega\)-agatoxin IVA (939). The most prominent channels, therefore, appear to be the Q type and the N type, with the L type next in prevalence.

In cultured rat hippocampus, different types of channels are localized and colocalized in different boutons of the same axon; in 45% of the boutons, exocytosis is completely N type dependent, whereas in 55% of the boutons, L and P/Q type also contribute to the release process (700).

Evidence thus points to the P/Q-type channel as the most prominent in many different nerve terminals, with N type also playing a very significant role while the L type contributes little to the transmitter release process at many synapses. It should be noted, however, that this is not the case in all terminals. For example, in goldfish bipolar retinal neurons (332), L type was the only calcium current found and in the calyx-type synapse of the chick

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**TABLE 5. Distribution of different type of calcium channels in nerve terminals**

<table>
<thead>
<tr>
<th>Terminal</th>
<th>Monitor</th>
<th>(N_{(\omega\text{-CgTx})}) (%)</th>
<th>(L_{(DHP)}) (%)</th>
<th>(P/Q_{(\omega\text{-AgaTx, FTX})})</th>
<th>Sum (%)</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aplysia</em> buccal ganglion</td>
<td>(I_{Ca})</td>
<td>30–40%</td>
<td>40%</td>
<td>9%</td>
<td>79–89%</td>
<td>266</td>
</tr>
<tr>
<td></td>
<td>EPSC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Torpedo</em> electric organ synaptosomes</td>
<td>ATP release</td>
<td>90%</td>
<td>No</td>
<td>35%</td>
<td>85–95%</td>
<td>244</td>
</tr>
<tr>
<td></td>
<td>ACh release</td>
<td>30%</td>
<td></td>
<td>30%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat tail artery</td>
<td>Norepinephrine release</td>
<td>69–82%</td>
<td>No</td>
<td>69–82%</td>
<td>272</td>
<td></td>
</tr>
<tr>
<td>Rat spinal thin slices*</td>
<td>IPSP</td>
<td>20%</td>
<td>No</td>
<td>55%</td>
<td>75%</td>
<td>862</td>
</tr>
<tr>
<td>Rat terminals of visceral sensory</td>
<td>EPSP</td>
<td>57%</td>
<td>11%</td>
<td>12%</td>
<td>80%</td>
<td>543</td>
</tr>
<tr>
<td>neurons</td>
<td>(I_{Ca})</td>
<td>–20%</td>
<td>–20%</td>
<td>–60%</td>
<td>–100%</td>
<td>938, 939, 943</td>
</tr>
<tr>
<td>Rat terminals of neurohypophysis</td>
<td>EPSC</td>
<td>50%</td>
<td>No</td>
<td>93%</td>
<td>143%</td>
<td></td>
</tr>
<tr>
<td>Rat granule to Purkinje cell synapse,</td>
<td>(I_{Ca})</td>
<td>27%</td>
<td>No</td>
<td>50%</td>
<td>87%</td>
<td>550</td>
</tr>
<tr>
<td>cerebellar slice</td>
<td>EPSC</td>
<td>50%</td>
<td>No</td>
<td>93%</td>
<td>143%</td>
<td></td>
</tr>
<tr>
<td>Rat inferior olive-Purkinje cell synapse,</td>
<td>EPSP</td>
<td>29%</td>
<td>No</td>
<td>77%</td>
<td>106%</td>
<td>695</td>
</tr>
<tr>
<td>cerebellar slice</td>
<td>IPSP</td>
<td>21%</td>
<td>No</td>
<td>73%</td>
<td>94%</td>
<td>862</td>
</tr>
<tr>
<td>Rat cerebellar thin slice*</td>
<td>IPSP  (glycine)</td>
<td>26%</td>
<td>No</td>
<td>50%</td>
<td>80%</td>
<td>905</td>
</tr>
<tr>
<td>Rat striatal midbrain synaptosomes</td>
<td>GABA release</td>
<td>48%</td>
<td>16%</td>
<td></td>
<td>64%</td>
<td>421</td>
</tr>
<tr>
<td>Rat striatal midbrain synaptosomes</td>
<td>Dopamine release</td>
<td>23–35%</td>
<td>–20%</td>
<td>20%</td>
<td>–85%</td>
<td>131</td>
</tr>
<tr>
<td>Rat striatal synaptosomes</td>
<td>Dopamine release</td>
<td>–10%</td>
<td></td>
<td>50–70%</td>
<td>60–80%</td>
<td>898</td>
</tr>
<tr>
<td></td>
<td>Glutamate release</td>
<td>No</td>
<td></td>
<td>50–70%</td>
<td>50–70%</td>
<td></td>
</tr>
<tr>
<td>Rat cultured hippocampal neurons</td>
<td>Exocytosis</td>
<td>100%</td>
<td>No</td>
<td>No</td>
<td>100%</td>
<td>700</td>
</tr>
<tr>
<td>in ~45% of the boutons</td>
<td></td>
<td>38%</td>
<td>23%</td>
<td>17%</td>
<td>88%</td>
<td></td>
</tr>
<tr>
<td>in ~55% of the boutons</td>
<td></td>
<td>50%</td>
<td>No</td>
<td>36%</td>
<td>95%</td>
<td>615</td>
</tr>
<tr>
<td>Rat cultured hippocampal neurons*</td>
<td>IPSP</td>
<td>50%</td>
<td>No</td>
<td>36%</td>
<td>95%</td>
<td>615</td>
</tr>
<tr>
<td>Rat hippocampal slice</td>
<td>(I_{Ca}) (postsynaptic)</td>
<td>–33%</td>
<td>No</td>
<td>–66%</td>
<td>100%</td>
<td>237</td>
</tr>
<tr>
<td>Rat hippocampal slice</td>
<td>Glutamate release</td>
<td>10%</td>
<td></td>
<td>40%</td>
<td>56%</td>
<td>489</td>
</tr>
<tr>
<td></td>
<td>EPSP</td>
<td>70%</td>
<td></td>
<td>100%</td>
<td>170%</td>
<td></td>
</tr>
<tr>
<td>Rat CA3-CA1 synapse hippocampal slice</td>
<td>EPSP</td>
<td>–40%</td>
<td>No</td>
<td>–90%</td>
<td>–130%</td>
<td>961</td>
</tr>
<tr>
<td>Rat hippocampal slice</td>
<td>IPSP (GABA)</td>
<td>–30%</td>
<td>No</td>
<td>–80%</td>
<td>–125%</td>
<td>215</td>
</tr>
<tr>
<td>Rat hippocampal slice*</td>
<td>EPSP</td>
<td>12%</td>
<td>No</td>
<td>46%</td>
<td>58%</td>
<td>862</td>
</tr>
<tr>
<td>Rat frontal cortex synaptosomes</td>
<td>Glutamate release</td>
<td>No</td>
<td>No</td>
<td>56%</td>
<td>56%</td>
<td>897</td>
</tr>
<tr>
<td>Guinea pig mossy fiber synapse,</td>
<td>EPSP</td>
<td>75%</td>
<td>13%</td>
<td>96%</td>
<td>184%</td>
<td>134</td>
</tr>
<tr>
<td>hippocampus slice</td>
<td></td>
<td>53%</td>
<td>13%</td>
<td>60%</td>
<td>126%</td>
<td></td>
</tr>
<tr>
<td>Guinea pig assoc-com synapse,</td>
<td>EPSP</td>
<td>40%</td>
<td>4–8%</td>
<td>&gt;20%</td>
<td>–70%</td>
<td>978</td>
</tr>
<tr>
<td>hippocampus slice</td>
<td></td>
<td>70%</td>
<td>No</td>
<td>61%</td>
<td>137%</td>
<td></td>
</tr>
<tr>
<td>Guinea pig CA3-CA1 synapse,</td>
<td>EPSP</td>
<td>+</td>
<td>–75%</td>
<td></td>
<td></td>
<td>983</td>
</tr>
<tr>
<td>hippocampus slice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Calculated assuming a power relationship between calcium and transmitter release. EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential; \(I_{Ca}\) calcium current; \(\omega\)-CgTx, \(\omega\)-conotoxin; DHP, dihydropyridine; \(\omega\)-AgaTx, \(\omega\)-agatoxin.

~26% are of the N type (905). In three types of synapses, both excitatory and inhibitory in rat cerebellum, there is a clear dominance of P/Q type (550, 695, 862). In rat hippocampal slice preparations, most of the channels are of the P/Q type (215, 237, 489, 862, 961). On the other hand, it should be noted that in cultured hippocampal neurons, the picture is quite the opposite, with the majority of the channels of the N type (615, 700). In guinea pig hippocampal slices, most of the channels are of the P/Q type, colocalized with the N type (134, 983); the same pattern is seen in rat frontal cortex synaptosomes (897). The N-like channel (\(N_t\)) described at the rat neurohypophysis (943) was thought to be responsible for the transient component of the calcium current. Later, it was found that a large part of this transient current is sensitive to FTX and \(\omega\)-agatoxin IVA (939). The most prominent channels, therefore, appear to be the Q type and the N type, with the L type next in prevalence.

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Evidence thus points to the P/Q-type channel as the most prominent in many different nerve terminals, with N type also playing a very significant role while the L type contributes little to the transmitter release process at many synapses. It should be noted, however, that this is not the case in all terminals. For example, in goldfish bipolar retinal neurons (332), L type was the only calcium current found and in the calyx-type synapse of the chick.
ciliary ganglion, and the N-like channel was the only type found both in the whole terminal and in single-channel recordings (328, 817–819, 821).

It has been suggested that the different types of channels are required for kinetically distinct phases of release (55, 470, 581, 646), depending on the electrophysiological characteristics of the type of channel involved. The density and distribution of calcium channels are probably developmentally regulated at some synapses (see Ref. 757). There may be important functional implications in the variations of the distribution of the different channels among terminals. This may reflect the differences in the strength of the synapse and may be responsible for the speed and duration of transmission and the ability of hormones and transmitters to modulate transmission (223).

Data on the fractions of the different channel types in the same terminal are summarized in Table 5.

F. Cooperativity of Action of Calcium Channels in Transmitter Release

1. Cooperativity in transmitter release

In previous sections we have shown that different types of calcium channels coexist on the same nerve terminals. Inhibition of the activity of each of calcium channel type in such cohabitation produces a substantial decline in transmitter release from the presynaptic nerve terminals. How is it possible that the sum of all the fractional inhibitions is larger than unity? This phenomenon can be attributed to the cooperative action of calcium ions in the process of transmitter release.

The cooperative relation between calcium and transmitter release was first demonstrated at the frog neuromuscular junction (167, 209). In this preparation, changes in the extracellular calcium concentration ([Ca\(^{2+}\)]\(_o\)) produced a significant change in the number of the transmitter quanta liberated by the nerve impulse. In certain concentration ranges of [Ca\(^{2+}\)]\(_o\), doubling the calcium concentration produced an almost 16-fold increase in quantal release. This observation was interpreted as a cooperative relation between calcium and quantal liberation of transmitter. Subsequently, it was found that a similar relation exists in many other synapses (220, 360, 407, 439, 481). The question remained whether the cooperativity exists between [Ca\(^{2+}\)]\(_i\) and calcium influx into the terminal, as suggested by some investigators (481), or between [Ca\(^{2+}\)]\(_i\) and transmitter release. A voltage-clamp experiment of the nerve terminals at the squid giant synapse examined the relation between transmitter release and calcium influx. In the squid preparation, it is difficult to detect individual transmitter quanta; hence, the relation between the calcium influx and the postsynaptic response was investigated. These studies showed a highly nonlinear dependence of postsynaptic response on calcium entry through the presynaptic membrane, which strengthens the notion of cooperative action of intracellular calcium ions in the process of transmitter release (42, 45) (see Ref. 462, 820).

More recently, this problem was approached by using photolabile calcium chelators that release their calcium upon light (440). Here the entry stage was bypassed, and hence it was possible to examine directly the relation between the [Ca\(^{2+}\)]\(_i\), and transmitter release, measured as the amplitude of the postsynaptic response. These experiments (440) showed that the cooperativity occurs between [Ca\(^{2+}\)]\(_i\), and the process of transmitter release.

2. Calcium channel cooperativity

The cooperativity of calcium ions in transmitter release is also highly relevant for understanding the interaction of different types of calcium channels in the release process. To illustrate the calcium channel interaction, we will cite the results of Mintz et al. (550). They measured the effects of two different calcium channel blockers on calcium currents (measured as calcium transients by imaging furaptra) and on the amplitude of the excitatory postsynaptic currents (EPSC) in granular cells of rat cerebellar slices. Because the calcium channel blockers had almost no postsynaptic effects, one can take the amplitude of the EPSC as a measure of transmitter release. Calcium current is reduced by 27% with \(\omega\)-conotoxin GIVA (that blocks specifically N-type calcium channels) and by 50% with \(\omega\)-agatoxin IVA (at concentrations of 50–400 nM that probably block both P- and Q-type calcium channels). These inhibitory effects account for 77% of the calcium currents. Addition of cadmium ions inhibited the remaining calcium currents. The summation of the different channel blockers was linear on the calcium signal. A completely different picture emerged when the actions of the same pharmacological agents were examined at the level of the EPSC. The EPSC were reduced by 50 and 93%, respectively, with the same toxin concentrations. The sum of the fractional inhibitions was greater than unity.

Two important conclusions can be drawn from these experiments. First, the cooperativity between calcium and release is at the level of [Ca\(^{2+}\)]\(_i\), and not at the level of calcium entry. Second, different types of calcium channels act on the same pool of transmitter quanta and are probably intermingled. This conclusion supports the involvement of many calcium channels types in the release of a single quantum of transmitter, discussed in section II. G.

We will illustrate this more than linear summation of the effect of two different toxins with the aid of Figure 3.

We assume that the relation between [Ca\(^{2+}\)]\(_i\), and transmitter release is sigmoidal in nature (for short
pulses) and that in the physiological range there is an almost linear relation between the activity of the calcium channels and the $[\text{Ca}^{2+}]_i$. The abscissa in Figure 3 is the normalized $[\text{Ca}^{2+}]_i$, and the ordinate is the normalized release. We assume that each one of the inhibitors reduces calcium entry by the same amount. The sum of the effects of the 2 inhibitors is much more than linear. For details, see text.

G. How Many Calcium Channels Have to Open to Release a Quantum of Transmitters?

The major role of calcium channels in nerve terminals is to induce the release of transmitter quanta (see Ref. 223). Therefore, it is of particular interest to determine how many calcium channels have to open within a short time frame to cause the liberation of a quantum of transmitter. Is one calcium channel in the open state sufficient to evoke release of a quantum, or do multiple calcium channels have to be open, almost simultaneously, to generate an exocytotic event?

Let us first examine some of the evidence for the “one channel” suggestion provided by Stanley (819). In Stanley’s experiments, single calcium channel openings were recorded at the ciliary ganglion presynaptic nerve terminal of the chick. After dissociation of these nerve terminals, the exposed presynaptic surface can be approached by a patch pipette and high-resistance seals formed. The cell-attached (on-cell) configuration of the patch-clamp technique was used to record single calcium channel openings. It is known that at this synapse, there is a quantal liberation of ACh (514), whereas a previous study has shown that the calcium channels are confined to the release face of the presynaptic calyx. The same patch pipette was used to monitor ACh release by a photon emitting reaction (375). It was found that the photon emission was frequently preceded by a calcium channel opening. This finding by itself is not yet complete evidence that a single calcium channel opening caused the release, since although there may be a single channel opening in the membrane facing the patch pipette, it cannot be excluded that there are many channel openings at the presynaptic membrane in the immediate vicinity outside the patch pipette. However, the association between calcium channel openings and photon emission was found in cases where the rate of channel openings was very low, and the probability of adjacent opening was negligibly low. (It is noteworthy that the current trace showed a clear peak after the photon emission, indicating that an additional channel opens after the fusion. Is this the vesicle channel discussed elsewhere in this review?)

The experimental results were compared to a single domain model (69). (The name of this model indicates that the quantal release machinery is the domain of influence of a single calcium channel.) These experiments provide a strong support that a single calcium channel opening can in principle cause the release of a quantum, but is this the situation in normal synaptic transmission?

There is evidence, however, for an alternative model: the model of overlapping domains, where the quantal release machinery is under the influence of many calcium channels. In two recent articles, Borst and co-workers (95, 96) provide evidence that under normal physiological conditions, many more channels are necessary, on the average, to release a quantum of transmitter at the synapse of Held. This synapse is at the rat medial nucleus of the trapezoid body. It is large enough to permit accurate measurements of both the presynaptic and the postsynaptic currents and to measure individual transmitter quanta. They found that the calcium influx generated by
the action potential occurs during the repolarizing phase of the action potential when the calcium conductance is large and the driving force increases (tail currents). Thereafter, they compared the number of calcium channels that open by the action potential, with the number of transmitter quanta that were liberated, and reach the conclusion that ~60 calcium channels have to open to cause the release of a quantum of transmitter. In principle, it can be argued that some of the calcium channels open in the presynaptic terminal at locations not associated with transmitter release. They address this argument by using “slow” and “fast” calcium buffers. The result is that even slow calcium buffers like EGTA are able to suppress quantal transmitter release, indicating that calcium ions have to travel a substantial distance before they trigger transmitter release. This distance is much larger than envisaged for a single, high calcium concentration domain (6, 484, 791, 842, 1012, 1013). Hence it appears that although a single calcium channel is able occasionally to trigger transmitter release, under normal circumstances at the synapse of Held, many calcium channels are involved in the liberation of a single transmitter quantum. This number of channels-to-number of quanta ratio is also very important in understanding the modulation and plasticity of synaptic transmission. If this ratio is always one, it is much more difficult to envisage the calcium channel cooperativity.

H. Other Routes for Calcium Entry Into the Nerve Terminal

1. Channels

A) NONSELECTIVE CHANNEL. One of the channels identified in the preparation of fused presynaptic nerve terminals of Torpedo electromotor nerve is a large, calcium-permeable and highly voltage-dependent ion channel (536). Single-channel conductance is on the average 846 pS. At voltages below 0 mV (inside negative), the probability of the channel to open is negligible but increases dramatically, within a very narrow voltage range, to >50% at −8 mV. In short pulse experiments from holding potential of −70 mV, it was found that the channel is activated shortly after depolarization and deactivated shortly after the membrane voltage returns to the resting potential. Therefore, it may be activated and deactivated by a typical action potential invading the nerve terminal.

On the basis of the transport number for calcium ions, the calculated driving force and the mean channel open time, we estimated the number of calcium ions entering the nerve terminal upon depolarization. This calculation led to rather surprising results. For example, when the terminal membrane is depolarized to −10 mV, 1,024 calcium ions will enter the terminal during the mean open time of the channel (~5 ms at −10 mV). For comparison, we calculated the number of calcium ions that flow through two types of calcium-selective voltage-activated channels. Wang et al. (944) recorded single calcium channels from the rat neurohypophysial terminals. They found N1-type and L-type channels. From their data, we calculated the number of calcium ions passing through each of these channels during a single opening. Their values correspond to the passage of 710 and 1,498 calcium ions per single mean opening of these two channels, respectively. Hence, the calcium entry through the nonselective channel is not substantially different from that through voltage-dependent and calcium-selective channels found in other nerve terminals. Applying the same calculations to potassium, sodium, and chloride, one can conclude that the opening of the large, nonselective channel does not cause a substantial dissipation of the ion gradients across the membrane.

Thus this nonselective ion channel may serve as a calcium entry route into the nerve terminal and may consequently be involved in transmitter release.

B) INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR CHANNEL. Inositol 1,4,5-trisphosphate (IP3) activates calcium channels in the plasma membranes of rat brain nerve terminals as was measured by 45Ca2+ influx into IP3-activated synaptosomes (753, 902). Thus IP3 can cause an entry of calcium through the surface membrane and release of calcium ions from intracellular stores (257).

