PRESYNAPTIC RECEPTORS

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ABSTRACT
Activation of different types of G-protein-linked and ionotropic presynaptic receptors has been shown to regulate neurotransmitter release throughout the central and peripheral nervous systems. In the case of G-protein-linked receptors, three major mechanisms have been suggested: (a) inhibition of Ca channels in the nerve terminal; (b) the activation of presynaptic K channels, resulting in a reduction in the effectiveness of the action potential; and (c) direct modulation of one or more components of the neurotransmitter vesicle release apparatus. In the case of ionotropic presynaptic receptors, inhibition of release may be achieved through depolarization of the terminal and inactivation of Na and Ca channels. Activation of presynaptic ionotropic receptors that are appreciably Ca permeable can also enhance the release of transmitters as a result of their ability to raise \([\text{Ca}^2+]_i\) in the terminal directly. Many transmitters employ several of these mechanisms, thus allowing considerable flexibility in the presynaptic regulation of transmitter release.

INTRODUCTION
Neurons communicate with each other through the release and subsequent action of neurotransmitters. The efficiency of the transmitter release process can be influenced by a number of factors that allow the strength of synaptic communication to be constantly modulated. Presynaptic receptors are particularly important in this regard. There are numerous studies in vertebrates and invertebrates demonstrating that the presynaptic terminal of a neuron may possess receptors for the same neurotransmitter that it releases. Activation of these...
receptors closes a feedback loop, which results in either the enhancement or inhibition of transmitter release. Such presynaptic receptors are usually known as autoreceptors. In addition, the presynaptic terminal can possess receptors for transmitters that are released from other neurons in the vicinity, and activation of these can also influence the release process. Such receptors are generally known as heteroreceptors. It is likely that activation of presynaptic receptors helps to shape the strength of neurotransmission at virtually every synapse (1–4). Thus, these receptors are of considerable importance both from the neurophysiological point of view and also as potential targets for therapeutic agents.

How does the activation of presynaptic receptors produce changes in the release of neurotransmitters? In this article, I examine some of the major possibilities. For reasons of space, I restrict the discussion to the vertebrate nervous system.

MOLECULAR ASPECTS OF TRANSMITTER RELEASE

In order to understand the way in which activation of presynaptic receptors modulates neurotransmitter release, it is important to review, at least in outline, some of the molecular events that underlie the release process (5–7).

Neurotransmitter-containing vesicles are thought to be localized at specialized release sites, or active zones, on the presynaptic membrane, in a state of readiness so that exocytosis can occur rapidly when required (5–8). Initially, vesicles are targeted (docked) at release sites, although the precise details as to how this happens are not well understood. Once docked, however, synaptic vesicles are primed so that they can respond by rapid exocytosis to the appropriate stimulus (normally a highly localized Ca influx). The priming process involves the formation of a multiprotein complex that tethers the secretory vesicle to the plasma membrane in close juxtaposition to other important signaling elements. This complex involves proteins from the vesicle membrane and the presynaptic neuronal plasma membrane, as well as other elements that help to link the two together (5, 6). Some of the proteins also have properties that allow them to respond to the Ca signal that triggers exocytosis (9). Although a complete discussion of all the components of the complex is beyond the scope of this article, several of the components are particularly relevant. Initially, a core complex is formed between the plasma membrane proteins syntaxin 1A and SNAP-25 and the vesicle membrane protein synaptobrevin with a 1:1:1 stoichiometry (5–7, 10). These three proteins provide a high-affinity receptor scaffold for the protein α-SNAP, and its arrival then allows the recruitment of NSF (N-ethylmaleimide-sensitive ATPase). ATP hydrolysis, together with dissociation of NSF/α-SNAP, may now cause partial fusion of the vesicle with the
plasma membrane, thus readying it for final release. Also brought into close juxtaposition at this point are the Ca channels that provide the final Ca trigger for exocytosis (10–15) and the putative Ca sensor molecule on the vesicle membrane—the protein synaptotagmin (16–18). Other elements that may be important in the final release of synaptic vesicles are the small GTP binding protein, rab3A (19–22), and the local generation of fusigenic inositol phospholipids that can bind to synaptotagmin (23).

It is particularly interesting to consider the interaction of Ca channels with the rest of the complex. At least six different $\alpha_1$ pore-forming Ca channel subunits have been identified (24, 25). All of these appear to share the same basic architecture, as well as many basic functional properties. They also exhibit important differences in the way they are regulated that are consistent with their diverse functions. Not all Ca channels appear to be concerned with the regulation of neurotransmitter release. However, the channels formed by the $\alpha_{1B}$ (N-type) and $\alpha_{1A}$ (P/Q-type) subunits, together with their appropriate ancillary subunits, are clearly important in this regard (4, 26). This being the case, it is interesting to note that both of these $\alpha_1$ subunits can form specific associations with the vesicle release complex (11–15). This interaction occurs between a specific region of the large intracellular loop connecting domains 2/3 of the $\alpha_1$-subunits and the presynaptic membrane protein syntaxin 1A. In particular, binding appears to occur between the transmembrane region of syntaxin 1A and a specific region of the intracellular loop of the $\alpha_1$-subunit designated the synprint region (synaptic protein interaction site) (27–31). This region can also interact with SNAP-25 (27–31), and possibly with other members of the vesicle secretory complex as well. Indeed, this same loop, which contains the most divergent sequences between $\alpha_1$-subunits, appears to be generally significant in determining downstream targeting of Ca channels to important effector molecules. For example, the targeting of Ca channel $\alpha_1$ subunits to ryanodine receptors in skeletal muscle is also provided by analogous interactions (32).