C) NICOTINIC ACETYLCHOLINE RECEPTOR CHANNEL. The nicotinic ACh receptor channels (nAChR) in nerve terminals is discussed in section vii. Here we want to stress that these channels also allow calcium ions in the nerve terminal when they are in an open state (603, 609, 828).

2. Calcium/sodium exchanger

At first glance, it may seem that any discussion of transporters at the presynaptic nerve terminal is outside the scope of this review, which deals with ion channels. However, because recently it has been shown that a number of transporters have channel like properties (197), including the calcium/sodium exchanger in heart (342, 343), we feel that a short discussion is necessary.

With the use of immunocytochemical staining, it was shown that the calcium/sodium exchanger is concentrated at presynaptic sites at the neuromuscular junctions of the rat diaphragm and in Xenopus laevis nerve and muscle cell cocultures. The high concentrations of calcium/sodium exchanger, at presynaptic nerve terminals, in vertebrate neuromuscular preparations suggest that the exchanger may participate in the calcium-dependent regulation of neurotransmitter release. The calcium/sodium exchanger is also abundant in developing neurites and growth cones where it may also be important for [Ca2+]i homeostasis (494). There are several functional isoforms (277, 278, 676) of the calcium/sodium exchanger.
The activity of the exchanger was demonstrated in vertebrate brain synaptosomes as sodium-dependent calcium fluxes (26, 83, 122, 263, 859, 860).

In nerve terminals of cultured hippocampal cells, immunocytochemical evidence for localized distribution of the calcium/sodium exchange protein in synaptic regions is shown. Removal of extracellular sodium produced enhanced and prolonged elevation of $[Ca^{2+}]_i$ in nerve terminals during and after electrical stimulation of the cells. Correspondingly, initial rates of exocytosis, measured by fluorescence changes of FM 1–43 during stimulation, were faster in LiCl-containing solution than in NaCl-containing solution. In contrast, endocytosis at 20 s was the same in both solutions (701).

It seems, therefore, that the calcium/sodium exchanger is an important pathway for calcium extrusion from the nerve terminal. The calcium/sodium exchanger can operate also in a reverse mode and admit calcium into the terminal, if the membrane potential is above the equilibrium potential of the exchanger. It is less clear whether the duration of the action potential in most nerve terminals is long enough for this entry mechanism to be of significance.

I. Molecular Interactions of Calcium Channels With Nerve Terminal Proteins

Lately there is an increasing amount of evidence indicating that the calcium channels in the nerve terminals interact with some intracellular proteins involved in transmitter release. The most convincing evidence exists regarding the interaction of syntaxin with N-type calcium channels in nerve terminals. When syntaxin interacts with the calcium channels, it depresses calcium currents and synaptic transmission (799), by stabilization of channel inactivation (71, 966). Molecular complexes between syntaxin and calcium channels were found in a number of species: Aplysia (799), electric ray (884), rat (65, 233, 463, 778), rabbit, and human (71). This was achieved mainly by using immunocytochemical methods, but also electrophysiology was used to assess the functional role of this interaction. In most cases, it was found that the N-type channels are the channels that are associated with parts of the fusion machinery.

In rat small cell lung cancer lines, which are secreting a wide variety of peptides, commmunoprecipitation studies show that syntaxin can form a complex with synaptotagmin and N-type channels (192). In bovine brain membranes, vesicle-associated membrane protein (VAMP)/synaptobrevin-2, rab3A/smg25A, and SNAP-25 form a complex with N-type channels (358).

In the chick ciliary ganglion calyx synapse, the cleavage of syntaxin by botulinum toxin C1 prevented the G protein modulation of voltage-dependent N-type calcium channels (826).

The interactions of calcium channels with synaptic proteins are summarized in Table 6.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Interaction</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aplysia buccal ganglion neurons</td>
<td>Syntaxin depressed $Ca^{2+}$ current and transmission</td>
<td>799</td>
</tr>
<tr>
<td>Electric ray electric organ synaptosomes</td>
<td>Syntaxin, forms a complex with calcium channel</td>
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</tr>
<tr>
<td>Chick ciliary ganglion calyx synapse</td>
<td>Cleavage of syntaxin, prevented the G protein modulation of N-type channels</td>
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</tr>
<tr>
<td>Rat small cell lung cancer lines</td>
<td>Syntaxin form a complex with synaptotagmin and N-type channels</td>
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<tr>
<td>Rat brain</td>
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<td>778</td>
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<tr>
<td>Rat brain synaptic membrane</td>
<td>Exocytotic complex where N type tightly interacts with a synaptic vesicle docking site (syntaxin)</td>
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<tr>
<td>Rat brain</td>
<td>P35 associating with N-type channel and synaptotagmin</td>
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<tr>
<td>Rat brain synaptosomes</td>
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<td>P35 or syntaxins interact with the synaptic vesicle protein p65 (synaptotagmin). p65 proteins is associated with N-type channels</td>
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<td>Bovine brain membranes</td>
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<tr>
<td>Expressed channels</td>
<td>Syntaxin 1A and SNAP-25 inhibit L and N type</td>
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</tr>
<tr>
<td>Expressed channels, cloned from human brain, rabbit muscle, and brain</td>
<td>Syntaxin 1A leads to stabilization of N-type and Q-type channel inactivation</td>
<td>71</td>
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</tbody>
</table>

J. Modulation of Calcium Channels in Nerve Terminals

The modulation of calcium channels in the nerve terminals is of key importance in the regulation of transmitter release and, thus, of synaptic transmission. There are at least nine different modes of modulation of calcium channels. The modulation can be achieved by the action of the transmitter released by the same nerve terminal; through actions on autoreceptors present on the releasing nerve terminal; by degradation products of the transmitter liberated; by transmitters released from other nerve
terminals; and by hormones secreted into the extracellular fluid, by antibodies, by drugs, and by changes in the chemical and physical environment of the nerve terminal. The modulation of calcium channels at nerve terminals is a huge topic; here, only some of the basic aspects are described.

Some of the modulatory actions are due to a direct effect on the channels at the nerve terminals, whereas others are achieved through the action of second messengers.

1. Modulation of calcium channels in nerve terminals by neurotransmitters: the involvement of G proteins

Many studies have indicated that a number of hormones and neurotransmitters cause a change in the amount of transmitter released from presynaptic nerve endings (see Refs. 1, 152, 362, 423, 922). The proposed mechanism, for at least some of these effects, is the activation of inhibitory G proteins by appropriate receptor cascade in the presynaptic cell membrane (212, 761). A subunit of the active G protein may directly suppress the activity of VDCC (for reviews, see Refs. 211, 346). The inhibited channels allow fewer calcium ions to enter the presynaptic terminal, and as a result, less transmitter is released.

Although the details of the interaction between the inhibitory G protein and the calcium channel are not in the scope of this review, it is known that the inhibition is voltage dependent (57) and is probably caused by the \( \beta \gamma \) G protein subunit (204, 334, 368). Several sites (on the calcium channel protein) for G protein subunit binding and mechanisms of inhibition have been suggested (see, for example, Refs. 121, 1001).

This modulatory set of processes is very widespread in the nervous system and involves at least 10 different transmitter molecules and a large number of receptors. This action is probably due to a change in the voltage dependence of VDCC (57) or in the single-channel conductance (437). On the single-channel level, it was shown that the only parameter that changes as a result of G protein modulation is the latency for first opening, which is increased by G protein (639). When measured directly in nerve terminals of the chick, calcium currents were inhibited by guanosine 5’-O-(3-thiotriphosphate) (GTP\( _{\gamma} \)S); this inhibition involved the presynaptic protein syntaxin (826). An altered activation of VDCC has been found for many neurotransmitters and hormones, including histamine (238, 207, 431, 661), 5-hydroxytryptamine (235), neuropeptide Y (829, 886), somatostatin (29, 305, 824, 857), opiates (80, 157, 254, 319, 545, 575, 829, 857, 867), and glutamate (914). Recently, it was shown that also the cannabinoids act on receptors inhibiting N- and Q-type calcium channels via G proteins (252, 497–499).

In the following sections we briefly describe this mode of inhibition for several transmitters and hormones, as found in nerve terminals according to the receptor that presumably participates in the control.

A) ACETYLCHOLINE RECEPTORS. Acetylcholine, which serves as a neurotransmitter in the vertebrate skeletal neuromuscular junctions and in many central synapses, can also, in some instances, modulate the neurotransmitter release via heteroreceptors (where another transmitter is released) or via autoreceptors. In rat slices of prefrontal cortex, cholinergic heteroreceptors on dopaminergic terminals modulate dopamine release. Carbachol, an ACh receptor agonist, in potassium stimulates dopamine release (384). In rat motor nerve terminals using focal extracellular electrodes ATP and ACh, which are released during exocytosis, may inhibit their own release through attenuation of the terminal calcium current via autoreceptors coupled to a G protein. Acetylcholine (100 \( \mu \)M) depressed the terminal calcium current, and pertussis toxin (10\(^{-5}\) g/ml) eliminates ACh-induced inhibition (317). On the other hand, in guinea pig bladder, nicotine induced ACh release that was partially inhibited by \( \omega \)-conotoxin (782). In chick ciliary ganglia, ACh receptors increase intracellular calcium by two distinct mechanisms (691).

B) ADRENORECEPTORS. Adrenoreceptors at the presynaptic surface membrane modulate the calcium currents in the nerve terminal and, thereby, the transmitter release process. In contrast to GABA, which modulates calcium channels by activation of a cascade that includes GABA\( _{\text{B}} \) receptors and G proteins that inhibit calcium channels, epinephrine and norepinephrine modulate the calcium current through various adrenoreceptors, sometimes with opposite actions. Adrenoreceptors were found both as autoreceptors and heteroreceptors, modulating the release of ACh and substance P.

C) ADENOSINE RECEPTORS. The adenosine receptors could be considered as autoreceptors in many systems, since adenosine in the extracellular compartment is formed by ATP breakdown after it is coreleased with the neurotransmitter. In mouse motoneurons, \( \text{A}_{1} \) receptors were found to act via G protein cascade to directly inhibit presynaptic N-type calcium channels (579). The inhibition via G protein was demonstrated also in rat motoneurons (317).

D) GABA RECEPTORS. \( \gamma \)-Aminobutyric acid is probably the most studied endogenous modulator of synaptic transmission causing inhibition. Its influence on other channels is discussed elsewhere; here we summarize its influence on calcium channels. \( \gamma \)-Aminobutyric acid acts on GABA\( _{\text{B}} \) receptors in the terminal surface membrane (207, 215, 354, 367, 438, 666, 871, 933, 979), which activates a G protein, inhibiting HVA calcium channels in the terminal (331, 354, 367, 666, 871). It is generally accepted that the action of GABA is on the N- and/or P/Q-type channels, but its influence on L-type channels has also been shown. An inhibitory action of GABA on presynaptic calcium channels has been demonstrated in several preparations. Such
an action that modulates the ability of cells to transmit information could be one of the ways for maintaining brain plasticity and an important mechanism for therapeutic use. γ-Aminobutyric acid also inhibits R-type calcium channels (976).

Modulation of calcium channels by adrenoreceptors, adenosine, ATP, and GABA is summarized in Table 7.

2. Modulation of calcium channels in nerve terminals by phosphorylation and dephosphorylation

In guinea pig hippocampal synaptosomes, protein kinase C (PKC) phosphorylation increases release of glutamate by increasing VDCC activity (875). In rat cerebral cortex synaptosomes, release of glutamate and calcium current are reduced by dephosphorylation effected by calcineurin (788), and in rat hippocampal synaptosomes, it was found by measuring \([\mathrm{Ca}^{2+}]_{\text{i}}\) that there is a dynamic balance between phosphorylation by PKC (activation) and dephosphorylation (inactivation) by protein phosphatase, both being tonically active in terminals (55). Protein kinase C phosphorylation occurs on an intracellular loop of the \(\alpha_1\)-subunit that binds the inhibiting G protein complex mentioned above (995).

3. Translocation of calcium channels

Most of the calcium channel regulation in presynaptic nerve terminals, described in the previous sections, was directed toward the probability of an opening of a calcium channel that is already present in the surface membrane of the nerve terminal. Recently, a very interesting possibility was suggested for human neuroblastoma and for rat pheochromocytoma cell lines (638), which if applicable to nerve terminals opens new directions for regulation of calcium channels and for frequency modulation (see Ref. 686) of synaptic transmission. It was found that secretagogues such as KCl, ionomycin, and phorbol esters (see Ref. 770) cause a translocation of N-type calcium channel from the intracellular pool to the surface membrane (see Ref. 638). If this mechanism exists also in the nerve terminals, then an appropriate stimulation that causes the insertion of new calcium channels in the surface membrane will increase the synaptic strength for subsequent stimuli. This increase in synaptic strength will last as long as the additional calcium channels dwell in the surface membrane in their active state.

4. Other modulators of calcium channels

The calcium channels in nerve terminals are probably subject to modulation by many other environmental signals in their vicinity. These signals can be either external or cytoplasmic and are chemical or physical in nature. Here, we present a partial list of modulators of calcium channel in presynaptic nerve terminals that either enhance or block the channels activity.

In rat corpora striata synaptosomes, extracellular, but not cytosolic, acidification inhibits the release of dopamine by blocking voltage-gated calcium channels (216).

In rat soleus muscle, voltage-dependent calcium influx of the nerve terminal could be increased by muscle stretching (985).

In the sympathetic nerve in kidney and spleen and in heart and blood vessels, PGE\(_2\) and PGI\(_2\) inhibit the release of norepinephrine, inhibitors of cyclooxygenase enhances norepinephrine release via \(\omega\)-conotoxin-sensitive channels (506).

In rat cerebral cortex synaptosomes, glucocorticoids promote calcium channel activity by enhancing calmodulin-dependent activation of the channels (853).

In rat brain stem synaptosomes, stimulation of presynaptic receptors coupled to the \(G_s\) adenylate cyclase system may lead to a facilitation of the release of neurotransmitters, through a cAMP-dependent enhancement of the opening of the calcium channels located on nerve terminals. Maitotoxin increases the \(^{45}\text{Ca}\) entry and \([\mathrm{Ca}^{2+}]_{\text{i}}\). These are significantly enhanced by addition of dibutyryl cAMP and by the pretreatment with cholera toxin (903).

In the buccal ganglion of Aplysia, FMRFamide facilitates ACh release by increasing the presynaptic calcium influx. These neuromodulators control only the influx of calcium through N-type channels, since their action on transmitter release can be prevented by \(\omega\)-conotoxin but not by funnel web spider toxin. FMRFamide increased calcium influx by shifting the voltage sensitivity to activation of the channels (267).

In lobster abdominal muscles, blockers of N-type channels enhanced excitatory junctional current suppression exerted by high pressure (310).

### TABLE 7. Modulation of calcium channels by adrenoreceptors, adenosine, ATP, and GABA

<table>
<thead>
<tr>
<th>Channel</th>
<th>Receptor and Direction</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adrenoreceptors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N type</td>
<td>Norepinephrine, (\alpha_2)-adrenoceptor ↓</td>
<td>488, 517, 661, 956</td>
</tr>
<tr>
<td>L type</td>
<td>(\alpha_1)- and (\alpha_2)-adrenoceptors ↓</td>
<td>53, 141, 885, 956</td>
</tr>
<tr>
<td><strong>Adenosine and ATP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N type</td>
<td>(A_1) and (A_2) receptors ↓</td>
<td>207, 317, 421, 445, 579, 655, 905, 988</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine ↓</td>
<td>905</td>
</tr>
<tr>
<td>L type</td>
<td>Adenosine ↓</td>
<td>399</td>
</tr>
<tr>
<td><strong>GABA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N type</td>
<td>GABA(_b) ↓ GABA ↓</td>
<td>215, 367, 488, 666, 933, 979</td>
</tr>
<tr>
<td>P/Q type</td>
<td>GABA(_b) ↓</td>
<td>215, 367</td>
</tr>
</tbody>
</table>
In *Torpedo* electric organ synaptosomes, lactate production by stimulated postsynaptic electroplaques may inhibit ACh release from presynaptic nerve terminals. Lactate reduced the release of ACh triggered by depolarization with potassium via inhibition of voltage-dependent calcium influx mediated by the natural calcium channel (286).

K. Intracellular Calcium Stores and Channels

Although the free \([\text{Ca}^{2+}]_i\) is very low, there is a very substantial amount of calcium in intracellular stores. These "stored" calcium ions are important in the intracellular calcium dynamics and, hence, in cell function. Stored calcium can be released upon an adequate stimulus through intracellular calcium channels and, thus, contribute to the increase in free \([\text{Ca}^{2+}]_i\). Conversely, calcium ions can be taken from the cytoplasm into the stores and thus participate in the reduction in the free \([\text{Ca}^{2+}]_i\) after stimulation. We present here some of the evidence on the existence of calcium stores in the presynaptic nerve terminals and their possible relevance in transmitter release.

A large number of different calcium stores have been described in several cells (for a review, see Ref. 667). They include mitochondria and the intracellular reticulum (endoplasmic and sarcoplasmic reticulum) where at least two different types of channels have been described, namely, IP$_3$ and ryanodine; the number of different channels has been described, (endoplasmic and sarcoplasmic reticulum) where at least a hundred different genes have been shown to encode the IP$_3$ channel. In addition, it was proposed that the following intracellular structures can serve as calcium stores: the Golgi complex, secretion vesicles and granules, endosomes and lysosomes, the nucleus, and cytosolic calcium-binding molecules (see Ref. 667). In the nerve terminal, there are no nuclei, but many of the other organelles have been suggested to take part in the regulation of transmitter release, by opening of calcium release channels and by uptake of the cytosolic calcium after nerve stimulation and at rest. We would like to dwell briefly on two intracellular organelles: the reticulum and the mitochondria.

The interest in the role of mitochondria in intracellular calcium homeostasis was very substantial in the 1970s (see Ref. 124). With regard to the presynaptic nerve terminal, it was shown then (15) that mitochondrial calcium uptake inhibitors augment transmitter release, suggesting that mitochondria may be part of the intracellular machinery regulating free \([\text{Ca}^{2+}]_i\). In situ morphological studies done on liver mitochondria (92, 810) suggested that the calcium content of mitochondria is rather low while calcium was found in the endoplasmic reticulum (529). These and other findings led to reduced efforts to show that the mitochondrion is an important component of \([\text{Ca}^{2+}]_i\) regulation under physiological conditions. The situation changed dramatically in the last 5 years when the calcium reporter aequorin was targeted to intact intracellular mitochondria and showed that not only the intramitochondrial calcium content is very substantial, but also that it responds rapidly to channel and receptor activation of the surface membrane (709–711, 735). Recently, it was shown that mitochondria take up and release calcium in physiological calcium loads (190, 868). Hence, it is tempting to predict that the interest in role of mitochondria in the function of the nerve terminal in physiological and pathophysiological conditions (hypoxia, neurotrauma, and aging) will regain momentum (see Ref. 540).

There is no doubt that the ryanodine and the IP$_3$ channels are expressed in many types of cells (270, 847), including cells in the nervous system (279, 335, 353, 359, 396, 415, 429, 478, 479, 610, 728, 783–785, 909). However, most of the studies do not deal specifically with the nerve terminal.

Recently, however, evidence was obtained on the existence of ryanodine-sensitive calcium stores that take part in transmitter release (801) at the sympathetic nerve terminals of the guinea pig. When the presynaptic nerve was stimulated at low frequencies, all the transmitter release was inhibited by calcium channel blockers acting on calcium channels at the surface membrane. At high frequencies (5 stimuli every 2 s at 20 Hz), a residual release was observed that was time-locked to the stimulus. This residual release was blocked by ryanodine. Frequency of stimulation-dependent calcium release through ryanodine calcium channels was also observed in cerebellar Purkinje neurons (396). These experiments are of potential interest regarding two different aspects. First, they suggest the functional presence of ryanodine calcium channels in the sympathetic nerve terminals. Second, the time locking shows that the communication between the surface and the ryanodine channels is very fast.