How is transmitter release finally achieved? The sequence of events appears to be approximately as follows. Upon depolarization of the nerve terminal, the Ca channels that have been appropriately targeted to release sites open, rapidly elevating the local submembrane [Ca], to values of $>100$ $\mu$M (5, 7). This Ca can then interact with the appropriate Ca-sensing molecule on the partially fused vesicle membrane (probably synaptotagmin), allowing the ultimate fusion of the vesicle and release of its contents. Ca simultaneously produces other effects on the complex of proteins assembled in association with the vesicle (9). For example, the interaction between Ca channels and syntaxin 1A is enhanced as the local Ca concentration climbs to about 10 $\mu$M or so but is then reduced at higher [Ca], which may facilitate final vesicle exocytosis (29, 30). The
association between syntaxin 1A and Ca channels is not only important from the point of view of bringing these elements into close juxtaposition at release sites, but it also has important functional effects on the behavior of Ca channels, as I discuss below.

MECHANISMS OF PRESYNAPTIC INHIBITION

How does activation of presynaptic receptors modulate the release process? Presynaptic receptors can be of several types, including members of both the G-protein-linked (metabotropic) (4, 33) and multisubunit ion channel (ionotropic) families (34). Activation of both types of receptors can regulate transmitter release in different circumstances. There are several major explanations of how presynaptic receptors could work.

1. Inhibition of Ca channels. We know that Ca influx through appropriately localized channels is the major trigger for the final release of synaptic vesicles. Thus, changes in Ca influx due to regulation of these channels should certainly influence release at some point (4). Indeed, as I discuss in detail below, activation of many G-protein-linked receptors has been shown to influence Ca channel activity.

2. Activation of presynaptic ion channels. Stimulation of a presynaptic receptor could activate a conductance in the nerve terminal (e.g. a K or Cl channel), thereby “shunting” the action potential in this region of the neuron (35–37). Activation of such a presynaptic conductance would reduce the duration and amplitude of the action potential in the presynaptic terminal, Ca influx would be reduced, and less transmitter would be released. Alternatively, activation of Na- or Cl-permeable presynaptic channels might depolarize the terminal sufficiently to inactivate voltage-dependent Na and Ca channels, thereby blocking propagation of the action potential into the terminal or its local influence (38).

3. Regulation of the vesicle release complex. As I have discussed above, the final steps that lead to the release of synaptic vesicles involve a complex of proteins, including several Ca-sensitive components. Activation of presynaptic receptors could signal to one or more of these proteins and influence the release process at a point distal to Ca entry.

Evidence exists that all of these mechanisms are actually used. Indeed, sometimes several strategies may be employed simultaneously.
G-PROTEIN-LINKED RECEPTORS

Here, I first consider the ways in which G-protein-linked receptors might influence transmitter release. The greatest variety of presynaptic receptors likely fit into this category. Even in cases in which important presynaptic actions of a transmitter are produced by activation of ionotropic receptors (e.g. GABA), presynaptic G-protein-linked receptors for the same transmitter are also likely to be important. In virtually all cases, activation of presynaptic receptors of this type leads to a reduction in evoked transmitter release, thereby closing a negative feedback loop.

Regulation of Presynaptic Ca Channels

THE MECHANISM OF Ca CHANNEL INHIBITION Ca influx is the ultimate trigger for the release of neurotransmitters. Although the relationship between Ca influx and release is complex (7), a reduction in Ca influx must, at some point, impact transmitter release. If Ca channels are to be targets for the inhibition of neurotransmitter release, it would be important for the relevant types of Ca channels to be inhibited. These would be the classes of Ca channels that have been shown to be localized at release sites and most closely connected with the release process (see above). In general the release of neurotransmitters appears to be under the influence of both N and P/Q types of Ca channels (4, 26, 34–65). The basic observations that support this contention concern the ability of toxins and drugs that selectively target these Ca channels (e.g. \(\omega\)-conotoxin GVIA for N-type and \(\omega\)-agatoxin IVA for P/Q-type) to inhibit transmitter release, whereas drugs and toxins that block L-type channels (e.g. dihydropyridines) are generally ineffective (4, 34–65). There are, naturally, some exceptions to this rule, and instances can be found where dihydropyridines inhibit release (46). Furthermore, in some circumstances Ca channels that have not yet been fully identified and characterized also seem to influence release (43). Nevertheless, the key roles of N and P/Q channels have been clearly illustrated in numerous instances and so will serve as the basis for discussion.

Activation of many G-protein-linked receptors has been shown to produce inhibition of N- or P/Q-type Ca currents in whole-cell voltage-clamp experiments (e.g. 3, 4, 47–49). Several mechanisms can apparently transduce the signal between the receptor and these Ca channels. Let us start by discussing the pathway that has been most fully characterized. In this situation, activation of a receptor changes the gating properties of Ca channels so that they are no longer opened by depolarizations in the normal range, although very strong depolarizations can still produce channel opening (49–52). Depending on the paradigm employed, this results in slowing of the rate of current activation and/or steady state inhibition of the current. The kinetic slowing and inhibition
can be relieved by a strongly depolarizing prepulse, and it has been suggested that this may be of physiological significance. For example, it raises the possibility that inhibition might be relieved during a rapid train of action potentials (53). Such a phenomenon would help to explain the “use dependence” commonly associated with presynaptic inhibition (54, 55). Thus, it has frequently been demonstrated that presynaptic inhibition is less effective when neurons are firing more rapidly. However, when this proposal has been formally tested, the data indicate that Ca channel inhibition would not be significantly alleviated by firing rates in the normal range (56, 57). Thus, the use dependence of presynaptic inhibition probably has a different explanation (58).

How is Ca channel inhibition produced? Activation of the receptor initially leads to activation of heterotrimeric G proteins. The first question to be answered therefore is how the G protein influences the behavior of the Ca channel. Given the fact that no diffusible second messenger seems to be involved (47), it is likely that the G protein exerts its influence by directly binding to the channel. Experiments using heterologous expression systems suggest that it is the β/γ subunits of G proteins that are responsible for transducing the signal (59–61). Thus, the G-protein-mediated modulation of Ca channels is similar in this regard to the regulation of the GIRK family of K channels by receptors and G proteins—a process that also involves signal transduction via G-protein β/γ subunits (62). Indeed, several G-protein-mediated effects are now known to be produced in this way (63).

Initial attempts to identify the point of interaction between β/γ subunits and Ca channels have suggested that the binding domain is located in the first intracellular loop that connects domains 1 and 2 of the α1 subunit, the major pore-forming subunit of the Ca channel (64, 65). Indeed, there is little doubt that β/γ subunits can interact with Ca channels at this site, as suggested by both mutagenesis and biochemical studies (64–66). This intracellular loop contains a motif (QXXER) that has been shown to be involved in the binding of G-protein β/γ subunits to several other effectors as well, including GIRK-like K channels (63). This motif is present in α1B, α1A, and α1E, the dihydropyridine-insensitive Ca channels, all of which seem to be modulated by G-protein β/γ subunits to some extent (59–61). On the other hand, the motif is absent from the dihydropyridine-sensitive Ca channels (α1C, α1D, and α1S), which do not seem to be directly modulated by G proteins (67, 68). Synthetic peptides from the 1/2 loop that include this sequence seem to reduce G-protein-mediated inhibition of α1A- or α1B-based Ca channels in the predicted manner, and, in some reports, mutations in this sequence interfere with the effects of G proteins. This supports the proposal that β/γ binding at this site mediates G-protein inhibition (64–66).

Nevertheless, the story appears to be far from complete. One should consider, for example, experiments in which the 1/2 linker has been exchanged between
different $\alpha_1$ subunits. It has been shown that although $\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1E}$ are all inhibited by G proteins, they differ considerably in terms of their sensitivity. Thus, $\alpha_{1B}$ is the most sensitive and $\alpha_{1E}$ the least (59–61, 67–69). Chimeric Ca channels have been tested in which the 1/2 linker from $\alpha_{1A}$ or $\alpha_{1B}$ has been inserted into an $\alpha_{1E}$ background (70), the 1/2 linker from $\alpha_{1B}$ has been inserted into an $\alpha_{1A}$ background (64), or the 1/2 linker of $\alpha_{1A}$ or $\alpha_{1C}$ has been inserted into an $\alpha_{1B}$ background (68). The results of these experiments are quite inconsistent with one another and range from the inserted 1/2 linker resulting in a large change in G-protein inhibition (64), a small change (70), or no change at all (68). Furthermore, $\beta/\gamma$ subunits also seem to bind to more than one site on responsive Ca channel $\alpha_1$ subunits (65). Thus, DeWaard et al (65) demonstrated that there is a second binding site in the 1/2 linker that has an even higher affinity for the $\beta/\gamma$ subunit. Interestingly, this site does not possess the QXXER binding motif. In addition, Zhang et al (68) demonstrated that portions of the $\alpha_1$ subunit that are outside the 1/2 linker also appear to be important in mediating G-protein inhibition. Consistent with this, a $\beta/\gamma$ binding site has now been identified in the C-terminal tail of $\alpha_1$-subunits (71). Thus, the precise way in which $\beta/\gamma$ binding is transduced into channel inhibition is still incompletely understood.

The idea that the QXXER-containing binding site in the 1/2 linker is an important element in the $\beta/\gamma$ effect is interesting for at least two reasons. First, this site overlaps with the binding site for the Ca channel $\beta$-subunit (72). This suggests that Ca channel $\beta$-subunits and G-protein $\beta/\gamma$ subunits may be able to interfere with their respective interactions with Ca channel $\alpha_1$-subunits. Such a proposal is consistent with observations that the effects of Ca channel $\beta$-subunits and of G proteins on channel function are, in many ways, the opposite of one another (65, 73, 74). Secondly, Zamponi et al (64) have demonstrated that this putative $\beta/\gamma$ binding site is a substrate for protein kinase C–mediated phosphorylation. Phosphorylation at this site may be responsible for the observed uncoupling of receptor-mediated inhibition of N channels by phorbol esters (75) and may be of physiological significance in this regard.

The specific localization and clustering of Ca channels at sites of neurotransmitter release (see above) may also have important implications for Ca channel regulation. For example, there is evidence that the interaction with the protein syntaxin 1A, in addition to helping to locate Ca channels to the appropriate place, has a functional effect. Coexpression studies have demonstrated that syntaxin 1A can exert an inhibitory effect on N- and P/Q-type Ca channels through an interaction between the membrane spanning domain of the syntaxin 1A molecule and the large intracellular loop connecting domains 2 and 3 of Ca channel $\alpha_1$ subunits (76, 77). This interaction may be modulated by other components of the vesicle docking complex, such as synaptotagmin and SNAP-25 (77, 78) or by phosphorylation (31). It has also recently been demonstrated
that cleavage of syntaxin 1A using botulinum toxin prevents the regulation of N channels by G proteins (73). Such functional interactions between docking complex proteins and Ca channels may represent a mechanism by which these proteins not only help to juxtapose vesicles and the source of incoming Ca, but also ensure that channels behave in the appropriate manner, i.e. receptors will modulate only appropriately located channels.

The mechanism of Ca channel modulation described above represents a tightly coupled arrangement that makes excellent sense in terms of organizing the local feedback inhibition of release from the nerve terminal. It is clear, however, that other mechanisms of Ca channel regulation can occur (47). In these instances Ca current inhibition does not necessarily exhibit the kinetic slowing and voltage dependence described above. In some cases one or more diffusible second messengers have been shown to be involved in the signal transduction pathway, although the identities of these messengers have not been generally determined. A receptor may modulate Ca channels by recruiting several pathways involving the activation of more than one G protein. The case of bradykinin serves as a good example of this. It has been shown that bradykinin can inhibit Ca currents in dorsal root ganglion (DRG) neurons and in certain neuronal cell lines (80, 81). Belardetti and colleagues demonstrated that in the NG108-15 cell line, bradykinin inhibits Ca channels using at least three different pathways, two of which seem to require the participation of intracellular Ca (81). In addition, a third pathway inhibits Ca channels using at least three different pathways, two of which seem to require the participation of intracellular Ca (81). In addition, a third pathway seems to signal through activation of the heterotrimeric G-protein G_{13} and subsequently through small G proteins of the Rac1 type (82). It is likely that this then feeds into the MAP kinase (MAPK) pathway, but exactly what happens after that is unclear. The situation with bradykinin also highlights the fact that the receptor regulation of neuronal Ca channels is not necessarily solely concerned with the feedback inhibition of transmitter release. In DRG neurons, for example, the effects of bradykinin likely lead to a reduction in spike accommodation through effects onIK_{Ca} and subsequently to increased transmitter release in the spinal cord. In this way effects on Ca channels may underlie the hyperalgesic actions of bradykinin (80).