The intracellular IP$_3$ receptor-channel complex is present in many tissues, including the nervous system (see, for example, Ref. 275). This provides possible links among surface membrane receptors (such as metabotropic glutamate receptors), changes in \([\text{Ca}^{2+}]_i\), transmitter release, and synaptic plasticity (2, 25, 72, 185, 274, 426, 476, 780, 874). There are also pathophysiological implications since mice deficient in this receptor show ataxia and epilepsy (520).

L. Pathophysiology of Calcium Channels

Calcium channels are probably involved in a number of diseases and pathophysiological states. Here, we summarize several instances where calcium channels are probably implicated.
1. Lambert-Eaton myasthenic syndrome

A) Clinical characteristics of Lambert-Eaton myasthenic syndrome. Lambert-Eaton myasthenic syndrome (LEMS) is an autoimmune disease that often occurs in association with malignancy. The syndrome is characterized by weakness, myalgias, and a tendency to develop fatigue. The disorder affects men more than women. The incidence of associated malignancy is 70% in men and 25% in women. In most cases of either sex, the tumor is a small cell carcinoma of the lung. Other tumors associated with LEMS affect the breasts, the prostate, and the stomach. The diagnosis of LEMS may be suspected clinically but must be confirmed with electrophysiological testing (526), which involves the recording of the electrical response of a muscle to supramaximal stimulation of its motor nerve by repetitive (2–3 Hz) shocks.

B) Pathophysiology of LEMS. Lambert-Eaton syndrome is associated with diminished quantal release of ACh at the neuromuscular junction (see Ref. 442). In paraneoplastic LEMS, physiological data incriminate antibodies directed against VDCC present both on the tumor and at distal motor nerve terminals (779). The physiological features of the syndrome can be passively transferred to mice by the patients’ sera, consistent with an autoantibody interfering with the function of VDCC (713).

Kim and Neher (418) have shown that when IgG antibodies from patients with LEMS were applied to bovine adrenal chromaffin cells, the VDCC currents declined by ~40%. The pathogenic IgG modified neither kinetics of calcium channel activation nor elementary channel activity, suggesting that a reduction in the number of functional calcium channels underlies the IgG-induced LEMS autoantibodies and seems to be specific against calcium channels. Sera from LEMS patients reduce calcium currents more strongly in motoneurons than in sensory neurons. In motoneurons, both LVA and HVA components of calcium current decreased, demonstrating that the sera target more than one calcium channel type (282). Whether the anti-calcium channel antibody titers correlate with the clinical disease severity is still debatable (see Ref. 443).

It should be noted that LEMS antibodies can also attack calcium channels outside the presynaptic terminal. Serum samples from three LEMS patients reduced both the maximal LVA and HVA calcium conductances in murine dorsal root ganglion neurons. Thus, even though LEMS is expressed as a neuromuscular and autonomic disorder, calcium channels at different sites may be broadly affected in LEMS patients (283).

C) What types of calcium channels are targeted by LEMS antibodies? In recent years, evidence has accumulated that LEMS antibodies can target several types of calcium channels in the presynaptic nerve terminal.

Grassi et al. (304) reported that IgG from LEMS patients applied to human neuroblastoma IMR32 cells reduced the density of low- (LVA; T) and high-threshold (HVA; L and N) barium currents by 36% (LVA) and 56% (HVA). Lambert-Eaton myasthenic syndrome IgG selectively reduced sustained (L-type) calcium channel current amplitudes in a rodent neuroblastoma × glioma cell line (642) and in bovine adrenal chromaffin cells (84). Reports have also shown that LEMS autoantibodies react with ω-conotoxin-sensitive calcium channels of the P/Q type (442, 840). In addition to ω-conotoxin MVIIIC-labeled channels, LEMS patients’ sera also immunoprecipitated ω-conotoxin GVIA-labeled N-type calcium channels (863). Anti-P/Q-type calcium channel antibodies were found in serum from 100% of patients with LEMS and a diagnosis of cancer (n = 32) and in 91% of the patients with LEMS without cancer (n = 33). Anti-N-type calcium-channel antibodies were found in only 49% of patients with LEMS (n = 65) (460). It was found that LEMS IgG downregulates mainly P-type calcium channels and to a lesser extent L-type channels (282).

Autoantibodies were detected by immunoprecipitation assays (233) using solubilized receptors labeled with ligands selective for N-type and L-type calcium channels. Sera with a high antibody titer (>3 nM) against rat brain N-type channels also contained autoantibodies that immunoprecipitated neuronal and muscle L-type channels. Lambert-Eaton myasthenic syndrome antibodies that recognize the β-subunit of calcium channel complexes were also described previously by Rosenfeld et al. (729).

D) Other presynaptic binding sites for LEMS antibodies. The question of whether LEMS antibodies bind also to other sites on the presynaptic terminal is still debatable. Sera from LEMS patients have a heterogeneous spectrum of antibodies, and some studies have suggested that this heterogeneity reflects the immune response to various synaptic proteins, including not only multiple voltage-gated calcium channels but also synaptotransmitter complex proteins (863). It has been shown that plasma from patients with LEMS contains antibodies that bind to the synaptic vesicle protein synaptotagmin (234). This led to the suggestion that since the interaction between synaptotagmin and ω-conotoxin-sensitive calcium channels may play a role in the docking synaptic vesicles at the plasma membrane before rapid neurotransmitter release, autoantibody binding to a synaptotagmin-calcium channel complex may be involved in the etiology of LEMS (464, 515).

E) Microscopic findings. Electron microscopy of presynaptic motor nerve terminals at the neuromuscular junction in LEMS revealed a decrease in the number of structures believed to correspond to voltage-sensitive calcium channels. The same picture was found by passive transfer of LEMS IgG from human to mouse (276). In addition, it was found that the active particles, normally arranged in double parallel rows, move closer together, form clusters, and are reduced in number (583).
2. Amyotrophic lateral sclerosis

A) CLINICAL CHARACTERISTICS OF AMYOTROPHIC LATERAL SCLEROSIS.

Amyotrophic lateral sclerosis (ALS) is a motoneuron disease, manifested by weakness and wasting of muscles. Although reflexes are relatively preserved, the wasting and fasciculation of the muscles is prominent. In the progressive muscular atrophy form of ALS, the anterior horn cells of the spinal cord are primarily affected. There are no sensory changes. Frequently in ALS, there is a combination of spasticity and hyperreflexia in the lower limbs and weakness, wasting, and fasciculation in the upper limbs. In the progressive form of the disease, there is difficulty in swallowing, which may lead to aspiration and pulmonary disease. Intellectual function is usually spared.

B) ETIOLOGY OF ALS. The cause of ALS is not fully understood. There are four main hypotheses: excitotoxicity linked to glutamate receptor overactivation, mutation of the superoxide dismutase gene, neurofilament accumulation, and production of autoantibodies to calcium channels (see Ref. 364). Only the latter alternative is reviewed here.

C) ANTIBODIES TO CALCIUM CHANNELS IN ALS. During the last decade, evidence has accumulated on the involvement of autoantibodies in ALS that lead to motoneuron degeneration and death (27). In animal models, inflammatory foci within the spinal cord and IgG at the neuromuscular junction as well as within upper and lower motoneurons support the role of autoimmune mechanisms in motoneuron destruction (804). Immunoglobulin G fraction from ALS patients can passively induce physiological changes at the neuromuscular junction in mice. The interaction of autoantibodies with calcium channels and, therefore, the alteration of their function have been shown both by biochemical and electrophysiological assays. Furthermore, it has been documented that motoneurons may be selectively vulnerable, since they have a low calcium-buffering capacity (14).

Prior studies have demonstrated that titers of antivoltage-gated calcium channel antibodies correlate with disease progression rates and that patient-derived antibodies produce electrophysiological changes in the function of these channels (419). Moreover, ALS patients possess immunoglobulins that bind to purified L-type voltage-gated calcium channels (419).

Antibodies to voltage-gated calcium channels in ALS patients’ IgG were shown to enhance calcium current and cause cell injury and death in a motoneuron cell line in vitro. Furthermore, it has been demonstrated that antibodies to calcium channels from ALS patients passively transferred to mice, selectively increased intracellular calcium and increased vesicle number in spinal motoneuron axon terminals, and in boutons making synapses on spinal motoneurons (240). These antibodies produced a dose-dependent intracellular increase in calcium concentration in spinal motoneurons and frontal cortex cells. The observed changes in ultrastructure and calcium distribution were predominant in motoneurons.

It has been shown that the α1-subunit of the voltage-gated calcium channel is the major voltage-gated calcium channel antigen to which ALS IgG binds. In addition, the binding of an L-type voltage-gated calcium channel α1-subunit-directed monoclonal antibody, which itself mimics the effects of ALS IgG on skeletal muscle voltage-gated calcium channel currents, is selectively prevented by the preaddition of ALS IgG. The antibody titers correlated with the rate of disease progression (419).

Electrophysiological studies in animals indicate that immunoglobulins from patients with this disease alter presynaptic voltage-dependent L-type calcium currents and calcium-dependent release of neurotransmitters (803). Furthermore, antibodies purified from ALS patient sera have been found to alter the physiological function of voltage-gated calcium channels in nonmotoneuron cell types (997). Using whole cell patch-clamp techniques, immunoglobulins purified from the sera of patients with ALS were shown to increase calcium currents in a hybrid motoneuron cell line, in mammalian cerebellar Purkinje cells, and in isolated channel protein in lipid bilayers (482, 802). These calcium currents were blocked by the polyamine funnel web spider toxin FTX, which has previously been shown to block calcium currents, and evoked transmitter release at mammalian motoneuron terminals (573).

Amyotrophic lateral sclerosis IgG kills 40–70% of a hybrid motoneuron cell line within 2 days. Amyotrophic lateral sclerosis IgG-mediated cytotoxicity is dependent on extracellular calcium concentration and is prevented by antagonists of N- or P-type voltage-gated calcium. Preincubating IgG with purified intact L-type VGCC or with isolated VGCC α1-subunit also blocks ALS IgG-mediated cytotoxicity (802).

It is tempting to speculate that antibodies found in ALS bind with a subunit of the calcium channel, increase calcium entry, and cause cell death. However, not all the evidence is in complete agreement with such a speculation (see Ref. 879). For example, nimodipine and verapamil (L-type calcium channel blocking drugs) were ineffective in slowing the progress of ALS (548, 549). One may argue, of course, that because the action of the ALS IgG occurs on more than one type of calcium channels, the inhibition of L-type calcium channels is not effective.

3. Hibernation

Hibernation is a very puzzling physiological condition. An animal survives a prolonged almost total ischemia of the brain without major deficit. Recently, it was shown that synaptosomes isolated from hibernating ground squirrels exhibit a reduced calcium entry upon
depolarization (289). A pharmacological dissection of the phenomenon indicates that it is probably associated with a reduction in the activity of Q-type calcium channels (289). The interaction between hypoxia and the activity of calcium channels occurs not only in synaptosomes but also in smooth muscle (697). It will be of interest to see whether mechanisms operating in hibernation can be used as neuroprotection.

III. POTASSIUM CHANNELS
IN NERVE TERMINALS

A. Function of Potassium Channels

Potassium channels are responsible for setting the resting potential of nerve endings and for repolarizing their membranes during action potentials. They also participate in afterhyperpolarizations, ending periods of strong activity, and modulate firing rates. As a result of these actions, potassium channels take part in regulating transmitter release.

B. Classification of Potassium Channels

Gating properties, rather than ionic selectivity or pharmacology, are best at distinguishing among the various types of potassium channels. The major classes of potassium channels in nerve terminals are listed below.

1. Delayed-rectifier potassium channels, K channels

Prevalent in most excitable cells with short (1–10 ms) action potentials, K channels are activated upon depolarization and exhibit little or no inactivation within hundreds of milliseconds to seconds.

2. Fast transient A channels

These are potassium channels that are activated upon depolarization, especially after a period of hyperpolarization, and then inactivate rapidly, usually within 1–100 ms.

3. Slowly activating K_s channels

Potassium channels, mostly found in heart cells, that activate very slowly upon depolarization are named K_s channels. These channels, whose activation lasts for several seconds, are less selective than other voltage-activated potassium channels.

4. Calcium-gated potassium channels, K(Ca) channels

Potassium channels that are activated by either internal micromolar concentrations of calcium alone, or by both internal calcium and depolarization, are called calcium-activated small-conductance (SK) and big-conductance (BK) channels, respectively.

5. ATP-dependent [K(ATP)] channels

These are voltage-insensitive potassium channels, normally closed, which are activated by a decrease in intracellular ATP level.

6. S channels

Only weakly sensitive to voltage, these potassium channels, turned off by the transmitter serotonin (5-HT), are commonly found in sensory neurons of Aplysia, and presumably in their nerve terminals as well.

C. Functional Properties According to Channel Type

1. Delayed-rectifier potassium channels, K channels

The outward potassium current in giant axons of the squid, described by Hodgkin and Huxley (348, 349), was called the delayed rectifier, because it activated, with a delay, upon depolarization and rose more slowly than the sodium current. Ever since, voltage-dependent potassium currents with similar kinetics, lacking rapid inactivation, and exhibiting no activation by intracellular calcium have been referred to as delayed rectifiers. On the basis of their activation threshold, gating kinetics and sensitivity to pharmacological agents such as aminopyridines and tetraethylammonium (TEA), it appears that K channels are not homogeneous and include several subtypes, the determinants of which are not yet fully known.

Table 8 presents nerve endings (with the exception of the Aplysia sensory neuron) in which delayed rectifier channels and currents were directly detected and measured. In the motor nerve terminals of squid, crayfish, frog, lizard, and mouse, K channels contribute to keeping the action potentials short. The high potassium permeability originating from the rapidly activating K channels repolarizes the presynaptic terminal after an action potential. By regulating the action potential duration, the delayed rectifier channels play a critical role in transmitter release. Thus Augustine (42) showed that in squid nerve terminals, the channel blocker 3,4-diaminopyridine (DAP) causes broadening of the presynaptic action potential leading to an increase in calcium entry and an enhancement of transmitter release. Likewise, action potentials in the excitor axon terminals of the crayfish are kept short by a delayed rectifier. Sivaramakrishnan et al. (794) showed that 4-aminopyridine (4-AP), which blocked this current in crayfish terminals, led to action potential broadening and an increase in transmitter release. In frog motor nerve terminals, where similar actions were seen,
Hevron et al. (341) and Shakiryanova et al. (769) found in addition that ACh suppressed the delayed rectifier, and it was suggested that this modulation may be significant in cases of acetylcholinesterase inhibition, accounting for nerve terminal membrane hypersensitivity. In mouse motor nerve endings, two delayed rectifiers were distinguished, one fast and one slow. The fast component is responsible for action potential repolarization as evident from its blockade by external TEA, while the slow component may play a role in regulating transmitter release since its selective blockade by the snake venom dendrotoxin (DTX) leads to facilitation (217, 854). Action potentials are kept short by K channels in CNS nerve terminals as well. Thus, in rat calyx of Held nerve endings, which utilize glutamate as their transmitter, a fast-activating delayed rectifier was recorded by Forsythe (264). This K channel permits short action potentials and high firing rates and participates in producing prominent afterhyperpolarizations, and its block by 4-AP leads to potentiated release.

Some neuronal cells, with much longer action potentials than those characteristic of motor axons, also employ delayed rectifiers to aid repolarization, but use a different K channel subtype whose gating kinetics are much slower. Thus, in sensory neurons of Aplysia, K channels activate within 100–200 ms and inactivate within 0.5 s (422). Byrne and Kandel (117) summarized the contribution of such delayed rectifiers to spike broadening. Another relatively slow K channel, with an activation time of 100 ms, was recorded by Bennett and Ho (66) in chick calyciform nerve terminals. Finally, the patch-clamp technique was used to study posterior pituitary nerve endings of axons originating in the hypothalamus, and a delayed rectifier was found with a single-channel conductance of 27 pS (76). This channel, too, has relatively slow activation kinetics and is selectively blocked by DTX. Because of its slow activation, it cannot contribute to action potential repolarization; rather, this delayed rectifier may be activated during long bursts of activity known to occur in the hypothalamus-pituitary system.

**Table 8. Delayed rectifier potassium channels in nerve terminals**

<table>
<thead>
<tr>
<th>Animal and Tissue (Recording Method)</th>
<th>Voltage Dependence and Kinetics</th>
<th>Aminopyridine Block</th>
<th>TEA(out) Effect</th>
<th>Other Blocking Agents</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squid giant synapse nerve terminal (intracellular voltage clamp)</td>
<td>Activation threshold, −60 mV; activation time, 1–15 ms; sigmoid current rise power, 1–2</td>
<td>DAP, ( K_d = 7 \mu M )</td>
<td>Little sensitivity</td>
<td>TEA(in)</td>
<td>42</td>
</tr>
<tr>
<td>Crayfish motor nerve endings (intracellular)</td>
<td>4-AP, DAP</td>
<td>May not block this current but just ( I_{Ca} )</td>
<td></td>
<td></td>
<td>794</td>
</tr>
<tr>
<td>Frog motor nerve endings (focal extracellular)</td>
<td>DAP</td>
<td>Blocks at 0.5–5 mM</td>
<td>Reversible block by 0.5–2 mM ACh; zinc ions</td>
<td></td>
<td>341, 552, 769</td>
</tr>
<tr>
<td>Lizard motor nerve endings (focal extracellular)</td>
<td></td>
<td>Blocks</td>
<td></td>
<td></td>
<td>473</td>
</tr>
<tr>
<td>Mouse motor nerve endings (focal extracellular)</td>
<td>Two current components: ( I_{kf} ); fast current; ( I_{ks} ); slow current</td>
<td>DAP blocks both; 4-AP blocks fast current</td>
<td>Slow component resistant to up to 30 nM TEA; blocks fast component</td>
<td>Uranyl ions and guanidine block both components; DTX blocks slow current; neostigmine blocks</td>
<td>104, 106, 217, 507, 854</td>
</tr>
<tr>
<td>Aplysia sensory neurons (intracellular voltage clamp)*</td>
<td>Called ( I_{kv} ); slow activation, 100–200 ms; inactivates within 0.5 s</td>
<td>4-AP</td>
<td>Blocks at 10 mM</td>
<td></td>
<td>117, 422</td>
</tr>
<tr>
<td>Chick calyciform nerve terminals of ciliary ganglion (voltage clamp)</td>
<td>Activation threshold, above −50 mV; activation time, 100 ms</td>
<td>Blocks</td>
<td>Cs⁺ (in)</td>
<td></td>
<td>66</td>
</tr>
<tr>
<td>Rat calyx of Held nerve terminal (whole cell patch)</td>
<td>Activation threshold, above −60 mV; activation time, 2 ms</td>
<td>4-AP</td>
<td></td>
<td></td>
<td>264</td>
</tr>
<tr>
<td>Rat posterior pituitary nerve endings (cell-attached patch clamp)</td>
<td>Called “D channel”; high activation threshold, above −30 mV; slow activation, 65 ms; ( \gamma ); 27 pS</td>
<td>Blocks</td>
<td>DTX blocks at 20 nM</td>
<td></td>
<td>76</td>
</tr>
</tbody>
</table>

DAP, 3,4-diaminopyridine; 4-AP, 4-aminopyridine; TEA, tetraethylammonium; DTX, snake venom dendrotoxin; \( \gamma \), single-channel conductance; \( K_d \), dissociation constant. * Not directly recorded from nerve terminal.
Opening of the K channel would raise the threshold for generating action potentials and hence limit secretion at the end of a long burst.

2. Fast transient A channels

A channels are voltage-gated potassium channels giving rise to currents that have been termed fast transient, transient outward, early outward, and rapidly inactivating potassium currents. They were first observed in molluscan neurons by Connor and Stevens (170), who called them the A current. A channels function in different neurons by regulating firing rates, determining first spike latencies and in some cases contributing to action potential repolarization. Compared with other potassium channels, these channels activate and inactivate more rapidly. Their steady-state inactivation is usually almost complete near the resting potential, and most important, their activation threshold is lower than that of other potassium channels. Therefore, A channels function in the sub-threshold range for generating action potentials, opening transiently upon small depolarizations that start from hyperpolarized potentials. Because of these characteristics, A channels are able to regulate repetitive firing at low rates, and they are prevalent in sensory cells that fire at rates that reflect stimulus intensity, in axons with low-frequency firing rates (169), and in other cells (733). After an action potential, the ensuing hyperpolarization removes A channel inactivation, and the transient outward current flowing through these channels slows down the return of the membrane potential toward action potential threshold so that the interval between the past action potential and the next one is prolonged. The upshot is that A channels serve as dampeners of interspike intervals.

A channels are presumed to be present in the presynaptic terminals of a number of sensory cells as well as in the nerve endings of CNS neurons, but to date, in most cases they have only been recorded in the soma of these cells. Among others, they were found in sensory neurons of *Aplysia* (422, 781) and guinea pig (398). In the latter, a single-channel conductance of 20 pS was found, and the channels inactivated with two time constants of 100 ms and 4 s. The slow inactivation component was suggested to provide the basis for building central delays into neuronal networks (186). In the CNS, a notable example of A channels was recorded by Dolly et al. (210) in guinea pig hippocampal slices, where an A current was specifically attenuated by DTX, leading to facilitation and spontaneous epileptiform activity in intact cell populations. It is also noteworthy that in cultured hippocampal neurons, an A current was found to be modulated by GABA<sub>B</sub> agonists, which induced a positive shift in the voltage dependence of channel inactivation (738). If similar A channels were present in the nerve terminals, GABA<sub>B</sub> agonists could make action potentials briefer by potentiating the A current and hence depressing transmitter release; at the resting potential, no change in potassium conductance would be expected, leaving the excitability of the nerve terminal intact.

Table 9 presents the only two prominent instances in which A channels were recorded directly from “native” nerve endings. Ironically, in these two cases, the classical functional role of the A channel is hardly evident. In the axon terminals of retina horizontal cells (which receive synaptic inputs from photoreceptors), A channels were detected and characterized, although their role remains unclear (296). A channels and A currents have also been detected in rat posterior pituitary nerve endings (76, 380, 382, 882). These channels are thought to participate in repolarizing the terminal after a spike. In addition, during

### Table 9. Fast transient A channels in nerve terminals

<table>
<thead>
<tr>
<th>Animal and Tissue (Recording Method)</th>
<th>Voltage Dependence and Kinetics</th>
<th>Aminopyridine Block</th>
<th>TEA(out) Effect</th>
<th>Other Blocking Agents</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turtle retina horizontal cell axon terminals (whole cell patch)</td>
<td>Activation threshold below −70 mV; exponential activation with τ = 1–2 ms; inactivation τ = 10–15 ms; full recovery from inactivation takes 2 min</td>
<td>5 mM 4-AP</td>
<td>Only partially blocked by 20 mM</td>
<td>20 μM capsaicin reduces current</td>
<td>296</td>
</tr>
<tr>
<td>Rat posterior pituitary nerve endings (cell-attached and/or whole cell patches)</td>
<td>Activation threshold below −30 mV; activation time, 1.4 ms at +60 mV; inactivation τ = 18 ms; γ = 32 pS (76) Voltage midpoint for steady-state inactivation, −48 mV; full recovery takes more than 16 s (882)</td>
<td>3 mM 4-AP</td>
<td>1–2 mM (76); in contrast, no effect by 100 mM (882)</td>
<td>Cs&lt;sup&gt;+&lt;/sup&gt;</td>
<td>76, 382, 882</td>
</tr>
</tbody>
</table>

τ, time constant; γ, single-channel conductance; 4-AP, 4-aminopyridine; TEA, tetraethylammonium.
high-frequency stimulation, reduction of the A current by inactivation may lead to action potential broadening.

Another noteworthy A channel was detected in fused synaptosomes prepared from the electric organ of Torpedo (228, 229). These pinched off cholinergic nerve terminals were patch-clamped in the cell-attached and inside-out configurations, and an A channel was recorded and found to have a single-channel conductance of 24 pS, a bursting activity and voltage-dependent activation, and inactivation kinetics that conferred upon it a “statistical memory” (114, 681) and oscillatory behavior (682). The channel may be important in frequency modulation phenomena such as facilitation, tetanic potentiation, and posttetanic potentiation.

Recently, evidence was obtained for the presence of an inactivating potassium current in the motor nerve terminals of the frog (553). Thus an A-type potassium channel may be directly involved in synaptic facilitation.

3. Slowly activating $K_s$ channels

Delayed rectifier potassium currents with extremely slow activation kinetics were originally described in Purkinje fibers of the heart (597). The $K_s$ channels underlying these currents were further characterized in atrial and ventricular myocytes (521, 790). It appears that the first demonstration of such a current in a neuron was in a nerve terminal. A novel slowly activating $K_s$ channel was found in isolated rat pituitary nerve endings that are a part of magnocellular neurons (417). The activation time constant of the current was 4 s at $-50$ mV, decreasing to 700 ms at $-40$ mV, and the half-activation occurred at $-37$ mV. Magnocellular neurons secrete oxytocin following bursts of activity that are sustained by plateau currents that last for seconds (22). Repetitive stimulation for more than 5 s was found to lead to inactivation of excitability (99). A long-lasting potassium current with kinetics of seconds would be required for this phenomenon; indeed, a calcium-gated potassium current of suitable properties was found and suggested to be involved in terminating action potential bursts or regulating the duration of interburst intervals (943). The novel slowly activating $K_s$ channel may be another potassium channel that contributes to the modulation of excitability and release, through its activation kinetics (395).

4. Other potassium channels

A) $K_{(Ca)}$ channels. The functional properties of calcium-gated potassium channels are described in section IV.

B) $K_{(ATP)}$ channels. Potassium channels that are voltage insensitive and shut by direct binding to cytosolic ATP are called K(ATP) channels. They were found in pancreatic $\beta$-cells, various muscle cells, and several neurons (727). At physiological glucose levels, K(ATP) channels are shut, but a decrease in intracellular ATP concentration increases their open probability so that these channels hyperpolarize cells when energy reserves dwindle. Deist et al. (198) found that mouse motor nerve terminals probably contain such channels, since the antidiabetic drug sulfonylurea, which is a specific blocker of K(ATP) channels, reduces part of the potassium current in these terminals. Furthermore, bathing the terminals in glucose-free solution increases the size of the sulfonylurea-sensitive current. It was suggested that under hypoglycemic conditions, when the cytosolic ATP level decreases and the ATP-dependent pumps become less effective, the opening of K(ATP) channels becomes essential for maintaining the resting potential by preventing membrane depolarization. With normal glucose levels, on the other hand, these channels do not contribute to the resting membrane potential.

Recently, Lee et al. (453) detected K(ATP) channels in patch-clamped fused nerve terminals from the motor cortex while the same channels could not be seen in the neuronal cell bodies. They suggested that the nerve terminal K(ATP) channels serve to limit glutamate release during metabolic stress.

C) $S$ channels. These potassium channels, which are shut by 5-HT, are only weakly sensitive to voltage if at all and provide a voltage-independent background conductance to Aplysia sensory neurons in which they are found. Although not directly detected in nerve terminals, Klein et al. (422) showed that in Aplysia sensory neurons, the S channels contribute substantially to the total outward current and that S current reduction causes action potential broadening, leading to presynaptic facilitation.

Belardetti et al. (61) employed whole cell and cell-attached patch configurations to record S-channel activity in growth cones of cultured Aplysia sensory neurons, which are the immediate precursors of mature terminals. They found that 5-HT produced long closures of the channel, resulting in slow depolarizations and prolongation of the action potential. Byrne and Kandel (117) reviewed how, in addition to spike broadening, reduction of S current enhances membrane excitability as reflected by a lower threshold for action potentials and less frequency adaptation to prolonged stimuli. Serotonin shuts S channels acting through the GTP-binding regulatory protein $G_{s}$, cAMP, and protein kinase A (PKA), as described by Siegelbaum et al. (786), leading to facilitated release of transmitter from the sensory neuron. Conversely, when more S channels are opened by the peptide transmitter FMRFamide, which acts via arachidonic acid metabolites, synaptic depression ensues (656).

5. Types of potassium channels in various nerve terminals

As noted by Hille (345), each excitable membrane uses a mix of several potassium channels to fulfill its
functional needs. Table 10 presents the particular mixes employed by various nerve endings. The most conspicuous facts that emerge from Table 10 are the presence of K(Ca) channels in nearly all nerve terminals, the presence of at least two potassium channel types in most nerve endings, and the cohabitation of delayed rectifier and calcium-activated potassium channels at motor nerve terminals (see also Ref. 380).

The potassium channels employed by each particular terminal have different gating kinetics and sensitivities. These usually ensure action potential repolarization, a link between calcium-triggered secretion and cessation of release, and in many cases, the possibility for frequency modulation of transmitter liberation.

D. Pharmacology of Potassium Channels

The pharmacological agents commonly used in the study of potassium channels are not very useful tools for distinguishing unambiguously among the various channel types. Tetraethylammonium and cesium ions, for example, block most potassium channels, at least to some degree. Nevertheless, certain agents, particularly toxins, appear to be relatively selective in their actions, although few instances of absolute specificity for one channel type may be noted (for general reviews of potassium channel pharmacology, see Refs. 171, 321, 555, 733).

Delayed-rectifier channels may usually be blocked by externally applied TEA and 4-AP. Tetraethylammonium is impermeant, and its blocking effects differ when applied externally or internally, suggesting two distinct binding sites (33, 345, 815). Indeed, extensive in vitro mutagenesis studies have identified the two separate sites and the K channel protein residues that participate in TEA binding (330, 502). A more specific K channel blocker is the scorpion peptide noxiustoxin, which was found to act on the delayed rectifier current in the squid axon (127).

In guinea pig hippocampal cells, Dolly et al. (210) demonstrated that the snake venom DTX blocked K channels. In nerve terminals, DTX was shown to block the D channel of posterior pituitary nerve endings (76) and the slower delayed rectifier component of the mouse motor nerve terminal (217). Other blockers of the two delayed rectifiers in mouse motor nerve terminals are guanidine and uranyl ions (854), although a later study reported that while uranyl ions do partially block the faster K channel, the slower delayed rectifier current is actually enhanced by these ions (147).

Fast transient A channels in nerve terminals are blocked, at least partially, by external application of TEA and millimolar concentrations of 4-AP (76, 296). No evidence for their block by DTX has been reported in nerve terminals, unlike that found in hippocampal neurons (316). Capsaicin, a plant toxin, blocks the A current of horizontal cell axon terminals at 20 mM (296).

Calcium-gated potassium channels of the SK type are usually TEA resistant and are blocked by the peptide apamin, a component of bee venom (112), at nanomolar concentrations. Less than 1 mM of externally applied TEA usually blocks BK channels as do nanomolar levels of the scorpion venom charybdotoxin (CTX) (547). The action of CTX was shown to be both voltage and state dependent (21). At the frog motor nerve terminal, CTX was found to block the K(Ca) channel at a nanomolar concentration (20). Recently, Harvey et al. (322) reported K(Ca) channel block in mouse motor nerve terminals by a series of CTX homologs and unveiled some of the critical peptide residues responsible for the block. The site of CTX binding and the particular amino acids participating in the binding have been identified in cloned potassium channels (500,

TABLE 10. POTASSIUM CHANNEL TYPES IN DIFFERENT NERVE ENDS

<table>
<thead>
<tr>
<th>Nerve Terminal</th>
<th>Delayed Rectifier</th>
<th>Calcium-Gated K(Ca) Channel</th>
<th>ATP-Sensitive K(ATP) Channel</th>
<th>Fast Transient A Channel</th>
<th>Slowly Activating K&lt;sub&gt;a&lt;/sub&gt;</th>
<th>Serotonin-Sensitive S Channel</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squid, motor</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>41, 42</td>
</tr>
<tr>
<td>Crayfish, motor</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>794</td>
</tr>
<tr>
<td>Frog, motor</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>341</td>
</tr>
<tr>
<td>Lizard, motor</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>473, 569</td>
</tr>
<tr>
<td>Mouse, motor</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td>200, 854</td>
</tr>
<tr>
<td>Chick, calyform</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>66</td>
</tr>
<tr>
<td>Rat, calyx of Held</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>264</td>
</tr>
<tr>
<td>Rat, posterior pituitary</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>76, 417</td>
</tr>
<tr>
<td>Turtle, horizontal cell</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td>296</td>
</tr>
<tr>
<td>Aplysia, sensory*</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>422</td>
</tr>
<tr>
<td>Mammalian, hippocampal*</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>210, 738</td>
</tr>
<tr>
<td>Torpedo electric organ, fused synaptosomes</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>252, 254, 757</td>
</tr>
</tbody>
</table>

+, One type of this channel present; ++, two types of this channel present. *Not directly recorded from nerve terminal. K(Ca) channel, Ca<sup>2+</sup>-dependent K channel; K(ATP), ATP-sensitive K channel. See text for further explanation.
501). Further pharmacological details of K(Ca) channels are presented in section ivE.

The K(ATP) channel does have a specific blocker, namely, the antidiabetic drugs sulfonylureas (171). Indeed, on the basis of its effect on the outward potassium current, Deist et al. (198) proceeded to detect the K(ATP) channel in mouse motor nerve terminals.

S channels have, as their “natural blocker,” the transmitter serotonin. These channels are turned off by cAMP-dependent protein kinase phosphorylation, resulting from cAMP level increases produced by 5-HT.

E. Methodology of Studying Potassium Channels

When several types of potassium channels are present in a cell or its terminal, it is rarely easy to dissect their individual properties, their particular parts in making up the total potassium current, or their specific contributions to function. Consequently, it is still not uncommon to be left with the meager knowledge that an outward current in a given nerve terminal is carried by potassium ions with no further identification of the channels involved. Many nerve terminals are too small to study with intracellular microelectrodes. Many others, due to their geometrics, are not suitable for proper voltage clamping, yet some others are not amenable to patch recordings. Hence, not surprisingly, our information about ion channels of nerve terminals in general is sparse compared with what we know of channels in the cell soma. Thus many nerve endings have not been probed to date, and in some nerve terminals that have been investigated, evidence concerning the presence of channels is still rather indirect, relying on focal recording of external current signals. When intracellular recording and voltage clamping are possible, the two main difficulties encountered in studying potassium channels are their similarities in gating and pharmacological responses. A systematic strategy for analyzing potassium currents cannot be presented here, but any appropriate design of experiment must clearly rely on suitable choices of voltage protocols and applications of blocking agents. In certain instances, a fortunate combination of such choices enabled the relatively direct demonstration of particular potassium channel types in nerve terminals.

Thus, for example, in the squid nerve terminal, which is large enough to study with intracellular voltage clamping, a K channel and a K(Ca) channel were found. Augustine (41) showed that the calcium-activated potassium current could be suppressed by blocking calcium current using cadmium or by external application of TEA, to which the delayed rectifier in this terminal was found to be resistant. Furthermore, the slow activation of the K(Ca) current during depolarization pulses, in combination with external TEA, enabled them to characterize the voltage properties and kinetics of the delayed rectifier. Augustine (42) showed further that by employing TEA and the aminopyridine DAP, which blocks both current components, the functions of the two channels in the terminal could be demonstrated.

Another prominent example is the nerve terminal of posterior pituitary axons. Here, patch clamping was successfully carried out by Bielefeldt et al. (76), and three different potassium channels were recorded: a K channel, an A channel, and an unusual CTX-resistant BK-type K(Ca) channel with single-channel conductances of 37, 32, and 134 pS, respectively. The unusual K(Ca) channel in this terminal was found to be extremely sensitive to calcium, activating during depolarizing pulses even at the low resting calcium levels. Consequently, its separation from the other two potassium current components in whole cell current measurements was not readily achieved by cadmium. Fortunately, in this terminal, the delayed rectifier could selectively be blocked by DTX. An additional separation problem arose from the fact that the BK current inactivation, although slower, was similar to the inactivation of the A current. Luckily, again, the currents could be separated on the basis of their activation properties: the A current activated within 1 ms by strong depolarizations, while the activation of the K(Ca) channel was much slower. In cell-attached patches, the individual activities of the three channel types could more readily be distinguished on the basis of their amplitudes and thresholds for activation.

The lack of a clear-cut means of distinguishing among K channel types does not only cause methodological problems. As pointed out by Rudy (733), it has also furnished considerable confusion in the literature; for example, A currents, often described as such in certain cells, turn out to be very similar to currents identified as delayed rectifiers in other cells. Molecular biology has begun to shed some light on the structural features of potassium channels, and the ongoing research in this area may alter our classical view of channel classification. There is growing evidence that rather than envisaging K channels and A channels as two discrete channel types, they should be viewed as placed on a continuum.

F. Molecular Biology of Potassium Channels

1. Genetic classification of potassium channels

Voltage-gated potassium channels are composed of integral membrane α-subunits (388, 746) that form the channel pore, and these are sometimes associated with accessory cytoplasmic β-subunits. Each α-subunit contains six hydrophobic segments, S1—S6, that are assumed to span the membrane, with a special pore-forming domain, called H5 or P, between S5 and S6, and a positively charged S4 segment that acts as a voltage sensor. The
α-subunits cloned so far appear to belong to two superfamilies that are denoted Kv and Kir for voltage-activated and inwardly rectifying, respectively (721). Only the former is of interest here, since just one case of detecting a Kir channel in nerve endings has so far been reported. Usually, four α-subunits are required to form a functioning Kv potassium channel. The β-subunits, when present, can modify the channel gating properties (531, 699).

On the basis of mutants exhibiting abnormal motor behavior, different genes encoding for potassium channel subunits were originally characterized in Drosophila (reviewed by Papazian et al., Ref. 632): Shaker, encoding for an α-subunit a Kv channel; hyperkinetic (Hk), encoding for a Kv channel β-subunit; ether-a-go-go (eag), for a different Kv channel α-subunit; and slow-poke (slo), for a calcium-activated potassium channel subunit. Changes in A current were found to be associated in Drosophila with Shaker, changes in delayed rectifier current with eag, and changes in IK(Ca) with slow-poke. Subsequently, four separate Shaker-like genes, mapped to different chromosomal sites, were classified in Drosophila and were called Shaker, Shal, Shaw, and Shaw (116). In Drosophila, expressed channels of Shaker inactivate rapidly, those of Shal less rapidly, while expressed channels of Shaw exhibit little inactivation and those of Shaw virtually none.

The Shaker probes from Drosophila led the way to cloning-related potassium channels from other invertebrate and vertebrate neurons, and Shaker, Shal, Shaw, and Shaw-like genes were classified in several animals (951). Today, it is recognized that each of the four members of the Drosophila gene family has one or more mammalian homologs and thus defines a subfamily (525, 745, 951). The sequence identity within the four genes in Drosophila is ~40%, whereas significantly higher identity (70%) is found among Drosophila Shaker and its homologs across the phyla. The same holds true for each of the Drosophila Shal, Shaw, and Shaw genes and its homologs (745). The channels coded for by homologous genes in different animals may or may not share the same biophysical properties. Thus the Shab-related delayed rectifiers in Drosophila and mouse are very similar (626), whereas most mammalian channels of Shaker homologs inactivate slowly in contrast to the Drosophila Shaker coded channel, which acts as a rapidly inactivating A channel (951).