LOCALIZATION OF Ca CHANNEL INHIBITION The vast majority of electrophysiological studies on Ca channels have been carried out on cultured neurons or acutely isolated nerve cell bodies. In some instances attempts have been made to correlate effects on cell body Ca currents with transmitter release from the same neurons (83, 84). However, the question has naturally arisen as to whether observations in the cell body really reflect events occurring in the nerve terminal, which is, after all, the principal site of neurotransmitter release. Although it is much harder to perform studies on nerve terminals, data of various types
indicate that Ca channels in terminals are modulated by receptors in the same way as indicated above for the cell soma.

Evidence suggests that there are different types of Ca channels in nerve terminals. The most direct evidence comes from studies using specific antibodies that indicate the presence of the \( \alpha_{1A} \) and \( \alpha_{1B} \) Ca channel subunits in nerve terminals (85, 86). Similar conclusions come from studies using fluorescently labeled toxins that target different channel types (87). In addition, many studies have examined the effects of specific drugs and toxins on transmitter release from intact neurons and synaptosomes (3, 26). The results of these studies generally show that either \( \omega \)-conotoxin-GVIA or \( \omega \)-agatoxin-IVA can block release, to varying degrees, in different preparations, whereas dihydropyridines are usually ineffective. Some studies have sought to determine how channels are distributed among different synapses. For example, Reuter examined this question at synapses between cultured rat hippocampal pyramidal neurons by using the dye FM1-43 to measure the exocytosis of vesicles from individual nerve terminals together with \( \omega \)-conotoxin-GVIA and \( \omega \)-agatoxin-IVA to block N and P/Q channels, respectively (88). Although both of the toxins used were partially effective at blocking evoked glutamatergic excitatory postsynaptic potentials (EPSPs) at these synapses, the two types of Ca channels proved to be arranged differently. Whereas P/Q channels were fairly evenly distributed at most nerve terminals, the distribution of N channels was much more heterogeneous. Thus, the ratio of channel types differed considerably at individual terminals. Although there are some quantitative differences between the results of this study and more conventional electrophysiological investigations (e.g. see 89), there is a general consensus that the distribution of Ca channels among terminals is not homogeneous and that most terminals contain mixtures of channels rather than a single type. The use of selective channel blockers has also indicated that the efficiency of coupling of different types of Ca channels to transmitter release depends on the situation. For example, Mintz et al concluded that at the parallel fiber/Purkinje cell synapse in the cerebellum, P/Q channels were more “efficiently” coupled to transmitter release than were N channels (62); whereas at the CA3/CA1 synapses of the hippocampus, Wu & Saggau found that the two channel types were equally well coupled to transmitter release (90).

Are Ca channels in nerve terminals modulated by receptors in the same way as channels in the cell body? This is not an easy question to answer, as it is obviously difficult to access nerve terminals in the same way as the soma. Nevertheless, such experiments have been performed by using advantageous preparations. Channel function in terminals has been assessed both electrophysiologically and by measuring Ca flux directly through channels using imaging paradigms. At calyx-like giant synapses in chick ciliary ganglia (15, 79, 91) and the rat brain stem calyx of Held (92, 93), measurement of Ca currents in
presynaptic terminals has been possible. Transmitter release has been found to be mostly dependent on N and P/Q channels, respectively, at these two synapses. Activation of presynaptic receptors for somatostatin (92), adenosine A1 (91), or glutamate (mGluRs class 3) (92) inhibited the Ca currents and transmitter release in each instance. At rat sympathetic neuroeffector junctions between sympathetic neurons and cardiac myocytes, presynaptic Ca influx (mostly via N channels) at single nerve terminals was inhibited by neuropeptide Y (NPY), as was transmitter release at the same synapses (95). At populations of guinea-pig hippocampal CA3/CA1 synapses (96–99) and granule cell/parallel fiber–Purkinje cell synapses in the cerebellum (100), presynaptic Ca influx via N and P/Q channels, as well as the “residual” Ca influx, was inhibited by activation of adenosine A1, GABA_B, or NPY receptors, whereas activation of muscarinic receptors specifically targeted Ca influx via N channels. In all of these instances transmitter release was also shown to be inhibited in parallel with inhibition of Ca flux. Results of studies on the hippocampus demonstrate that activation of adenosine A1 receptors and GABA_B receptors inhibits Ca currents in the neuronal soma as well as transmitter release (83, 84). Thus, in the hippocampus, at any rate, a correlation exists among effects of A1 and GABA_B agonists in the soma, the presynaptic terminal, and the release of transmitter.