In Drosophila, alternative splicing of primary Shaker-related RNA transcripts is extensive, leading to great potassium channel diversity, whereas in vertebrates, diversity is created by mRNA expression from separate genes (663). The number of α-subunit coassembly combinations is enormous, since both homomultimers and heteromultimers can be expressed. This great diversity may be useful for a vast number of cells, since it allows for the blending of multiple potassium channel types in a fine-tuned manner according to particular needs. As pointed out by several workers (663, 745), however, an unrestrained mixing of subunits would be undesirable for cells that require functionally independent and discrete potassium currents, and indeed, a molecular barrier to heteromeric association of α-subunits of different subfamilies seems to exist (179).

The Shaker-Shal-Shab-Shaw genes place the A channels and K channels within one group of voltage-activated potassium channels with intermediate features. Furthermore, originally shown by Zagotta et al. (994), inactivation could be restored to a noninactivating mutant Shaker-related potassium channel in vitro, by adding to the mutant channel a synthetic peptide “ball” that blocked the internal channel mouth in a “ball-and-chain” mechanism. Later, Covarrubias et al. (180) showed that rapidly inactivating A channels can be converted into noninactivating delayed rectifier K channels by phosphorylation of the inactivation domain. In parallel, Rettig et al. (699) demonstrated how association of a Kv β-subunit with α-subunits conferred rapid inactivation on delayed rectifiers.

A Babel-like confusion afflicts the terminology of potassium channel genes. For example, virtually identical rat, mouse, and human cDNA clones of the Shaker subfamily have been called RCK1 (or RBK1), MBK1 (or MK1), and HK1, respectively, and similarly, the Shab subfamily-related genes in mouse and rat have been termed Msab and DRK1, respectively. As cloning proceeded, “conversion tables” were needed, and several have indeed been put forward to make sense of the various voltage-gated potassium channel genes (386, 663). Table 11 presents the simplified nomenclature for gene subfamilies that was proposed by Chandy (144), which seems to be the one currently used by most investigators. Here, the Shaker-related genes are classified as Kv x,y, where x is a number denoting a subfamily and y a number denoting the specific member of the subfamily.

Chandy and Gutman (145) reviewed the six subfamilies Kv1 to Kv6, and recently new subfamilies have emerged (363). It was found that α-subunits of the same subfamily, but not of different subfamilies, can coassemble in forming channels as summarized by Shen and Pfaffinger (771). It was also found that cloned β-subunits assemble specifically with Kv1 α-subunits (768).

A detailed structural model of voltage-gated potassium channels was proposed by Durrell and Guy (224), and many molecular biological studies have provided insights into the factors that determine channel properties.

<table>
<thead>
<tr>
<th>Table 11. Potassium channel gene homologs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drosophila Genes</strong></td>
</tr>
<tr>
<td>Shaker</td>
</tr>
<tr>
<td>Shal</td>
</tr>
<tr>
<td>Shaw</td>
</tr>
<tr>
<td>Shal</td>
</tr>
</tbody>
</table>
such as ionic selectivity, voltage gating, kinetics of activation and inactivation, and binding of blockers (for review, see Ref. 663). Unfortunately, however, the pharmacological investigation of clonal potassium channels has not yielded simple selective tools for identifying particular potassium channel types in native cells.

2. Kv channel proteins detected in brain nerve terminals

Several investigators have combined in situ hybridization techniques and immunohistochemistry to follow the localization of potassium channels in mammalian brain. Site-specific polyclonal antibodies were prepared for the different α-subunits of many members of the Kv subfamilies encoding for a variety of both A-type and K-type channels. Various channel subunits that were found to be directed to presynaptic nerve terminals in a number of brain structures are summarized in Table 12.

Subunit localization in nerve terminals and other subcellular areas vary from neuron to neuron, and no stereotypic patterns emerge from the data collected so far (918). The differential expression of Kv proteins, however, as well as their targeting to contrasting subcellular compartments and their particular colocalizations in different neurons, obviously contribute to channel mixing diversity, which may be tailored to specific functional requirements. Thus, for example, Sheng et al. (775) found that two A channels, assembled from Kv1.4 and Kv4.2 subunits, were segregated in vivo to different subcellular locations, with the former concentrated in axon terminals and the latter in somatodendritic domains of rat CNS neurons. They suggested that a Kv1.4 channel is involved in the regulation of presynaptic transmission, whereas the Kv4.2 channel regulates postsynaptic signals. Veh et al. (918) pointed out that the presynaptic colocalization of the A-type channel subunits Kv1.4 and Kv3.4 in particular hippocampal terminals may be related to the long-term potentiation known to occur at these presynaptic points, which is probably related to memory mechanisms (87).

The localization of subunits that encode for delayed rectifier channels in various CNS terminals (e.g., Kv3.2 and many of the Kv1 subfamily members) most probably contributes to action potential repolarization and regulation of transmitter release (566, 918, 953).

Finally, it should be noted that only one Kv β-subunit has so far been detected in brain nerve endings (704). This β-subunit was found to be concentrated in both hippocampal perforant path terminals and in cerebellar basket cell terminals. Similarly, just one instance has been reported on a slo-related subunit of the maxi calcium-activated potassium channel in rat brain. In this case, the slo protein was found to be concentrated in terminal areas of several major projection tracts (424).

3. A Kir potassium channel protein detected in brain nerve terminals

As mentioned in section III, most potassium channels are members of either the voltage-gated superfamily Kv or the inward-rectifying superfamily Kir. The Kir protein subunits appear to have two membrane-spanning sections, M1 and M2, and a P domain, but they lack an S4-like voltage sensor (721). The Kir superfamily, which includes G protein-gated inward rectifiers and K(ATP) channels, may have arisen from a deletion of four of the six membrane-spanning segments of the more ancient Kv channels (160). Inward rectifying potassium channels are prevalent in skeletal muscle, heart cells, and central neurons, where they are important mediators of transmitter actions (345). There is no electrophysiological evidence for the presence of Kir channels in nerve terminals, but in one recent immunohistochemical study, Ponce et al. (662) reported that inward rectifier potassium channel proteins have been detected in rat brain nerve endings. The authors, who were surprised to find Kir channels in thalamocortical and hippocampal nerve terminals, suggested that the channels may mediate the actions of μ-opiate receptors, which are known to be present in these terminals.

<table>
<thead>
<tr>
<th>Terminal</th>
<th>Kv1.1</th>
<th>Kv1.2</th>
<th>Kv1.3</th>
<th>Kv1.4</th>
<th>Kv3.1</th>
<th>Kv3.2</th>
<th>Kv3.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellar parallel fiber terminals</td>
<td></td>
<td></td>
<td>918</td>
<td></td>
<td></td>
<td></td>
<td>953</td>
</tr>
<tr>
<td>Cerebellar basket cell terminals</td>
<td>918</td>
<td></td>
<td>532,533,776,918</td>
<td></td>
<td></td>
<td></td>
<td>918</td>
</tr>
<tr>
<td>Cerebellar Purkinje cell axon terminals</td>
<td>942*</td>
<td>942</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampal mossy fiber terminals</td>
<td></td>
<td></td>
<td>532,774</td>
<td></td>
<td>774,775,918</td>
<td></td>
<td>918</td>
</tr>
<tr>
<td>Hippocampal tractus perforans terminals</td>
<td>942</td>
<td>774,918,942</td>
<td></td>
<td>774,775,918</td>
<td></td>
<td>918</td>
<td></td>
</tr>
<tr>
<td>Thalamocortical projection terminals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>566</td>
</tr>
<tr>
<td>Olfactory bulb mitral cell axons</td>
<td>918,942</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* In mouse brain. Values given are reference numbers.
IV. CALCIUM-GATED POTASSIUM CURRENTS AND CHANNELS

A. Distribution of Calcium-Gated Potassium Channels in Nerve Terminals

After presenting the calcium and the potassium channels in nerve terminals, we want to discuss one specific type of potassium channels that combines these two topics: the calcium-gated potassium channels.

Calcium-gated potassium currents are consistently found at the presynaptic nerve terminals in almost every preparation studied, including neuromuscular junction of crayfish (88, 794), frog (20, 409), lizard (23, 473, 569), and mouse (20, 217, 507, 513, 890, 916); rat brain synaptosomes (54, 245, 598); cochlear nerve terminals (948); rat posterior pituitary (neurohypophysis) (73, 76, 214, 937, 940); avian ciliary ganglion (66); barnacle photoreceptor (327); and goldfish retinal bipolar cells (741). Based on their biophysical and pharmacological properties, they can be subdivided into fast and slow current \( I_{K(Ca)} \) (569); and goldfish retinal bipolar cells (741). Based on their biophysical and pharmacological properties, they can be subdivided into fast and slow current \( I_{K(Ca)} \) (569); and goldfish retinal bipolar cells (741).

Calcium-gated potassium currents can also be selectively blocked by CTX (569). Slow \( I_{K(Ca)} \) (507, 535, 569) activate within milliseconds and account in part for the hyperpolarizing afterpotential. This current in lizard neuromuscular junction can be selectively blocked by apamin (569). In rat neurohypophysis, it is insensitive to apamin (940).

Calcium-gated potassium currents (reviewed in Ref. 85) can be divided into two groups based on their single-channel conductance: BK channels and SK channels. Large-conductance calcium-gated potassium channels typically have single-channel conductance of >200 pS and are blocked by CTX and TEA (see e.g., Refs. 214, 245, 598, 948). Presynaptic SK channels generally have unitary conductance <100 pS and are less sensitive to TEA (245).

Both types of calcium-gated potassium channels are highly selective for potassium ions and their activity is determined by two factors: membrane potential and intracellular calcium levels. Calcium concentrations required for the activation of the channels range from 10 nM to 10 \( \mu \)M for different channels (73, 245, 598). Membrane depolarization is also required for activation. In rat brain synaptosomes, calcium-gated potassium channels open at \(-40 \text{ to } -30 \text{ mV}\) when calcium concentration is 10–100 \( \mu \)M (245, 598). In rat neurohypophysis, the channels open at a potential of \(-30 \text{ mV}\) at calcium concentrations of 0.1 \( \mu \)M (73). The channels are voltage dependent, and the open probability increases e-fold per 8–15 mV of depolarization (73, 245, 598). Increase in intracellular calcium shifts the voltage dependence of activation to the negative side, i.e., less depolarization is required to activate calcium-gated potassium channels at higher calcium concentrations. This action of calcium ions is cooperative with a Hill coefficient of 1.6–1.8 (73, 598).

Single-channel properties of calcium-gated potassium channels at presynaptic nerve terminals are summarized in Table 13.

### Table 13. Calcium-gated potassium channels in the presynaptic nerve terminals

<table>
<thead>
<tr>
<th>( \gamma ), pS</th>
<th>Preparation and Recording</th>
<th>Gating and Modulation</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>258 ± 13</td>
<td>Rat brain synaptosomes incorporated into planar lipid bilayers</td>
<td>Blocked by aminoglycosides (0.195–3 mM) and by gallamin (0.3 mM)</td>
<td>598</td>
</tr>
<tr>
<td>221 ± 5</td>
<td>Efferent cochlear nerve terminals/patch clamp</td>
<td>( P_{\text{open}} ) ↑ with depolarization</td>
<td>948</td>
</tr>
<tr>
<td>193 ± 5</td>
<td>Rat posterior pituitary nerve terminals/patch clamp</td>
<td>( P_{\text{open}} ) ↑ upon depolarization</td>
<td>73</td>
</tr>
<tr>
<td>(range 130–305)</td>
<td></td>
<td>Blocked by extracellular TEA (20 mM) and charybdotoxin (0.1 ( \mu )M)</td>
<td></td>
</tr>
<tr>
<td>231 ± 9</td>
<td>Rat posterior pituitary/patch clamp</td>
<td>( P_{\text{open}} ) ↑ upon depolarization</td>
<td>937, 940</td>
</tr>
<tr>
<td>134 ± 18</td>
<td>Rat posterior pituitary/patch clamp</td>
<td>Not blocked by extracellular apamin (20 nM) or charybdotoxin (100 nM)</td>
<td></td>
</tr>
<tr>
<td>218 ± 5</td>
<td>Posterior pituitary/patch clamp</td>
<td>Rapid (2–30 ms) activation, no inactivation during 5-s pulse</td>
<td>76</td>
</tr>
<tr>
<td>75–80 120–125</td>
<td>Channels from rat brain synaptosomes inserted into phospholipid bilayer/patch clamp</td>
<td>Activates rapidly (&lt;1.5 ms at 60 mV) upon depolarization and inactivates slowly (( \gamma = 71 \text{ ms} ))</td>
<td></td>
</tr>
<tr>
<td>(range 65–145)</td>
<td></td>
<td>Blocked by extracellular TEA (5 mM); insensitive to CTX (100 ( \mu )M)</td>
<td></td>
</tr>
<tr>
<td>221 ± 12</td>
<td></td>
<td>( P_{\text{open}} ) ↑ upon depolarization at ( \text{Ca}^{2+} ) concentration &gt; 0.1 ( \mu )M</td>
<td></td>
</tr>
</tbody>
</table>

\( \gamma \), Single-channel conductance; ↑, increases; 4-AP, 4-aminopyridine; CTX, charybdotoxin; IC\(_{50}\), concentration that causes 50% inhibition; \( P_{\text{open}} \), open probability; TEA, tetraethylammonium.
B. Possible Physiological Roles

To understand the functions of calcium-gated potassium channels, it is helpful to look first at the location of these channels relative to the sites of transmitter release. Calcium-gated potassium channels have been demonstrated to be clustered at the active zones in the vicinity of calcium channels in hair cells (376, 714, 715), in presynaptic terminals of barnacle photoreceptor (830), and in frog neuromuscular junction (718, 720). This strategic location of the channels allows them to activate rapidly in response to the rise in calcium concentration (720) and thus makes them likely candidates to be involved in the local regulation of the duration of depolarization at the neurotransmitter release sites (718).

Blockade of calcium-gated potassium channels causes two notable changes in the shape of the action potential invading the nerve terminal. The first is a slight prolongation of the depolarization (88, 473, 569, 794), and the second is disappearance of the hyperpolarizing afterpotential several milliseconds after the action potential (66, 88, 217, 569, 794). The first effect is attributed to the fast calcium-gated potassium current, which activates almost immediately after depolarization, and the second to the slow current (569).

The role of calcium-gated potassium channels in transmitter release is not firmly established. An intriguing hypothesis has been proposed by Stanley and Ehrenstein (822) whereby calcium-gated potassium channels in synaptic vesicles open in response to the rise in intracellular calcium concentration and allow potassium into the synaptic vesicle. The entry of potassium and concomitantly of water causes swelling of the synaptic vesicles and fusion with the plasma membrane. However, this does not seem to be the case (44, 1009).

Although it is conceivable that calcium-gated potassium channels may regulate the duration of the action potential invading the nerve terminal and, thus, limit calcium entry, studies on the effects of blockade of calcium-gated potassium channels on transmitter release are inconclusive. Some investigators report an increase in transmitter release when a selective blocker of calcium-gated potassium channels, CTX (513, 718, 719), or its homolog CTX-2 (513), is applied to the nerve terminal, whereas several other reports show that selective inhibition of calcium-gated potassium channels produces little effect on transmitter release (20, 473, 559). Furthermore, it is argued that the effect of CTX and CTX-2 is due to their action on other potassium channels rather than on calcium-gated potassium channels (513).

Calcium-gated potassium channels are likely to come into play during the repetitive activity of the nerve terminal. Both a significant increase in intracellular calcium concentration (105, 382) and a rise in calcium-activated potassium current (327, 432) during repetitive stimulation have been demonstrated. Activation of the calcium-gated potassium conductance may cause hyperpolarization of the membrane, limit calcium entry, and subsequently reduce transmitter release. At least two observations support this hypothesis. The first is calcium-dependent hyperpolarization of the terminal membrane and action potential failures at high-frequency stimulation (73). The second is a decrease in paired pulse facilitation at crayfish neuromuscular junction when calcium-gated potassium channels are not blocked (795). Consequently, calcium-gated potassium channels have been hypothesized to be involved in termination of bursts of activity and regulation of duration of interburst intervals (940) as well as in the fatigue of secretion observed at the rat neurohypophysis (73).

In auditory hair cells, which are stimulated at different frequencies in vivo, calcium-gated potassium channels have been proposed to be involved in frequency tuning (34, 361, 376, 715) and in protection against overstimulation (948).

C. Molecular Biology and Structure

To the best of our knowledge, gene or genes encoding the calcium-activated potassium channels at the presynaptic nerve terminals have not been cloned, and the structure of the channel has not yet been unraveled. However, there is some information about the molecular properties of the small calcium-gated potassium channels blocked by apamin (446, 724, 984).

In different tissue preparations, including brain synaptosomes, apamin binds a 250-kDa protein (448, 724). Affinity labeling with $^{125}$I-apamine results in a covalent labeling of a single-chain polypeptide of ~30 kDa (448, 724). Thus the apamin receptor is postulated to be either a part of the calcium-gated potassium channel macromolecule (448) or a protein associated with calcium-gated potassium channel (807), and the calcium-gated potassium channel is proposed to be a large oligomeric structure containing a 30-kDa subunit (724). Sokol et al. (807) used antibodies against apamin-binding protein from bovine brain synaptosomes to clone a cDNA from a porcine vascular smooth muscle expression library. The gene encoding the apamin receptor (Kcal 1.8) encodes the protein of 438 amino acids, which has four potential transmembrane domains, one putative calcium binding site, a PKC phosphorylation site, and a leucine zipper motif. It does not possess any significant sequence homologies with known ion channels or receptors (807).

Mutations of Drosophila slow-poke (slo) locus, located on the third chromosome (40), specifically abolish calcium-gated potassium currents in adult and larval muscles and in larval neurons (236, 425, 739, 792). The molecular analysis of this locus provided information about the structural components of the calcium-gated potas-
sium channels. The slo locus contains an open reading frame of 3,552 nucleotides, which encodes a protein of 1,184 amino acids. Hydropathy analysis of this protein reveals seven hydrophobic domains near the NH2 terminal. This arrangement resembles the structure of other potassium channels (40). The fourth hydrophobic region (S4) and the region between the fifth and the sixth hydrophobic domains (H5) show sequence similarity with voltage-gated potassium channels (40). Other regions of the protein also show some degree of sequence similarity with voltage- and nucleotide-gated channels (387). Thus this putative calcium-gated potassium channel is thought to be a member of the superfamily that includes voltage-gated sodium, calcium, and potassium channels and second messenger-gated ion channels (387). Several cDNA derived from the slo locus can be found in the fly head cDNA library. These cDNA encode closely related proteins of ~1,200 amino acids (4). The RNA transcribed from the slo cDNA expressed in oocytes gives rise to several functionally diverse calcium-gated potassium channels (4, 789), thus suggesting that alternative splicing of slo mRNA gives rise to a large family of calcium-gated potassium channels (4). Similar genes encoding calcium-gated potassium channels in mice (115) and humans (892) have also been cloned. It is noteworthy that the expression pattern of slo protein in rat brain is consistent with the targeting of the protein to the presynaptic nerve terminals (424).

Recently, another gene and protein associated with calcium-gated potassium channels (the β-subunit) have been characterized (893). The gene encodes a polypeptide of 191 amino acids, which may contain 2 transmembrane domains. No evidence for alternative splicing of the gene product has been found. Coexpression of this protein with the α-subunit results in a channel with increased calcium and voltage sensitivity. The function of the β-subunit, its relationship to the channel molecule, and whether it is expressed to a significant extent at presynaptic terminals are unclear.

D. Modulation of Calcium-Gated Potassium Channels by Phosphorylation

Similar to many intracellular processes, the activity of calcium-gated potassium channels can be modulated by phosphorylation and dephosphorylation. In rat posterior pituitary nerve terminals, macroscopic calcium-gated potassium current disappears in ~5 min when the ATP is omitted from the pipette filling solution. The open probability of calcium-gated potassium channels in excised patches is dependent on the ATP concentration at the intracellular side of the channels. Adenosine 5′-triphosphate prevents the decline (rundown) in the channels’ activity and restores it after rundown occurs (75). Furthermore, addition of the catalytic subunits of the ATP-dependent protein kinase increases the activity of calcium-gated potassium channels from rat brain synaptosomes incorporated into lipid bilayers (245). Studies with a nonhydrolyzable ATP analog, nonspecific kinase inhibitor IQMP, and magnesium-free solutions show that ATP acts as a phosphate donor rather than a ligand directly affecting the channels (75). In excised patches, okadaic acid blocks the rundown at 50 nM, but not at 1 nM, thus indicating that the rundown is caused by dephosphorylation by phosphatase 1. The addition of GTP/βS to the cytoplasmic face of the excised patches accelerates the rundown in the absence of okadaic acid, suggesting that G protein is present in excised patches and may activate phosphatase 1 (75). Kinetic and statistical analyses of phosphorylation and dephosphorylation, as well as the voltage dependence of phosphorylation (74, 75), indicate that the calcium-gated potassium channel and its kinase form a macromolecular complex, whereas phosphatase is probably a separate protein that diffuses in a membrane and must encounter the channel for the dephosphorylation to occur (74).

Calcium-gated potassium channels play a role in action potential failures at high-frequency stimulation of the pituitary nerve terminals (73). Agents that interfere with phosphorylation and dephosphorylation influence action potential failures in a manner consistent with the enzymatic modulation of calcium-gated potassium channels, suggesting that this modulation may play a role under physiological conditions (75).

E. Pharmacology

Calcium-gated potassium channels can be modified by various toxins, neurotransmitters, drugs, and ions. The substances affecting the calcium-gated potassium channels may be subdivided into two broad categories: 1) agents that inhibit or block calcium-gated potassium channels and 2) agents that increase the activity of the channels. Toxins and several drugs belong to the first category, alcohols to the second. Actions of various substances on calcium-gated potassium channels and currents are summarized in Table 14.

1. TEA

Tetraethylammonium specifically blocks BK and SK calcium-gated potassium channels when applied extracellularly or intracellularly (23, 76, 88, 245, 473, 599, 794, 890, 940, 948). Large-conductance calcium-gated potassium channels seem to be more sensitive to internal application of TEA than SK channels (245).

2. Toxins

Several toxins have been used in the study of calcium-gated potassium channels. These include the scorpion
toxins CTX (20, 73, 76, 88, 245, 473, 513, 569, 741, 794, 916, 940, 948) and iberiotoxin (88, 916), the bee venom toxin apamin (20, 73, 217, 446, 569, 724, 940, 984), and the red tide toxin brevetoxin B (890). Kaliotoxin (322, 444, 725) may block presynaptic calcium-gated potassium channels; its effect, however, has not been definitely established.

3. Drugs

Clinically used drugs that are known to block calcium-gated potassium channels include aminoglycoside antibiotics (598), antipsychotics (63), and the antimalarial drug quinine sulfate (54). Aminoglycoside antibiotics block calcium-gated potassium channels, when applied at the cytosolic side, in a dose- and voltage-dependent manner. The potency of different antibiotics depends on the number of positively charged amino residues, indicating electrostatic interactions between the agent and the receptor site at the channel (598). Extracellularly applied tetrandrine, a drug used in China for the treatment of hypertension and cardiac arrhythmias, blocks presynaptic calcium-gated potassium channels with high affinity (936, 937).

4. Alcohols

Alcohols such as ethanol, propanol, and butanol increase the activity of calcium-gated potassium channels (214, 984). Studies on excised patches show that ethanol alters the gating properties of calcium-gated potassium channels, causing them to spend more time in an open state (214). The fact that ethanol exerts its effect in excised patches suggests that it probably affects the channels directly, rather than via second messengers (214).

Calcium-stimulated potassium current probably plays an important role in alcohol intoxication, since intraventricular injection of apamin inhibits ethanol-induced narcosis in mice (984).

5. Ions

Zinc ions have been reported to affect presynaptic calcium-gated potassium channels (552). At frog neuromuscular junction, zinc ions block voltage-dependent and calcium-activated potassium channels, when applied extracellularly (552). The effect of zinc is not specific, and prolonged exposure to the ion leads to an irreversible disruption of all ionic conductances of the terminal (552). Barium ions also block presynaptic calcium-gated potassium channels when applied to the extracellular or the intracellular side of the channels (23, 409, 507, 940).

F. Pathophysiology of Potassium Channels

1. Acquired neuromyotonia (Isaacs’ syndrome)

Myotonia is a disorder in which sustained contraction of muscle is triggered by either a voluntary contraction or percussion. Its electrophysiological equivalent is the occurrence of repetitive discharges after activation of muscle, with these discharges then diminishing in amplitude or frequency. Acquired neuromyotonia (Isaacs’ syndrome) is a disorder characterized by hyperexcitable motor nerves and sometimes by central abnormalities (320). There are several lines of evidence suggesting that this syndrome is due to an immunologically mediated injury causing hyperexcitability, since it responds well to plasmapheresis (373), azathioprine (706), methylprednisolone (231), phenytoin (146), and valproic acid (608).

In Isaacs’ syndrome, a number of specific antibodies were detected to human brain voltage-gated potassium channels expressed in Xenopus oocytes (320), as well as to potassium channels of a neuronal cell line (PC-12) (31, 811). Thus Isaacs’s syndrome nerve hyperexcitability is probably the result of the immunological attack on the voltage-dependent potassium channels located along the motor nerve or at the nerve terminal.

V. SODIUM CHANNELS IN NERVE TERMINALS

A. Functions of Sodium Channels in Nerve Terminals

The main function of presynaptic nerve terminals is to release transmitter upon the arrival of an appropriate signal, which in most cases is the action potential. In most terminals, the action potential is generated by voltage-dependent sodium channels. The number of transmitter
quanta liberated from the presynaptic nerve ending depends on the amount of calcium ions entering the terminal. This in turn depends on the amplitude and duration of the depolarizing pulse, as shown in the pioneering articles of Katz and Miledi (401–403, 405). Here we deal only briefly with the presynaptic excitability of nerve terminals; other aspects are well described in the review by Jackson (380).

In addition to the primary role of generating the action potential, sodium channels also have a role in determining the intracellular sodium concentration ([Na\(^{+}\)]\(_{i}\)), which in turn affects the [Ca\(^{2+}\)]\(_{i}\). The initial observations pointed to a paradox. If the amplitude and the duration of the action potential are of importance in determining the number of quanta liberated by the nerve impulse \(m\), then reduction of extracellular sodium concentration should cause a reduction in \(m\). However, the opposite was observed (167, 677). The discovery of the sodium/calcium exchanger resolved this paradox. The activity of this protein is controlled in most cases by five parameters: the intracellular and extracellular concentrations of sodium and calcium as well as the membrane potential. Hence, sodium channels can affect the function of the nerve terminal by several of these parameters. It was found repeatedly that elevation of the [Na\(^{+}\)]\(_{i}\), causes a marked increase in transmitter release (98, 129, 219, 246, 280, 284, 465, 494, 542, 558, 577, 602, 641, 684, 701, 708, 833, 1012). The accumulation of sodium ions (and the resulting accumulation of calcium ions) has been implicated in a number of pathophysiological conditions, including aging and cell death (77, 86, 122, 129, 218, 450, 455, 494, 503, 838, 919).

The third functional aspect of the sodium channels in the presynaptic nerve terminals is their role as targets of the action of toxins and drugs. Some of the toxins are among the most potent biological weapons. By blocking the sodium channels they abolish transmitter release from the nerve terminals and thus paralyze the prey.

B. Cellular and Molecular Biology of Sodium Channels

The reader is referred to several recent reviews regarding the cellular and molecular biology of sodium channels (268, 269, 298, 586, 835, 836, 908).

C. Presynaptic Localization of Sodium Channels

Sodium channels were found in the nerve terminal of almost all the preparations studied. They were first demonstrated in the pioneering work of Katz and Miledi at the neuromuscular synapse of the frog and thereafter in many other species (23, 24, 168, 187, 188, 191, 308, 401, 403–405, 427, 535, 643, 734). Sodium channels were found in many other nerve terminals (49, 138, 162, 172, 246, 454, 457, 468, 518, 748).

D. Toxins Acting on Sodium Channels

The gating of sodium channels is characterized by four processes: activation upon depolarization, inactivation after longer depolarizations, deactivation after a repolarization, and reactivation when the channel exits from the inactivated state. It is of interest that during evolution different animals developed toxins aimed at different processes of sodium channel gating. Some of the toxins prevent the sodium channel from activation and thus cause paralysis of the prey. Other toxins produce a persistent activation of sodium channels in the prey, causing convulsions and preventing normal motility. It was suggested to us that the evolutionary selection of the molecular target on the sodium channel may be related to the visual acuity of the predator (E. Zlotkin, personal communication). Predators with poor visual acuity will have difficulty finding an inmobile prey.

1. Toxins preventing the passage of sodium ions

Tetrodotoxin is the best-known toxin in this group. Found in pufferfish and porcupinefish (572), it is an alkaloid containing a guanidino moiety. Its binding to the sodium channel prevents the passage of ions. The blocking effect of tetrodotoxin at nerve terminals has been known for more than three decades (402, 403, 405, 596). Other toxins, such as saxitoxin, neosaxitoxin, and gonautoxins, also block sodium channels (136). Further details on tetrodotoxin and saxitoxin binding and use can be found in a number of reviews (51, 136, 137, 831).

Another group of sodium channel-blocking toxins, the \(\mu\)-cono-toxins, was discovered in the venom of marine snails from the *Conus* family (183, 306, 530). \(\mu\)-Conotoxins do not block sodium channels in frog or mammalian motor nerves or in rat brain (183), but they bind to sodium channels in electric eel electroplax (986) and *Aplysia* neurons (530). The \(\mu\)-conotoxins bind to the same site on the sodium channel as tetrodotoxin and saxitoxin. Conotoxin GS isolated from the venom of *Conus geographus* is a toxin with similar binding properties to those of \(\mu\)-conotoxins (987).

2. Toxins affecting activation and inactivation of sodium channels

The first compound of natural origin found to affect sodium open channel characteristics is the plant alkaloid veratridine. The affected channels open at normal levels of membrane potential, but their inactivation is inhibited (135). Activation of sodium channels in synaptoneuro-
from the red-tide dinoflagellate sodium channel receptor site 5 (887) and can be isolated and evoked release (389). The release of ACh, followed by a block of both spontaneous chotoxin causes a transient increase in the spontaneous (941). At the mammalian neuromuscular junction, batrachotoxin affects sodium channel inactivation. In contrast, sodium channel activation is negligibly delayed, and the deactivation is unaffected (888).

Batrachotoxin from the dart-poison frogs (Dendrobatidae) is an alkaloid, which acts by preventing voltage-dependent sodium channels from closing or inactivating (941). At the mammalian neuromuscular junction, batrachotoxin causes a transient increase in the spontaneous release of ACh, followed by a block of both spontaneous and evoked release (389).

The brevetoxins are lipid-soluble toxins acting on sodium channel receptor site 5 (887) and can be isolated from the red-tide dinoflagellate Ptychodiscus paresis. Brevetoxin-B has been shown to depolarize giant axons of squid (38) and nerve terminals (975). The depolarization is sodium dependent and can be blocked by tetrodotoxin (142).

Ciguatoxin enhances quantal transmitter release at the neuromuscular junction (556), probably due to the prolonged sodium channel opening in nerve membranes (120). Ciguatoxin-1b induces a tetrodotoxin-sensitive swelling of the nodes of Ranvier by modifying sodium currents and increasing intracellular sodium concentration (67).

A series of polypeptide toxins was isolated from the sea anemone Anemonia sulcata (68), the most abundant of which is ATxII (143). Similar toxins have been isolated from a number of other organisms (412, 449). ATxII has been shown to prolong nerve action potentials and to cause spontaneous and repetitive activity (723). The toxin ATxII causes depolarization (which can be prevented by tetrodotoxin) and repetitive firing of motor nerves by prolonging the inactivation phase of sodium currents (557, 723).

Another class of protein toxins affecting sodium channels has been discovered in the venom of the spider Agelenopsis aperta. Termed μ-agatoxins, they cause gradual and irreversible spastic paralysis in flies. This is probably due to an effect on neuronal sodium channels, which leads to repetitive action potentials originating in presynaptic axons and nerve terminals (3).

E. Persistent Sodium Channels

Most sodium channels activate rapidly and thereafter inactivate (348, 349). The inactivation is usually a rapid process and occurs in a time scale of milliseconds, unless toxins are present as described in a previous section. Recently, however, persistent sodium channels have been described in a number of cell types, and they may have a substantial physiological and pathophysiological importance (48, 182, 260, 329, 394, 618, 675, 837). They usually activate at more negative potentials than the “normal” sodium channels, and their activity persists for long periods. There is presently no direct evidence that these channels exist at the nerve terminals. If they do exist, they may affect the membrane potential of the terminal and thus affect tonic release of transmitters (see Ref. 634).

VI. CHLORIDE CHANNELS IN NERVE TERMINALS

A. Function of Chloride Channels

Chloride channels are located both in the plasma and intracellular organelle membranes of animal cells. They play major roles in the regulation of intracellular pH (110), cell volume (350, 594), nerve excitability, and determination of the resting potential (12). Chloride channels were found in fused synaptic vesicles (980), mitochondria (81, 510), microsomes (441), and other intracellular organelles (10). This review deals only with chloride channels known to be present in the presynaptic terminals. Excellent reviews dealing with the structure and function of other chloride channels can be found elsewhere (390, 594).

Chloride is the most abundant extracellular physiological anion. In animal cells, the cytoplasmic chloride concentration is lower than the plasma concentration, and in most nerve cells, the chloride equilibrium potential ($E_{Cl}$) is near the resting potential. The contribution of chloride channels to the resting potential of the nerve terminal depends on their relative number and conductance. Chloride channels would be expected, when activated, to reduce normal excitability and help repolarize the cell during an action potential (345). In nerve terminals, chloride channels were found to be involved in presynaptic inhibition (222). Opening of these channels causes an increase in the total membrane conductance; this leads to decreased efficiency of subsequent inward currents to produce a depolarization and, hence, transmitter release (731). A mathematical model was developed suggesting that depolarization associated with the increase in chloride conductance (when the $E_{Cl}$ is above the resting potential) may inactivate some calcium and
sodium channels in the nerve terminal, contributing to presynaptic inhibition (303).

Chloride channels may also be involved in the determination of the size of the fraction of the nerve terminal arborization activated at any given time (934). Usually nerves branch extensively, and branches innervate many different postsynaptic targets. At the branch points, there is a lower safety factor of impulse conduction (694). By increasing the chloride conductance, the safety factor of impulse conduction is decreased even further. Thus an increase in chloride conductance (by activation of GABA_A receptors, see Ref. 1004) may determine which nerve terminals will be functional (230). It should be noted that not only chloride channels may serve this function (606).

B. Classification of Chloride Channels

At the surface membrane of the presynaptic nerve terminal, three chloride channel types were found: 1) ligand-gated chloride channels composed of GABA-gated chloride channels (490, 1004; for reviews, see Refs. 392, 496, 963) and glycine-gated chloride channels (932) (for a general review, see Ref. 688); 2) calcium-dependent or calcium-activated chloride channels (619) (for a general review, see Ref. 763); and 3) voltage-dependent chloride channels in fused synaptosomes (227).

C. Overview of Ligand-Gated Chloride Channels

The ligand-gated chloride channels belong to a large family of channel molecules that include also the nicotinic ACh (397), 5-HT_3 (512), glycine (436), and GABA (588) receptor channels (70). The functional channels are composed of five homologous membrane-spanning subunits that form ion-conducting channels across the membrane. They contain ligand-binding sites in the extracellular domains and modulatory sites in the cytoplasmic domains of the pentamer. The deduced amino acid sequences show significant homology within the members of the family (20–30%). Moreover, the general structural characteristics of the subunits are very similar.

1. GABA-activated chloride channels

γ-Aminobutyric acid is an important inhibitory neurotransmitter in the CNS (94). Its role was first described in the invertebrate nervous system (377, 625). In the CNS, it can modulate synaptic transmission by the activation of three classes of receptors: GABA_A, GABA_B, and GABA_C. Of those, only GABA_A and GABA_C are ion channels.

a) GABA_A receptor-channel complex. I) Functional properties and localization. Binding of an agonist to the GABA_A receptor-channel complex leads to the opening of an anion channel permeable to chloride and to a lesser extent to bicarbonate (94). In presynaptic nerve terminals, two mechanisms were proposed for the inhibition of transmitter release by activation of chloride channels (732): retardation in the propagation of action potentials into the nerve terminal arborization (934) and a decrease in the amount of transmitter released by each action potential which succeeds in reaching the terminal. γ-Aminobutyric acid-mediated chloride currents may produce both forms of inhibition by shunting membrane currents and by reducing the probability of opening of other ion channels (380).

The opening of the GABA_A receptor channels results in the inhibition of transmitter release in preparations such as spinal cord (593, 732), olfactory cortex (653), peptidergic nerve in posterior pituitary (380), central nucleus of the inferior colliculus (627), retina (628, 855), and the crayfish neuromuscular junction (258) (for a review dealing with activation of GABA_A in preparations other than presynaptic nerve terminals, see Ref. 1006).

II) Molecular biology of GABA_A receptor-channel complex. The functional heteromeric protein complex consists of five homologous membrane-spanning subunits. The pentameric structure of GABA_A channels was first inferred by analogy with other ligand-gated ion channels and confirmed by electron microscopy and rotational spin analysis of isolated native channels (588). There are five subunit classes, each of them having different types: α1-α6, β1-β4, γ1-γ4, δ, and ε. In addition, the γ-subunit has two forms, short and long, created by alternative splicing. These subunit subtypes allow for a great variety of possible combinations and, therefore, huge pharmacological differences (for reviews, see Refs. 496, 764, 963).

III) Modulation and pharmacology. The activity of GABA_A channels can be modulated by nitric oxide (225, 712), phosphorylation by PKA (664, 765), and calcium/phospholipid-dependent PKC (111, 456).

Natural and synthetic compounds that can modulate GABA_A channel include benzodiazepines, steroids, barbiturates, picrotoxin, ethanol, loreclezole, general anesthetics, zinc, lanthanides, pesticides, and nonsteroidal anti-inflammatory drugs. The affinity of each ligand is dependent on different channel subunit combinations, allowing one to distinguish between them (for general reviews, see Refs. 570, 963).

a) Benzodiazepines. The GABA_A channels are often called benzodiazepines (BZ) receptors because of their high affinity for BZ. The localization of the BZ binding site at nerve terminals was shown in posterior pituitary nerve endings (1007) and in rat brain synaptoneuroses (571). Benzodiazepines potentiate the effect of GABA by decreasing the half-maximal effect concentration without changing the maximal effect produced by GABA (571).

b) Barbiturates. The anesthetic and sedative properties of barbiturates are, at least in part, due to their agonist effect on GABA_A channel-dependent chloride...
fluxes. In presynaptic nerve preparations (synaptoneurosomes), it was found that their potency as an anesthetic agent is directly related to their ability to increase GABA_A channel-mediated chloride fluxes (758).

c) Steroids. Neuroactive steroids modulate transmitter release in peptidergic nerve terminals (1005) and rat hippocampal and spinal cord neurons (505, 877) by enhancing chloride currents elicited by GABA. At high concentrations, they activate the receptor-channel complex directly by increasing both the frequency and the duration of channel opening (670).

d) Picrotoxin. Picrotoxin is a noncompetitive antagonist of the GABA_A channel (1004). It binds preferentially to the agonist-bound receptor conformation at or near the chloride pore and stabilizes the desensitized state (798) in both presynaptic and postsynaptic forms of the channel (622, 1004). Another group of compounds interacting at least partially with the same binding site are the γ-butyrolactones. They act as agonists or partial antagonists (352, 519).

e) Ethanol. Ethanol and other short-chain alcohols potentiate GABA_A-mediated chloride currents in presynaptic nerve terminal preparations (850). During chronic intake of ethanol, there is a reduction of ~30% in the agonist-induced chloride influx (571).

f) Zinc and lanthanum. Zinc, a noncompetitive antagonist to GABA_A channel, was shown to increase transmitter release on prepyriform neurons in vitro (797). It blocks preferentially embryonic over adult channels by reducing the frequency of their opening (797). Lanthanides have been shown to potentiate GABA-mediated currents with a subunit dependence similar to that of BZ without being blocked by BZ antagonists (369, 495).