Regulation of Presynaptic K Channels

Another mechanism by which presynaptic G-protein-linked receptors might influence transmitter release is through the activation of some conductance (e.g. a K or Cl channel) in the presynaptic terminal. Sufficient numbers of open channels could have the effect of “shunting” the incoming action potential so that it was less effective in depolarizing the terminal; thus, fewer Ca channels would open, thereby shortening the Ca spike, and less Ca influx would ensue (35–38). A variation on this theme is that activation of presynaptic currents would result in an actual failure of the action potential to invade the terminal. However, whether activation of presynaptic K currents by G-protein-linked receptors is really a widely used mechanism for producing presynaptic inhibition by this, or any other mechanism, is less clear—at least in the vertebrate nervous system. In contrast, the participation of K channels in the presynaptic regulation of transmitter release at invertebrate synapses is much better established (101). It is certainly the case that many G-protein-linked receptors can activate inwardly rectifying K conductances in neurons and possibly other types of K channels as well (42, 102). Such effects often underlie neurotransmitter-induced neuronal hyperpolarization/inhibition, of which there are many examples (103). The inwardly rectifying K channels involved are probably members of the GIRK/CIR family (104). Activation of these channels by neurotransmitters is probably an important method for controlling neuronal excitability (103).
Indeed, animals lacking GIRK-like channels are prone to seizures (105). The mechanism by which receptors activate GIRK-like channels has been shown to involve G-protein β/γ subunits that bind directly to one or more sites on the GIRK molecule (106). Thus, the molecular mechanisms involved appear to have much in common with the receptor-induced inhibition of Ca channels, discussed above. Indeed, at least one of the β/γ binding sites on the GIRK channel also contains the same motif (QXXER) as the β/γ binding site on the first intracellular loop of N and P/Q Ca channel α₁ subunits (see above; 63). One study has suggested that the GIRK1 subunits in the hippocampus are predominantly found in the dendrites and dendritic spines of pyramidal cells, rather than in presynaptic terminals (107), although other studies with GIRKs 1–4 have also localized them to terminal fields (108–110).

The possible role of K current activation in presynaptic inhibition has been mostly studied in the hippocampus, but evidence for such a mechanism here and elsewhere is limited. The type of experiment most frequently performed is to examine the effects of Ba. The idea here is that because GIRKs are inhibited by Ba, presynaptic inhibition should also be inhibited by Ba if these K channels are involved. In some studies, such as those on the effects of the GABA_B receptor agonist baclofen in the hippocampus, this is the case (111). Thus, inhibition of GABAergic inhibitory postsynaptic potentials (IPSPs) by baclofen in the CA3 (but not in CA1) and by dopamine in the nucleus accumbens is blocked by Ba (112, 113). In contrast, the effects of baclofen on EPSPs, and of several other neurotransmitters on excitatory and inhibitory synaptic events throughout the hippocampus (and other parts of the brain), are unaffected by Ba (111, 113, 114, but see 115). Furthermore, Luscher et al (116) showed that presynaptic inhibition produced by GABA_B and several other types of agonists was unaltered in GIRK2 knockout mice. In a slightly different example, Simmons & Chavkin demonstrated that κ-opioid agonist–mediated inhibition of neurotransmitter release from mossy fiber terminals in the hippocampus was unaffected by Ba but was inhibited by dendrotoxin, which is a blocker of the Shaker Kv1 class of K channels (117). Whether this latter mechanism is widely used in the nervous system and what the signal transduction pathway leading to channel activation might be is unclear at this time. In summary, data using K channel blockers have not really supported the idea that K channels play a wide-ranging role in mediating presynaptic inhibition.

A further argument suggesting that effects on K channels may not be generally important comes from observations on the selectivity of the effects of receptors on different components of the presynaptic Ca flux. Thus, presynaptic Ca influx can be divided into different components based on the effects of different Ca channel blockers (see above). Activation of presynaptic receptors inhibits these different components to varying degrees. For example, in the
hippocampus, activation of adenosine A1, GABA_{B}, or muscarinic receptors seems to more effectively inhibit Ca influx through N rather than through P/Q channels (4, 96, 97, 118). At cerebellar parallel fiber/Purkinje cell synapses, activation of adenosine A1 or GABA_{B} receptors preferentially targets N and P channels rather than the “residual” Ca influx (4, 98). A similar result was obtained for the effects of NPY at sympathetic neuroeffector junctions (95). In contrast, inhibition of the residual Ca influx by presynaptic metabotropic glutamate receptors was found to be favored at excitatory synapses in the nucleus of the solitary tract (119). It has been argued (e.g. in 4) that if the major effect of presynaptic receptor activation was to hyperpolarize the presynaptic terminal, then each component of the Ca influx should be equally inhibited. Because this was not found to be the case, it follows that, at least in the instances investigated to date, activation of a K conductance does not play a major role.

In conclusion, considering the anatomical data on the localization of GIRK1, the data on Ba sensitivity, and the data on Ca influx, not much evidence links presynaptic inhibition at vertebrate synapses with the activation of K conductances.

**Direct Modulation of the Release Machinery**

A third possible way in which activation of presynaptic receptors might influence transmitter release is by directly influencing some component of the vesicle release/exocytosis complex. As I discussed above, the release complex contains many proteins that seem to be important in the docking, priming, and ultimate release of neurotransmitter vesicles. Activation of a receptor could alter the properties of one or more of these proteins and inhibit release in this manner. That this can indeed be the case was first demonstrated by Silinsky, studying transmission at frog motor neuron/skeletal muscle synapses (120). Silinsky observed that the evoked release of the transmitter (acetylcholine) at this synapse could be reduced by activation of adenosine A1 receptors acting by a presynaptic mechanism. Further analysis led to the conclusion that the major site of action of adenosine analogues was intracellular, i.e. beyond or downstream of the point of Ca entry, rather than on Ca entry per se. For example, Silinsky was able to increase the frequency of miniature end plate potentials (MEPPs) by using Ca containing liposomes to deliver Ca directly to the nerve terminal, thus bypassing the normal route of Ca entry. He also used the polyvalent cation La, which increases the frequency of release by some mechanism that is independent of Ca influx. As A1 agonists were still effective in reducing MEPP frequency, but not amplitude, in both of these cases, Silinsky concluded that they must produce their effects downstream of Ca entry. Moreover, that the effects of Ca itself could be blocked implied that the site of inhibition was actually downstream of the site of Ca action on the release process.
These observations have subsequently been confirmed in several more recent studies of both the vertebrate and invertebrate nervous systems. Scholz & Miller (121) studied the inhibitory effects of A1 agonists on evoked glutamate release at excitatory hippocampal pyramidal neuron synapses. Following the demonstration that A1 agonists blocked the evoked glutamatergic excitatory postsynaptic current (EPSC) at these synapses and the Ca current in the cell soma (83), these authors showed that the frequency but not the amplitude of miniature excitatory postsynaptic currents (mEPSCs) recorded in the presence of tetrodotoxin (TTX) and Cd (a nonselective blocker of all types of high-threshold Ca channels) was also blocked. All three of these effects were absent following pertussis toxin treatment. mEPSCs are usually thought to result from the spontaneous exocytosis of transmitter-containing vesicles occurring in the absence of Ca influx. Thus, these results also imply that A1 agonists can inhibit release at hippocampal nerve terminals downstream of Ca entry. Similar effects have been reported for several other presynaptic receptor agonists acting on mEPSCs and inhibitory postsynaptic currents (mIPSCs) in various regions of the hippocampus, including further studies on A1 agonists (111, 122–124) and also baclofen (GABA_B) (123, 124), somatostatin (125), acetylcholine (muscarinic) (126), glutamate (metabotropic) (126, 127), and opioid (mu) agonists (128). In all of these instances, the authors demonstrated that receptor activation produced a reduction in the frequency of mEPSCs under conditions allowing them to conclude that release was inhibited at a point beyond Ca entry. Similar results have been reported in other parts of the brain as well (100, 113). As in the case of Silinsky’s original report, several studies have attempted to increase transmitter release by employing various agents that enhance secretion while bypassing Ca channels. These agents include polycations such as Gd (123) and ruthenium red (122) as well as the Ca ionophore ionomycin (123) and α-latrotoxin from black widow spider venom (123). In all of these instances, agonists at presynaptic receptors (µ-opioid, A1, and GABA_B) were still effective at reducing the frequency of mEPSCs and mIPSCs, further supporting a site of action downstream of Ca entry. Nevertheless, not all investigations have reached the same conclusion. There are studies on the effects of mGluR (128), A1 (129), and GABA_B (130) agonists as well as NPY (131) in the hippocampus that found no effect on mEPSC and mIPSC frequency, although each agonist strongly suppressed evoked transmitter release. Curiously, these studies all utilized slice preparations, whereas the former group of studies, reporting a downstream point of action, all utilized cultured neurons. Whether this is merely a coincidence or whether it indicates something more significant is unclear. In cerebellar slices, however, Dittman & Regehr (100) did show that a component of the presynaptic inhibition caused by baclofen at parallel fiber/Purkinje cell synapses involved a downstream effect, and Nicola
& Malenka (113) came to similar conclusions in their study of inhibition of EPSPs by dopamine in nucleus accumbens slices. Recent observations in hypothalamic slices have also noted a downstream component in the presynaptic inhibition of glutamate release produced by the peptide galanin in the arcuate nucleus (G Kinney & RJ Miller, unpublished observations).

Results obtained on the mechanism of hormone secretion from different types of endocrine cells parallel the results discussed here for neurons. Just as with neurons, stimulation of different types of G-protein-linked receptors has often been shown to inhibit the evoked release of hormone-containing vesicles. As release in these cases is usually the result of Ca influx through voltage-dependent Ca channels, there are obvious similarities between the two processes. Indeed, it has often been found that activation of receptors will inhibit Ca currents in these cells (132). Nevertheless, several studies have demonstrated that activation of receptors can still inhibit hormone release from "permeabilized" endocrine cells in which release is no longer dependent on Ca influx via Ca channels. Thus, in these cases an inhibitory mechanism downstream of Ca entry has also been invoked (133, 134). At any rate, whether one considers the situation in endocrine cells or neurons, nobody has any idea what the molecular basis for such effects might be. Recently, Krasnoperov et al (135) made an intriguing observation about the mechanism of action of α-latrotoxin (135). This toxin increases transmitter release by two mechanisms, one Ca dependent and one Ca independent. These mechanisms involve the binding of the toxin to two receptors. The Ca-independent effects of the toxin appear to be mediated by a G-protein-linked receptor that is a member of the extended secretin receptor family. It appears that this receptor can interact with syntaxin 1A and synaptotagmin. Thus, there is now a precedent for a direct interaction between a G-protein-linked receptor and protein components of the transmitter release apparatus. Might this be an archetype for how downstream regulation of release occurs? In keeping with this possibility, it has recently been demonstrated that synaptosomal muscarinic receptors can directly interact with several components of the release apparatus, including syntaxin, synaptotagmin, and SNAP-25 (136).

Quantitative Aspects of Release
The above discussion indicates that there is evidence for two or possibly three types of effects contributing to presynaptic inhibition produced by G-protein-linked receptors at vertebrate synapses. Some attempts have been made to try to estimate which effects are quantitatively the most important. Given the fact that little evidence exists supporting a role for K channels, except in certain instances, most of the arguments concern the relative importance of inhibitory mechanisms operating directly on or downstream of Ca entry. As might be expected, there is no consensus on the answer. In studies in which downstream
effects on transmitter release have not been observed, conclusions obviously favor inhibition of Ca channels as the sole mechanism of importance (128, 130). The opposing view has also been put forward. Thompson and colleagues (111, 124, 126) have demonstrated that although activation of several types of presynaptic receptors in the hippocampus reduced mEPSC and mIPSC frequency, Cd produced no effect under the same conditions. The same group also demonstrated through the use of selective toxins, such as botulinum toxin, that release leading to the production of mIPSCs and mEPSCs appears to involve the same proteins and mechanisms as evoked release (123). Hence, it is argued that inhibition of presynaptic Ca channels is unnecessary for presynaptic inhibition to occur and that it can be completely accounted for by downstream mechanisms. In support of this view are instances in which the activation of receptors produced quantitatively equivalent inhibition of evoked and mIPSCs and mEPSCs. However, even though it is clear that effects on Ca influx are not a sine qua non for presynaptic inhibition to occur, this does not necessarily mean that inhibition of Ca influx is not normally an important factor. Indeed, other estimates have concluded that although the downstream mechanism may play a role (4, 100), an effect on Ca influx is of primary importance, although precise quantitative estimates as to the relative contributions of the two mechanisms differ depending on the situation.