B) GABA_C RECEPTOR-CHANNEL COMPLEX. I) Functional properties. Although GABA_C receptor-channel complex was first described in 1975 (393), its classification as a GABA channel subtype was confirmed only in recent years. The channel and pharmacological properties differ from those of the GABA_A family of channels (93, 392, 952). The GABA_C channels were found in presynaptic nerve terminals in vertebrate retina (491) and seem to be involved in visual information processing by the modulation of transmitter release from bipolar cells (490, 672, 974, 1002; for review, see Ref. 490). The GABA_C channels were also found in rat anterior pituitary (97) and brain (391) and lobster thoracic ganglia (378).

II) Molecular biology of GABA_C receptor-channel complex. The functional channel is a pentamer consisting of five homologous membrane-spanning subunits. Three different subunits, named c 1–2, were cloned from different sources (13, 241, 612, 999). However, only two were shown to be involved in the functional channel, c 1–2. A third ρ-subunit was localized in brain by immunocytochemical methods, but its function is still unclear (241). Both ρ1 and ρ2 can form functional channels when expressed in Xenopus oocytes. Whether physiologically the channel is formed by an heteromeric or homomeric composition is controversial (392, 490).

III) Regulation of GABA_C receptor-channel complex. In rat retinal bipolar cells, the regulation of GABA_C channels was shown to include the phosphorylation by PKC via G proteins in the signal transduction cascade. Some of the metabotropic glutamate receptors, or the 5-HT2 receptor, activating G_o or G_i, appear to be linked to this regulatory pathway (250). Another study demonstrated that dopamine and adenylyl cyclase activators, such as forskolin, selectively reduced the GABA_C channel current, indicating that dopamine might modulate GABA_C channel function in the vertebrate nervous system (213).

IV) Pharmacology. The activity of GABA-dependent chloride channels, which are insensitive to the traditional GABA_A agonist and antagonists, was important in the discovery and characterization of the GABA_C channels family. The GABA_C channels are sensitive to picrotoxin (671) and insensitive to both bicuculline and baclofen (for general reviews, see Refs. 93, 391) but are sensitive to the GABA analogs cis-4-amino crotoconic acid (CACA) and trans-4-amino crotoconic acid (TACA). In general, the overall agonist potencies are TACA > CACA > GABA. Competitive antagonists include 3-aminopropyl(methyl)phosphinic acid, 3-aminopropylphosphonic acid, and 3-aminopropylphosphonic acid.

Most of the pharmacological studies on GABA_C channels have been performed in retina preparations or in Xenopus oocytes expressing the recombinant p1–2 subunits. On average, higher affinity for GABA was found in the C- than the A-subtype family (249, 251, 490, 671, 673). In hybrid bass retinal bipolar cells, the GABA_C channel-mediated presynaptic inhibition is decreased by zinc (672). A comparison between GABA_A and GABA_C properties is summarized in Table 15.

2. Glycine-activated chloride channels

It has been known for more than three decades that glycine is present in the CNS and can serve as an inhibitory transmitter (193, 954). Several lines of evidence support the existence of glycine-activated chloride channels at presynaptic nerve terminals. Glycine was shown to inhibit potassium-evoked transmitter release in cultured neurons (378).

<table>
<thead>
<tr>
<th>TABLE 15. Comparison of GABA_A and GABA_C properties</th>
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<tr>
<td>Channel Characteristics</td>
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<tr>
<td>Mean open time, ms</td>
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<tr>
<td>Desensitization</td>
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<td>Single-channel current, pA</td>
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* Measured in preparations other than nerve terminals.
Cerebellar granule cells (932). Glycine-gated chloride channels have been demonstrated in synaptoneurosomes (239). The application of glycine induced the influx of chloride, which was inhibited by the glycine postsynaptic inhibitor strychnine. The effects of strychnine and picrotoxin, a specific inhibitor of GABA_A channel, were additive, indicating the coexistence of the two channels.

3. Calcium-activated chloride channels

Calcium-activated or -dependent chloride currents were shown to be involved in the regulation of cell volume in a wide variety of cells (for review, see Ref. 351). In neurons, the activation of these channels may account for the decrease in axon excitability, thereby decreasing transmitter release and inhibiting repetitive firing. In presynaptic terminal of goldfish retinal bipolar cells, a tail current following depolarization was identified as a calcium-dependent chloride current, since it was suppressed by the intracellular application of the calcium chelator 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid and its reversal potential was very close to the equilibrium potential of chloride (619). In a detailed characterization of calcium-activated chloride channels in embryonic cultured spinal neurons from Xenopus, two kinds of calcium-activated chloride channels were described in the soma: the maxi- and the mini-chloride channels (365). The maxi-channels show numerous subconductance states, voltage-dependent inactivation above and below ±20 mV, mean conductance of 310 pS, and a reversal potential close to \( E_{\text{Cl}} \). The mini-channels show conductance ranging from 42 to 69 pS, and they are not voltage inactivated. It is suggested that the activation of these channels is mediated by a second messenger cascade activated initially by the elevation of intracellular calcium.

4. Chloride channels in fused synaptosomes

A voltage-dependent chloride channel was found in fused synaptosomes of the Torpedo electric organ. The mean conductance of this channel is 11.7 pS. The open probability of the channel increases with depolarization and acidity (227). Among the channels found in this preparation, the chloride channel is the second most abundant, with the first being the bursting potassium channel.

VII. PRESYNAPTIC LIGAND-GATED CHANNELS

A. Presynaptic Receptors

Receptors for neurotransmitters are not found exclusively on the postsynaptic membrane, where they mediate the response of the postsynaptic cell. Presynaptic nerve endings also possess a variety of receptors that are activated by the transmitter released from the same or adjacent terminals. The main function of these receptors is modulation of transmitter release and, consequently, modification of synaptic efficacy (for review, see Ref. 528).

Several criteria must be met to demonstrate clearly that a presynaptic receptor is involved in modulation of transmitter release. First, the receptor must be present at the presynaptic site. Obviously, the endogenous ligand needs to be present in the vicinity of the receptor to activate it. Furthermore, activation of the receptor must alter the release of neurotransmitter, and finally, this activation must be necessary and sufficient to account for the changes in transmitter release (528).

Various preparations and methods are being used in the study of presynaptic receptors. Anatomic localization of the receptors in different parts of the nervous system can be determined with the use of radiolabeled ligand or receptor-specific antibodies (291, 644). These techniques, however, do not always provide a resolution high enough to distinguish between presynaptic and postsynaptic structures.

The activity of ionotropic receptors can be assayed by measuring changes in \([Ca^{2+}]_i\) (130, 132) and ionic fluxes (466, 563, 564, 849) through the plasma membrane during pharmacological modulation of the receptors. With this approach, one cannot definitely rule out the effect of a retrograde messenger, diffusing from the postsynaptic site, unless the experiments are performed on the isolated nerve endings. In suitable preparations, electrical currents passing through the plasma membrane, following activation of the receptors, can be directly measured. This is feasible in large presynaptic terminals such as rat superior olivary complex (calyces of Held) (264), chick ciliary ganglion (827), secretory endings in neurohypophysis (380, 756, 1004–1007), cones in tiger salamander retina (652), or terminals of the goldfish bipolar neurons (47, 522).

Functions of the presynaptic receptors are usually assessed by the effect of a pharmacological activation or inhibition of the receptors on transmitter release. The release of neurotransmitter is usually estimated directly by measuring release of radiolabeled transmitter from isolated presynaptic terminals, referred to as synaptosomes (158, 261, 808), or in intact preparation (740, 869, 1008). Changes in neurotransmitter release can also be estimated indirectly from measurement of the postsynaptic response (52, 554, 645, 755, 772). In that case, presynaptic actions of applied pharmacological agents must be separated from their postsynaptic effects (e.g., alteration of the sensitivity of postsynaptic receptors). This is usually achieved by statistical analyses of the postsynaptic currents (295, 554, 645; see also Ref. 242).
1. Diversity of presynaptic receptors

Presynaptic receptors for virtually all neurotransmitters are found in the CNS (881). These can be classified as metabotropic and ionotropic receptors. Activation of metabotropic receptors triggers second messenger cascades, whereas ionotropic receptors are coupled to ion channels. We concentrate here on the latter category of receptors.

Ionotropic receptors to three neurotransmitters have been identified on the presynaptic nerve terminals: GABA receptors, glutamate receptors, and nAChR. An additional class of receptors, purinergic P₂ receptors, has also been demonstrated.

2. nAChR

Neuronal nAChR can be subdivided into two classes, based on their affinity to nicotine and to muscle nicotinic receptor antagonist α-bungarotoxin (α-BTX). One class binds nicotine with a high affinity and the other one has a higher affinity to α-BTX (528).

Neuronal receptors share many properties with their muscle siblings (originally described by Sakmann and co-workers, Refs. 590, 742). Both types are nonselective ion channels with similar electrophysiological characteristics (603, 828). These similarities suggest that two kinds of receptors may function in an analogous fashion.

Nicotinic receptors are widely distributed in the central and peripheral nervous systems. In the CNS they are not limited exclusively to cholinergic nerve endings but are also found on glutamatergic, dopaminergic, GABAergic, and serotonergic terminals. There is a strong evidence of the existence of presynaptic nicotinic receptors in rat striatum (195, 413, 690, 730, 744, 759), hippocampus (163, 203, 333, 873), hypothalamus (620, 621, 759), and cortex (344, 841). Presynaptic nicotinic receptors have also been demonstrated in goldfish retina (1011), mouse thalamus (459), and human cortical slices (599). In addition, axons in chick optic tectum may contain presynaptic nicotinic receptors (851).

In the peripheral nervous system, presynaptic nicotinic receptors have been demonstrated at neuromuscular junction (phrenic nerve terminals) of rat (177, 955, 957), mouse (420, 896, 925), and guinea pig (955); buccal ganglion of Aplysia (265); and nerve terminals in rat sublingual acini (1000).

A) MOLECULAR BIOLOGY AND STRUCTURE. Like their postsynaptic counterparts, the neuronal nicotinic receptors are proposed to consist of five subunits. These are mainly α- and β-subunits, with a ratio of 2 to 3 (173). Receptors composed of three different types of subunits, as well as homo-oligomers composed of only α-subunits, are also possible (301, 920). Eight α-subunits (α2–9) and three β-subunits (β2–4) have been characterized so far in different tissues and species (527, 751); human genes encoding α3,4,5,7- and β2–4-subunits have also been cloned (309). Thus the family of the neuronal nicotinic receptors seems to be quite diverse. With possible combinations of the subunits taken into account, the number of different receptors that may exist in the nervous system can reach thousands (722).

The subunits of the human receptor are polypeptides composed of 458–627 amino acids. They consist of four transmembrane domains with a long cytoplasmic region between domains 3 and 4. The whole structure is oriented so that the NH₂ terminal is on the outer side of the membrane (309). The three-dimensional structure of the neuronal (and presynaptic) nicotinic receptors has not yet been elucidated. On the basis of the similar electrophysiological properties, it is appealing to speculate that it may be similar to that of the muscle postsynaptic receptor, which has been determined at high resolution in both open (907) and closed states (906).

B) MODULATION. Various substances have been found to modulate the activity of the presynaptic nicotinic receptors. Both inhibitors and activators of the receptors have been described and are summarized in Table 16.

Neuronal nicotinic receptors can also be modulated by divalent cations calcium and lead. Extracellular calcium ions enhance the receptors activity (578, 921), whereas the effect of lead ions is twofold. Low levels (1 nM-100 μM) have an inhibitory effect, but higher concentrations tend to increase the activity of the receptors (1014). To the best of our knowledge, it is not known whether this modulation occurs at the presynaptic nerve terminals.

C) POSSIBLE PHYSIOLOGICAL ROLES. Unlike postsynaptic receptors, presynaptic nicotinic receptors most likely serve as modulators of transmitter release. Activation of several tens of receptors could significantly depolarize the terminal membrane and cause a significant change in intraterminal calcium concentration (828). Thus it seems likely that their main function both in central and peripheral nervous systems would be augmentation of transmitter release.

In the CNS, the majority of the nicotinic receptors are located presynaptically (970). Pharmacological activation of the receptors stimulates the release of various neurotransmitters in different anatomic locales: dopamine in rat striatum (195, 690, 730, 744), norepinephrine (163) and ACh (203) in rat hippocampus, GABA in mouse thalamus (459), and ACh in rat (344) and human (599) cortex. On the other hand, antibodies to nAChR from myasthenia gravis patients decrease ACh release from rat hippocampal nerve terminals (333). Acetylcholine may also act on nAChR located on corticotropin-releasing factor (CRF)-secreting terminals in rat median eminence to release CRF (621).

In the peripheral nervous system, the presynaptic nicotinic receptors are located at the cholinergic termi-
nals and hence serve as autoreceptors. They are likely to be involved in a positive-feedback regulation of ACh release. This is suggested by the effects of the pharmacological modulation of the presynaptic nicotinic receptors located at neuromuscular junction of rat (955, 957), mouse (925), and guinea pig (955); in rat sublingual mucous acini (1000); and in buccal ganglion of Aplysia (265).

3. Glutamate receptors

Glutamate activates three different classes of ionotropic receptors (reviewed in Ref. 285), each having a distinct pharmacology and physiology. The receptors are named according to the agonists that elicit specific physiological responses: N-methyl-D-aspartate (NMDA) receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and kainate receptors. In addition, glutamate can act on metabotropic receptors coupled to G proteins (307).

There are anatomic and physiological data supporting the existence of all three classes of presynaptic glutamate receptors in the CNS. Immunocytochemistry techniques have been used to demonstrate AMPA receptors on the nerve terminals in rat retina (644) and in hypothalamohypophysial tract in rat and monkey (291). Electrophysiological studies have shown that glutamate opens chloride channels in cones of tiger salamander and that this effect of glutamate is limited to presynaptic terminals (652, 750). Presynaptic kainate receptors in rat hippocampus have been shown to possess a dual action. Activation of the receptors causes a transient increase in transmitter release, followed by a depression of glutamatergic transmission (158). Activation of NMDA receptors in guinea pig cortex synaptosomes causes an increase in the release of glutamate and norepinephrine (562). Interestingly, this effect is decreased by inhibitors of nitric oxide synthase (562). This is not entirely surprising, however, since it is known that nitric oxide is necessary to sustain opening of the NMDA receptor ion channel (9). In rat hippocampus, AMPA and NMDA receptors probably coexist and increase norepinephrine release (687). The cooperation of these two receptors seems to be necessary for their proper function (658). In cultured chick retina cells, all three types are likely to coexist, since agonists to each type of ionotropic glutamate receptors increase GABA release (130).

A) MOLECULAR BIOLOGY OF A PRESYNAPTIC GLUTAMATE RECEPTOR. Similar to other ligand-gated ion channels, glutamate receptors are heteropentamers. The exact subunit composition is not completely known (for review, see Ref. 584). Recently, the transmembrane topology of the glutamate receptor subunit was elucidated. The subunit is a 439-amino acid polypeptide with a molecular mass of 48 kDa (969). The NH2 terminus is likely to be extracellular, and the COOH terminus is intracellular. The polypeptide contains three transmembrane domains M1, M3, and M4 (64, 969). The M2 domain forms a hairpin loop lining the intracellular pore of the channel. This configuration is similar to that of potassium channels (64, 971), suggesting the possibility of a common origin of these two superfamilies of ion channels (971).

B) PHARMACOLOGY OF PRESYNAPTIC GLUTAMATE RECEPTORS. The pharmacological properties of presynaptic glutamate receptors are studied with the agents known to affect postsynaptic receptors. We failed to find substantial differences between the pharmacology of post- and presynaptic receptors. The table in Reference 307 summarizes the pharmacological modulation of glutamate receptors.

C) POSSIBLE PHYSIOLOGICAL ROLES. Both facilitatory and inhibitory functions have been attributed to presynaptic glutamate receptors. The role played by a specific receptor is likely to depend on the species and on the location of that receptor in the CNS. The NMDA receptors have been demonstrated to increase release of ACh in mouse brain slices (1008), norepinephrine in rat hippocampus

<table>
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<tr>
<td>Trimetaphan</td>
<td>163</td>
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<tr>
<td>Chlorisodamine</td>
<td>163</td>
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</table>
synaptosomes (687) and slices (128), and norepinephrine and glutamate in guinea pig cortex synaptosomes (562). Of special interest is the possible involvement of NMDA receptors in long-term potentiation. Increased amounts of mRNA encoding a putative presynaptic NMDA receptors are detected for at least 5 h after induction of long-term potentiation in rat hippocampus (800).

The AMPA receptors cause an increase in the release of norepinephrine in rat hippocampal synaptosomes (659) and GABA in chick retina cells in culture (130).

Kainate receptors in rat hippocampus seem to play a dual role in the regulation of transmitter release. The initial effect of their activation is a transient increase in the release of glutamate, which is followed by a depression of neurotransmission (158).

In cones of tiger salamander retina, glutamate receptors cause a hyperpolarizing current (652), thus reducing the excitability of cells. Opposite effects are elicited by glutamate receptors in chick retina (130).

4. Purinergic P2 receptors

There is a growing body of evidence that ATP and other nucleotides can function as extracellular signaling molecules. They act on a large and diverse family of P2 purinoceptors, which can be divided into two distinct classes: ligand-gated ion channels (P2x receptors) and G protein-coupled receptors (P2y receptors) (155).

In rat hippocampal slices, the P2x receptor antagonist pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) reduces the amplitude of evoked EPSC. Inasmuch as PPADS did not reduce the amplitude of the postsynaptic currents elicited by direct application of glutamate, the presynaptic site of action for PPADS has been proposed (574). P2x receptors have been demonstrated in chick ciliary ganglion by Sun and Stanley (845). The P2x channels exhibited a conductance of 17 pS and increased by 10.220.33.2 on October 18, 2017 http://physrev.physiology.org/ Downloaded from

5-HT3 receptors has been demonstrated in various locations in the nervous system in different preparations. They were found in rat cortex (181), rat striatum (592), and human cortex (523). They have also been demonstrated on nonsynaptic cholecystokinin-releasing terminals in rat cortex and nucleus accumbens (640).

The pharmacological agents used to investigate presynaptic 5-HT3 receptors are those known to affect the receptors at other locations. We have failed to find substantial evidence of the agents specific for the presynaptic receptors. The classical agonists include 5-HT and its derivative 2-methyl-5-HT. Antagonists include medications used in the treatment of nausea and vomiting, such as tozacopride, ondasetron, tropisetron, dolasetron, and granisetron (for review, see Ref. 314). Newer agents include agonists 1-phenylbiguanide (523, 640) and antagonists (3a-tropanyl)-IH-indole-3-carboxylic acid ester and MDL-72222 (640).