IONOTROPIC RECEPTORS

In addition to the effects of G-protein-linked receptors, it is also clear that presynaptic ionotropic receptors exist and that these may be very influential in the regulation of neurotransmitter release. Evidence exists for presynaptic GABA<sub>A</sub>C, nicotinic cholinergic, and diverse types of glutamate receptors in various parts of the nervous system whose activation can modulate transmitter release. All of these receptors are multisubunit ligand–gated ion channels. Receptor activation leads directly to the opening of ion channels in the presynaptic terminal, and the subsequent redistribution of ions influences transmitter release. There are several ways this could happen. For example, as discussed above, activation of an ion channel that is permeable to K or Cl may produce shunting of the action potential in the presynaptic terminal, resulting in less effective opening of Ca channels and subsequent inhibition of release. Alternatively, and this is considered to be more likely, activation of channels leading to the redistribution of Cl or Na might depolarize the terminal, leading to inactivation of voltage-dependent Na and Ca channels and inhibition of the spread of the action potential into the terminal and the influx of Ca. On the other hand, if the channel activated is actually appreciably permeable to Ca, one might expect enhancement of transmitter release due to increased Ca influx.
directly into the terminal. This might subsequently be followed by an inhibition of release when stores of transmitter are exhausted. There is evidence that mixtures of all of these processes are actually utilized in practice. Although it is not a neurotransmitter, the drug capsaicin binds to a presynaptic receptor on small nonmyelinated sensory nociceptors, causing the opening of an ion channel that is permeable to both Ca and Na (137, 138). This opening results in increased neuronal excitability and Ca influx, initially producing increased transmitter (substance P) release in the spinal cord and pain. However, when stores of transmitter are exhausted, hypoalgesia results. Indeed, the drug is used topically in the treatment of disorders such as shingles for this very reason.

PRESYNAPTIC GABA$_{A/C}$ RECEPTORS

The first bona fide example of presynaptic inhibition was actually a reduction in Group 1A EPSPs in spinal motoneurons, described by Frank & Fuortes (139), that could not be accounted for by a postsynaptic mechanism. It has subsequently been determined that this effect is mediated by GABA and that similar GABA-mediated presynaptic inhibition is found throughout the nervous system (140). The receptors involved in the presynaptic inhibitory effects of GABA are of different types (36). G-protein-coupled baclofen-sensitive GABA$_B$ receptors clearly contribute to such effects at many synapses (see above). In addition, however, activation of ionotropic GABA$_A$ (141) and GABA$_C$ (142) receptors is believed to constitute a widely distributed mechanism that mediates presynaptic inhibition in, among other places, the hippocampus, striatum, substantia nigra, retina, spinal cord, and posterior pituitary (34). Such a role has also been suggested by the many anatomical and neurochemical studies on the effects of GABA$_A$ agonists in different neuronal preparations (34, 141, 142).

The ionotropic GABA receptors constitute a family of multisubunit heteromultimers containing diverse combinations of subunits. At this time, $\alpha 1$–6, $\beta 1$–4, $\gamma 1$–4, $\delta$, $\epsilon$ and $\rho 1$–3 subunits have been identified (141–144). They are homologous to nicotinic cholinergic receptor subunits as well as to each other. GABA$_A$ receptors are thought to minimally contain $\alpha \beta \gamma$, $\alpha \beta \delta$, or $\alpha \beta \epsilon$ subunits in what is probably a pentameric array. Numerous clinically important classes of drugs interact with these receptors, and the selectivity of these interactions is determined by receptor subunit combinations (141, 142). For example, the binding of benzodiazepines is dependent on the presence of the $\gamma$ subunit (141, 142). Receptors containing $\rho$ subunits are quite exceptional, however (142). First, it is thought that they may assemble into homooligomeric channels (GABA$_C$ receptors), and furthermore, they do not bind many of the drugs that define the pharmacology of classical GABA$_A$ receptors—they are not blocked by bicuculline, for example (142, 145).
The exact composition of the ionotropic GABA receptors that exist in nerve terminals is unknown. In the case of the terminals of retinal bipolar cells, pharmacological analysis has suggested that the receptors involved are GABA_C, and so are presumably made up only of ρ subunits (142, 146). These subunits have also been found in other parts of the brain as well, but whether they are presynaptic is unknown (147). Recordings made directly from the terminals of posterior pituitary neurosecretory neurons have indicated the presence of GABA_A receptors (38). The pharmacology and functional characteristics of these receptors suggest that they may have a different subunit composition from GABA_A receptors found in other parts of neurons, and this may be true for presynaptic GABA_A receptors in other parts of the nervous system as well (148, 149).

Activation of GABA_A/C receptors leads to the opening of anion-selective channels (141, 147, 150). The permeability of these channels to Cl and HCO_3 presumably underlies their ability to produce presynaptic inhibition, although the exact mechanism involved is not entirely clear. Although it was initially believed that the effect of GABA_A/C receptor activation resulted from action potential shunting, modeling studies (37) and direct recording from nerve terminals (38) have suggested that it may primarily result from depolarization of terminals and inactivation of Na and Ca channels.

PRESYNAPTIC NICOTINIC RECEPTORS

A wealth of data from the neurochemical literature suggests that presynaptic nicotinic receptors exist in the CNS and that their activation enhances transmitter release (34, 151, 152). Anatomical data on the localization of these receptors support this contention in several instances (34). Recent studies have started to analyze the mechanism of action of nicotinic agonists. It is known that a large family of nicotinic receptor subunits exist in the nervous system, including families of α_{(2–9)} and β_{(2–4)} subunits (34, 151, 152). It is believed that nicotinic receptors in the brain are made up of heterologous combinations of α and β subunits, although in some cases (e.g. α_7) homologous arrangements of subunits are likely to occur (153). By analogy with nicotinic receptors at the neuromuscular junction, it is also probable that receptors in the brain are pentameric (152). Receptors that contain α_7 are highly permeable to Ca, although other subunit combinations also retain an appreciable Ca permeability (154–157). It is also clear that CNS receptors that contain the α_7 (or α_8 or α_9) are blocked by α-bungarotoxin (α-βTX), whereas those that contain other α subunits are not (158). Pharmacological studies have indicated that both types of receptors may be involved in the regulation of transmitter release in different circumstances (34, 159, 160).
In some instances stimulatory effects of nicotinic agonists on transmitter release have been reported to be inhibited by TTX (161–163). This is true, for example, for nicotinic receptors on GABAergic terminals in the chick lateral spiriform nucleus (162) and rat interpeduncular nucleus (161). The inhibitory effects of TTX have been interpreted as indicating that the receptors in these cases are actually “preterminal” and that the effects are exerted by triggering action potentials that subsequently invade the nerve terminal. On the other hand, several other reports have demonstrated stimulatory effects of nicotinic agonists that are resistant to TTX, and it is thought that the receptors in these cases are truly presynaptic, i.e. in the nerve terminal itself. Nicotinic agonists enhance mPSCs in the rat olfactory bulb (OB) (164), interpeduncular nucleus (IPN), lumbar sympathetic ganglion (LSG) (165), and the CA3 region of the hippocampus (166). In these instances, block with $\alpha$-BTX and/or antisense knockout of $\alpha_7$ subunits have indicated their participation in the response (164–166). Experiments in the CA3, IPN, and LSG also showed that nicotinic agonists increased $[Ca]_i$ in nerve terminals from which transmitter release occurred. In the CA3 it was demonstrated that nicotinic agonists were still effective in stimulating transmitter release and raising $[Ca]_i$ in mossy fiber terminals in the presence of Cd as well as TTX, indicating that at least a component of the Ca flux into these terminals occurred directly through nicotinic receptors rather than through voltage-sensitive Ca channels. Studies in the dorsolateral geniculate (DLG) (167, 168) and the ventrobasal complex (VB) (167), however, demonstrated that the stimulation of GABA release (mIPSCs) produced by nicotinic agonists was insensitive to $\alpha$-BTX but was absent in $\beta_2$ subunit knockout mice, indicating that the receptors involved probably contained $\beta_2$ plus an $\alpha$ subunit such as $\alpha_3$ or $\alpha_4$ (167). Interestingly, the stimulation produced by nicotinic agonists was insensitive to Cd (which blocked the effects of high K) in the DLG but substantially blocked by Cd in the VB (167). Taken together, these results suggest that presynaptic nicotinic receptors of various types can stimulate the release of transmitters by causing direct Ca flux into presynaptic terminals and also causing influx through voltage-dependent Ca channels that open as a result of depolarization. The precise contributions of these two processes seems to differ according to the situation. One surprising observation, however, is that nicotinic agonists also increased the evoked release of transmitter in the VB and IPN (165, 167). This is interesting in view of the fact that if depolarization of the terminal had occurred in these situations, one would have expected to see an inhibition of evoked release as observed with presynaptic GABA$_A$ or kainate receptors (see below). At any rate, the facilitation of release produced by nicotinic agonists may be of considerable importance therapeutically in several neurodegenerative disorders such as Parkinson’s and Alzheimer’s disease in which
deficits in transmitter release are clearly a problem. Similar considerations to those discussed for nicotinic receptors may also apply to P2X ATP receptors, which have been shown to regulate transmitter release in the spinal cord (169).

PRESYNAPTIC GLUTAMATE RECEPTORS

Ionotropic glutamate receptors also constitute a large family of widely distributed subunits that can be organized into heterologous combinations of AMPA, kainate, and NMDA receptors (170–172). These receptors, which like nicotinic and GABA$_A$ receptors are likely to be pentamers, are ligand-gated cation channels that differ in their pharmacological specificity and in the details of their ion selectivity, particularly their permeability to Ca. It is clear that AMPA and NMDA receptors transduce excitatory transmission at many glutamate-utilizing synapses in the CNS (173). On the other hand, the physiological functions of kainate receptors in the CNS are far from clear (172, 173, but see 174, 175).

Neurochemical data obtained using synaptosomal preparations from different parts of the brain have suggested that all three types of glutamate receptors may also act as presynaptic receptors. Activation of synaptosomal AMPA and NMDA receptors has been generally linked to the enhancement of transmitter release from different parts of the brain (176–180). However, the best data supporting a presynaptic receptor function come from recent studies on the role of kainate receptors in the hippocampus. In contrast to the stimulatory effects of AMPA-selective agonists, kainate inhibited the release of glutamate from hippocampal synaptosomes, and these effects were suggestive of the activation of kainate rather than AMPA receptors (181). Electrophysiological studies in the hippocampal slice demonstrated that kainate had two effects on NMDA receptor–mediated EPSCs evoked in the CA1 (181). Following a brief enhancement of the EPSC, a long-term depression was observed. Here again, the selectivity of the effect suggested activation of kainate rather than AMPA receptors. Recently, Clarke et al (182) developed two drugs that are selective for GluR5 kainate receptors: the agonist A TPA and the antagonist LY294486. The authors used these tools to demonstrate that activation of presynaptic GluR5-like kainate receptors regulated the release of GABA from terminals in the CA1, as was also observed in another recent study (183). Both kainate and A TPA inhibited evoked GABAergic IPSCs in CA1, and their effects were blocked by LY294486. Thus, in the hippocampus, presynaptic kainate receptors appear to regulate both excitatory and inhibitory synapses. Although the mechanism of action of kainate receptors is still unclear, depolarization of terminals leading to the inactivation of Na and Ca channels is likely.
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