Similar to other ligand-gated ion channels, 5-HT3 receptors are oligomers that are probably composed of five subunits. The exact composition of the receptors is not yet completely understood, but there is evidence supporting the existence of both homo- and heteromers (366). The receptor subunit is probably a polypeptide of 487 amino acids with a molecular mass of ~56 kDa. Hydropathy analysis predicts four transmembrane domains named M1 through M4. The sequence of the subunit exhibits some homology with nicotinic, GABA, and glycine receptors (511).

The function of the presynaptic 5-HT3 receptors is not clear. These ligand-gated ion channels are likely to act as heteroreceptors in the modulation of transmitter release. In some preparations, pharmacological activation of the receptors can cause an increase in intracellular calcium levels (592) and the release of cholecystokinin (640), whereas in others, 5-HT3 receptors mediate inhibition of transmitter release (523). The extent and physiological significance of this modulation are yet to be established.

B. ATP-Gated Potassium Channels

The ATP-gated potassium channels are inhibited by millimolar levels of intracellular ATP (35) and therefore open when the ATP concentration decreases below a certain limit. Thus they provide the means of coupling the electrical activity of the cell to its energy content. These channels were demonstrated originally in pancreatic β-cells (726), where they play a key role in the regulation of insulin secretion (for review, see Refs. 103, 889). They have been demonstrated in other cells as well, including cardiac myocytes, skeletal and smooth muscle cells, and neurons (for review, see Refs. 447, 631). Presynaptic ATP-gated potassium channels have been demonstrated in...
noradrenergic nerve terminals of rat cortex (452, 453, 865), in GABAergic terminals of rat substantia nigra (816, 950, 991), and in cholinergic mouse motor nerve endings (205). In fused presynaptic terminals from rat motor cortex, one ATP-gated potassium channel has been characterized with the use of patch-clamp technique (452). This channel has a conductance of 52.5 pS and demonstrates an inward rectification at voltages positive to −20 mV. Intracellular ATP at the concentration of 1 mM is sufficient to inhibit the activity of this channel. The channel’s open probability is independent of intracellular calcium, and it is blocked by 100 μM tolbutamide.

1. Pharmacology

Several agents have been found to modulate the behavior of ATP-gated potassium channels. The activity of the channels is inhibited by TEA in high concentrations (205) and by sulfonylureas such as glibenclamide and related agents (205, 288, 452, 866, 950, 991). The activators of the channels include cromakalim and its L-enantiomer lemakalim, a benzothiadiazine compound diazoxide, and its pyridinic analogs (288, 614, 657, 816, 865, 991). Experimental evidence has shown that the CNS possesses an endogenous inhibitor of ATP-gated potassium channels. This molecule, called endosulfine, has been isolated from porcine (924) and ovine (923) brain.

2. Molecular biology

Knowledge about the molecular biology of presynaptic ATP-gated potassium channels is based on studies of the gene encoding a similar channel in rat heart. This gene may encode presynaptic channels as well, since in situ PCR demonstrates its mRNA in multiple areas of peripheral nervous system and CNS. The putative ATP-gated potassium channel (or its subunit) is a 417-amino acid peptide that contains two hydrophobic, most likely transmembrane segments. These segments flank a putative pore region, homologous to other potassium channel pore sequences. The protein is proposed to be oriented so that the NH₂ and COOH termini are on the cytosolic side of the membrane. Electrophysiological characterization of channels expressed in human or hamster epithelial cells demonstrates that several properties of cloned channels are similar to those of native channels. The channel exhibits bursts of activity, interspersed among long silent periods, and has a conductance of 70.2 ± 20 pS. Single-channel current shows inward rectification even in the absence of internal magnesium, which becomes more prominent with the increase in magnesium concentration on the intracellular side of the channel. The open probability of the channel shows no significant dependence on membrane potential. The activity of the channel is inhibited by intracellular ATP, with a half-maximal inhibitory concentration of <100 μM. On the other hand, the pharmacology of the cloned ATP-gated potassium channel is different from that of the native channels. The channel is activated by pinacidil (30 μM); however, the application of sulfonylureas has no effect on the open probability of the channel. One possible explanation for this fact is that the sulfonylurea receptor is not a part of the channel molecule, but rather represents a separate molecular entity (36).

3. Possible physiological and pathophysiological roles

The ATP-gated potassium channels are likely to be involved in maintaining the membrane potential and the reduction of transmitter release when the energy content of the nerve terminal is low, namely, during ischemia, hypoxia, and hypoglycemia. Several lines of evidence support this hypothesis. Hypoxia has been demonstrated to cause a decrease of evoked norepinephrine release from rat cortical (866) and substantia nigra (950) slices. This decrease is abolished by the application of sulfonylureas. Moreover, hypoxia also increases the ⁸⁶Rb efflux. Linear correlation is observed between the evoked norepinephrine release, intracellular ATP concentration, and ⁸⁶Rb efflux, suggesting the role of ATP-gated potassium channels in reduction of transmitter release (866). The sulfonylureas tolbutilamide and glibenclamide block fast potassium component of presynaptic current and enhance the release of norepinephrine in rat cortical slices. These effects are more prominent under hypoxic and glucose-free conditions (205, 865). Because the activation of ATP-gated potassium channels during metabolic stress causes an outward current that would tend to hyperpolarize the terminal plasma membrane, these channels may play a neuroprotective role during anoxia and ischemia. Indeed, pharmacological activation of the channels has been demonstrated to reduce anoxic depolarization in hippocampal neurons, probably by means of reduction of glutamate release (62).

The role of the ATP-gated potassium channels under normal metabolic conditions is less clear. In mouse motor nerve endings, ATP-gated potassium current contributes a little (~8%) to the total potassium current in the nerve terminal (205). However, in rat substantia nigra slices, the application of glucose or sulfonylureas induces an increase in release under normal conditions (18). This finding suggests that some of the ATP-gated potassium channels are active and therefore may play a role in the regulation of transmitter release, under normal conditions as well.

VIII. OTHER CHANNELS

A. Nonselective Channels

A large, nonselective channel has been described in fused synaptosomes from the Torpedo electric organ.
section II

entry into the nerve terminal. This channel is discussed in calcium ions and thus may serve as a route of calcium. It is permeable to 6 unitary conductance of 850 (536). This channel is highly voltage dependent, has a voltage-dependent, inward rectification 536

- Voltage dependent, inward rectification 536
- Activated by intracellular Na+, voltage independent, not blocked by 1 mM Cs+, 5 mM TTX, 10 mM SITS, 10 mM quinine, 40 mM TEA 834
- Activated by intracellular Na+ 457
- Activated by depolarization and intracellular Ca2+ 457
- Activated by depolarization and intracellular Ca2+, inactivated by ruthenium red (100 mM), blocked by Co2+ (1 mM), not blocked by ryanodine (10 mM) 870

γ. Single-channel conductance; TTX, tetrodotoxin; TEA, tetraethylammonium.

(536). This channel is highly voltage dependent, has a unitary conductance of 850 ± 18 pS, and does not distinguish between anions and cations. It is permeable to calcium ions and thus may serve as a route of calcium entry into the nerve terminal. This channel is discussed in section II.

Two nonselective and voltage-independent ion-gated channels have been identified in the peptidergic nerve terminals of the crustacean neurohemal organ, the sinus gland (457, 834). Both channels do not distinguish between sodium and potassium. One has a unitary conductance of 69 pS and is activated by intracellular sodium (834); the other channel has a conductance of 213 pS and is activated by [Ca2+]i above 1 μM (457). A different, voltage-dependent, and calcium-activated nonselective channel was found in insect ganglia synaptosomes fused with liposomes (870). The properties of the nonselective channels are summarized in Table 17.

The physiological role of these nonselective channels is largely unclear. It has been proposed that the channels participate in sustaining prolonged activation of the nerve terminal during the burst activity. Activation of the channels evokes changes in intracellular ion concentrations, which reinforce further openings of the channels. This may help the nerve terminal to stay depolarized during the period of burst of activity (834). Although this hypothesis seems appealing, experimental evidence to support it is insufficient.

B. Stretch-Activated Channels

It is well known from the early work of Fatt and Katz in 1952 (247) that a stretch of the nerve muscle synapse produces an increase in spontaneous quantal transmitter release. In many cells, including secretory cells, it was found that stretch-activated ion channels of low selectivity exist (100, 370, 737). This prompted the suggestion that stretch-activated ion channels couple the length of the terminal, with its ability to release transmitter (154). However, the situation may be more complicated and may involve the participation of the cytoskeleton (153).

IX. CHANNELS IN SYNAPTIC VESICLES

A. Intracellular Ion Channels

In recent years, ion channels were discovered in the membranes of many intracellular organelles, where they play a crucial role in numerous cellular functions. Thus intracellular ion channels were found in skeletal and heart muscles (176, 279, 965), where they take part in the excitation-contraction coupling, and in the kidney (5), bone (82, 754), taste buds (472), and many other cell types (10, 11, 347, 565). In this section, we briefly describe the ion channels in one specific type of intracellular organelle, namely, synaptic vesicles in the presynaptic nerve terminals.

B. Methods for Study of Ion Channels in Synaptic Vesicles

The main problem in the study of ion channels in the membrane of the synaptic vesicle is their small size. The typical size of a patch pipette is a little less than 1 μm (see Ref. 743). Because most synaptic vesicles have a diameter of 50–90 nm (see Ref. 1010), it is impossible with the current technology to create a seal between a single synaptic vesicle and the patch pipette. Two main methods were used to overcome this difficulty: fusion of many synaptic vesicles into a large structure amenable to patch-clamp recording (202, 680) and incorporation of synaptic vesicles into planar lipid membranes (972). Each of these methods permits the study of ion channels but has obvious disadvantages.

The disadvantages of the fusion method are the usage of fusogenic substances (such as polyethylene glycol 1500 and DMSO) and the marked change in curvature. In addition, the intravesicular content is largely diluted during the fusion procedure.

The disadvantages of the incorporation method are the necessity to incorporate the vesicle into a large membrane of usually foreign lipids and the large increase in

### TABLE 17. Nonselective channels at presynaptic nerve terminals

<table>
<thead>
<tr>
<th>Preparation and Recording</th>
<th>γ, pS</th>
<th>Gating and Modulation</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fused <em>Torpedo</em> synaptosomes/patch clamp</td>
<td>850 ± 18</td>
<td>Voltage dependent, inward rectification</td>
<td>536</td>
</tr>
<tr>
<td>Crustacean sinus gland/patch clamp</td>
<td>75</td>
<td></td>
<td>834</td>
</tr>
<tr>
<td>Crustacean sinus gland/patch clamp</td>
<td>69 ± 4</td>
<td>Activated by intracellular Na+</td>
<td>457</td>
</tr>
<tr>
<td>Crustacean sinus gland/patch clamp</td>
<td>213 ± 6</td>
<td>Activated by depolarization and intracellular Ca2+</td>
<td>457</td>
</tr>
<tr>
<td>Insect ganglia synaptosomes fused with liposomes/patch clamp</td>
<td>136 ± 24</td>
<td>Activated by depolarization and intracellular Ca2+, inactivated by ruthenium red (100 mM), blocked by Co2+ (1 mM), not blocked by ryanodine (10 mM)</td>
<td>870</td>
</tr>
</tbody>
</table>

...
the curvature and the surface area. Because membrane capacitance is proportional to the surface area, the time constant of the membrane is large. Hence, fast transients are frequently concealed during the recording procedure.

C. Types of Ion Channels in Synaptic Vesicles

Ion channels were found in synaptic vesicles isolated from nerve endings of the Torpedo electric organ (202, 410, 602, 678, 679, 973, 980) and in vesicles isolated from neurosecretory endings of the hypophysis (451). They were also found in the membranes of other secretory cells (32, 823).

Six different types of ion channels have been identified to date (see Ref. 973). For one type of channel, the molecular structure is known at present, and it corresponds to synaptophysin (878).

Before speculating about the physiological roles of the ion channels in synaptic vesicles, it is important to note the permeability properties of these ion channels. Most of the ion channels described in synaptic vesicles are nonselective in nature and allow for the transport of different ions (602, 678, 878, 973, 980). In addition, chloride channels were found in synaptic vesicles (10, 980). Some of the channels are voltage dependent (980), and the channel at the neurohypophysial endings is calcium dependent (451).

D. Possible Functions of Ion Channels in Synaptic Vesicles

Synaptic vesicles have a complex life cycle, which includes synthesis, filling with transmitter (or transmitters), transport to the release part of the nerve terminal, docking at the active zone, activation, fusion with the surface membrane, release of the stored transmitter, retrieval of the vesicle into the cytoplasm, and refilling with transmitters. One can envisage a role of ion channels in almost every stage in the life cycle of the synaptic vesicle (248, 255, 339, 340, 636, 678, 683, 717, 760, 814, 839, 913, 926, 931, 1009, 1010).

E. Postfusion Hypothesis of Transmitter Release

Transmitter is released from the nerve terminals as preformed, multimolecular quanta (200, 247; for summary, see Refs. 60, 400, 428, 915). After it was found that the presynaptic nerve terminals possess vesicles (716), it was proposed that the vesicles form the structural basis for quantal release (201). Many observations over the last four decades confirm this hypothesis (see Refs. 139, 140, 337–339). However, the vesicle hypothesis for secretion is not devoid of problems, as summarized recently (683). The three main problems are the hyperosmolarity of the contents of the synaptic vesicle, the slow rate of release as measured by amperometry in mast cells, and the lack of complete fusion of the vesicle membrane with the surface membrane during quantal transmitter release (16, 159, 560, 589, 607, 812, 929).

In the past, it was proposed (911, 912) that charged transmitter molecules are stored in the synaptic vesicles in a bound form, bound to an ion-exchange matrix. For positively charged transmitters, such as ACh and 5-HT, the ion-exchange matrix has a negative charge. Proteoglycans and other charged polymers that were found in the synaptic vesicle (416, 434, 585, 813, 814) can subserve this role. Hence, there is a “hunger for cations” in the releasing synaptic vesicle. The positively charged transmitter molecules have to be replaced with cations to be released through the fusion pore. If this is the case, then the nonselective ion channels in the vesicle membrane may have a crucial role in quantal transmitter release. During the fusion process, the membrane of the vesicle changes its membrane potential according to the potential of the surface membrane. If the voltage change is in the range of the activation of the nonselective ion channels, then they open and cations enter the vesicle, release the transmitter from the ion-exchange matrix, and make it available for release through the fusion pore. Such a mechanism will have a limited value if a complete fusion occurs between the vesicle membrane and the surface membrane but will be of great importance if a temporary fusion, nicknamed “kiss and run” (255), happens. If such a sequence of events does take place, then a postfusion control of transmitter release is possible. Many questions have to be clarified, however, before the generality of the postfusion hypothesis can be accepted, such as the similarities and the differences in the fusion machinery at different sequestering sites, the existence of ion exchange molecules at synapses releasing negatively charged transmitters, the relative contribution of soluble counter ions, and the regulation and modulation of vesicle channels.

X. EPILOGUE

In his inaugural lecture delivered at the University of London on 31 January 1952, Sir Bernard Katz stated that “The story of the nerve endings is in itself a most fascinating chapter of physiology and is full of mysteries.” Now, almost half a century later, one of the mysteries, namely, the control of transmitter release by ion channels, is rapidly unfolding before our eyes. However, with the unfolding, the plot thickens. Who would have imagined just a few years ago that presynaptic nerve terminals have at least 12 different major categories of ion channels, representing at present several dozens of different ion channel types. It should come as no surprise if in the very
near future this number increases to hundreds. Each channel type in turn is composed of many different ion channel molecules. This enormous number of ion channels molecules will no doubt have a significant place in understanding normal synaptic communication and plasticity. We venture to speculate that they will be of major significance in understanding pathophysiological processes as well. If this speculation turns out to be correct, the possibilities of pharmacological interventions are immense. But then we do not envy the future reviewers of this topic. The present review is based on over 7,000 articles, most of them published in the last several years. What will the number be in the near future?

We thank our colleagues Daphne Atlas, Annette Dolphin, Halina Meiri, Izhak Nussinovitch, and Hannah Rahamimoff for reading parts of this article and for very useful comments. We thank Eyal Dahan, Emanuel Harari, and Yaron Nazarian for help in bibliographic search and graphics. The editorial assistance of Heather Rockman is greatly appreciated. The unflinching secretarial assistance of Galit Bousidan, Sharon Benzeno, and Sarita Rotaru is very much appreciated.

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REFERENCES

29. ARAUJO, D. M., P. A. LAPCHAK, B. COLLIER, AND R. QUIRION. Evidence that somatostatin enhances endogenous acetylcholine


52. AZOUZ, R., M. S. JENSEN, AND Y. YAARI. Ionic basis of spike after-depolarization and burst generation in adult rat hippocampal CA1 pyramidal cells. J. Physiol. (Lond.) 492: 211–223, 1996.


363. Israel, M., and B. LesBats. Continuous determination by a chemiluminescent method of acetylcholine release and compart-

425. KOMATSU, A., S. SINGH, P. RATH, and C.-F. WU. Mutational and gene dosage analysis of calcium-activated potassium channels in Drosophila: correlation of micro- and macroscopic cur-


426. KOMATSU, Y. GABAₐ receptors, monoamine receptors, and postsynaptic inositol triphosphate-induced Ca²⁺ release are in-


429. KOSTYUK, P. G., F. V. BELAN, and A. V. TEPHIKN. Free calcium molecules in a quantum: an estimate from iontophoretic applica-

tion through N-type calcium channels. J. Physiol. (Lond.) 492: 45–57, 1996.

430. KOYANO, K., T. ABE, Y. NISHIUCHI, and S. SAKAKIBARA. Effects of synthetic omega-conotoxin on synaptic transmission. 


432. KRETZ, R., E. K. DIXON, and E. R. KANDEL. Post-tetanic poten-


433. KUHN, D. M., W. VOLKNANDT, H. STADLER, and H. ZIMMER-

mann. Cholinergic vesicle-specific proteoglycan: stability in iso-

lated vesicles and in synaptosomes during induced transmitter 


434. KUHN, D. M., W. VOLKNANDT, H. STADLER, and H. ZIMMER-

mann. Cholinergic vesicle-specific proteoglycan: stability in iso-

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435. KUHSE, J., H. BETZ, and J. KIRSCH. The inhibitory glycine recep-


436. KUHSE, J., H. BETZ, and J. KIRSCH. The inhibitory glycine recep-


437. KUO, C. C., and B. P. BEAN. G-protein modulation of ion perme-


438. KUHN, D. M., W. VOLKNANDT, H. STADLER, and H. ZIMMER-

mann. Cholinergic vesicle-specific proteoglycan: stability in iso-

lated vesicles and in synaptosomes during induced transmitter 


440. KUFUSS, W. S., and D. YOSHIMOTO. The number of transmitter molecules in a quantum: an estimate from iotophoretic applica-


441. KUHN, D. M., W. VOLKNANDT, H. STADLER, and H. ZIMMER-

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mann. Cholinergic vesicle-specific proteoglycan: stability in iso-

lated vesicles and in synaptosomes during induced transmitter 


638. PASSAFARO, M., P. ROSA, C. SALA, F. CLEMENTI, AND E. SHER. N-type Ca\(^{2+}\) channels are present in secretory granules and are transiently translocated to the plasma membrane during regulated exocytosis. J. Biol. Chem. 271: 30096–30104, 1996.


669. RAHAMIMOFF, R., AND F. COLOMO. Inhibitory action of sodium...


715. ROBITAILLE, R., M. L. GARCIA, G. J. KACZOROWSKI, AND M. P. CHARLTON. Functional colocalization of calcium and calcium-


TRUBE, G., J. HESCHELER, AND K. SCHROTER. Regulation and...


904. TSUDA, K., S. TSUDA, M. GOLDSTEIN, AND Y. MASUYAMA. Inhibition of the delayed outward \( K^+ \) current by 10.220.33.2 on October 18, 2017 http://physrev.physiology.org/ Downloaded from


