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Implications of G-Protein-Mediated Ca^{2+} Channel Inhibition for Neurotransmitter Release and Facilitation

RICHARD BERTRAM
Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306
bertram@sb.fsu.edu

MATTHEW BEHAN
School of Science and Center for Mathematical Biology, Pennsylvania State University, Erie, PA 16563

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Abstract. G-protein-mediated inhibition of Ca^{2+} current is ubiquitous in neurons, and in synaptic terminals it can lead to a reduction in transmitter release (presynaptic inhibition). This type of Ca^{2+} current inhibition can often be relieved by prepulse depolarization, so the disinhibition of Ca^{2+} current can combine with Ca^{2+}-dependent mechanisms for activity-induced synaptic facilitation to amplify this form of short-term plasticity. We combine a mathematical model of a G-protein-regulated Ca^{2+} channel with a model of transmitter secretion to study the potential effects of G-protein-mediated Ca^{2+} channel inhibition and disinhibition on transmitter release and facilitation. We investigate several scenarios, with the goal of observing a range of behaviors that may occur in different synapses. We find that the effects of Ca^{2+} channel disinhibition depend greatly on the location and distribution of inhibited channels. Facilitation can be greatly enhanced if all channels are subject to inhibition or if the subpopulation of channels subject to inhibition are located closer to release sites than those insensitive to inhibition, an arrangement that has been suggested by recent experiments (Stanley and Mirotznik, 1997). We also find that the effect of disinhibition on facilitation is greater for longer action potentials. Finally, in the case of homosynaptic inhibition, where Ca^{2+} channel inhibition occurs through the binding of transmitter molecules to presynaptic autoreceptors, there will be little reduction in transmitter release during the first of two successive bursts of impulses. The reduction of release during the second burst will be significantly greater, and if the unbinding rate of autoreceptors is relatively low, then the effects of G-protein-mediated channel inhibition become more pronounced as the duration of the interburst interval is increased up to a critical point, beyond which the inhibitory effects become less pronounced. This is in contrast to presynaptic depression due to the depletion of the releasable vesicle pool, where longer interburst intervals allow for a more complete replenishment of the pool. Thus, G-protein-mediated Ca^{2+} current inhibition leads to a reduction in transmitter release, while having a highly variable amplifying effect on synaptic facilitation. The dynamic properties of this form of presynaptic inhibition are very different from those of vesicle depletion.

Keywords: synapse, plasticity, presynaptic inhibition, secretion, mathematical model

Introduction

Many synapses are subject to presynaptic inhibition by a host of chemical messengers, including GABA, adenosine, glutamate, dopamine, and serotonin. These messengers typically inhibit transmitter release by activating presynaptic K^+ currents (Scholfield and Steel, 1988; Thompson and Gähwiler, 1992; Vaughan

et al., 1997), inhibiting presynaptic Ca^{2+} currents (Boehm and Betz, 1997; Chen and van den Pol, 1997, 1998; Dittman and Regehr, 1996, 1997; Qian et al., 1997; Takahashi et al., 1996, 1998; Toth et al., 1993; Wu and Saggau, 1994a, 1995), or affecting some process downstream of Ca^{2+} entry (Boehm and Betz, 1997; Dittman and Regehr, 1996). Activation of a K^+ current indirectly reduces Ca^{2+} entry and the subsequent release of neurotransmitter, either by blocking action potential propagation into the presynaptic terminal or by shortening the duration of the action potential. Inhibition of Ca^{2+} current acts directly by reducing the influx of Ca^{2+} during an impulse. Regardless of the mode of action, the chemical regulators are often linked to presynaptic inhibition through a G protein pathway, whereby the agonist binds to presynaptic receptors, leading to activation of G proteins that bind to and modify the behavior of one or more proteins linked to transmitter release.

The mechanism and properties of G-protein-mediated inhibition of Ca^{2+} channels have been the focus of much research in recent years, due largely to the apparent ubiquity of this modulatory pathway (Hille, 1994). Data suggest that $G_{\beta\gamma}$ subunits of activated G proteins bind to the $\alpha 1$ subunit of the Ca^{2+} channel in a voltage-dependent manner, so that binding is stabilized at low voltages and destabilized at depolarized voltages (see Dolphin, 1998, for review). This voltage dependence allows for the relief of Ca^{2+} channel inhibition by depolarizing prepulses. Such "disinhibition" has been observed in several preparations using long ($>$10 msec) prepulses (Bean, 1989; Grassi and Lux, 1989; Patil et al., 1996), and recent studies have shown that bursts of action potentials or short depolarizations are capable of relieving Ca^{2+} current inhibition (Brody et al., 1997; Williams et al., 1997), demonstrating that disinhibition can be induced by physiological stimuli.

Although the mechanism of Ca^{2+} current inhibition is still under investigation, data suggest that the binding of one or more G proteins to a Ca^{2+} channel puts the channel into a "reluctant" state, in which the probability of channel opening is greatly reduced (Bean, 1989). As a result, G-protein-bound channels are unlikely to open during a brief depolarization such as an action potential, and only those channels in an unbound or "willing" state contribute to the Ca^{2+} current. Depolarizing prepulses would then disinhibit the current by converting some of the channels from the reluctant state to a willing state by dislodging bound G proteins (Bean, 1989; Zamponi and Snutch, 1998).

Because synaptic vesicle exocytosis is activated by intraterminal Ca^{2+} (Katz and Miledi, 1968), neurotransmitter release will likely be reduced by inhibition of Ca^{2+} channels in the synaptic terminal. In addition, chemical messengers that activate inhibitory G proteins can in principle have a profound influence on short-term synaptic plasticity. Indeed, several studies have shown that paired-pulse facilitation, where evoked release is increased when preceded by an earlier stimulus, is greater in the presence of G protein agonists (Dittman and Regehr, 1997; Dunwiddie and Haas, 1985; Isaacson et al., 1993; Shen and Johnson, 1997). Although the mechanism of facilitation has not been established, two hypotheses propose that facilitation is due primarily to either a prepulse-induced elevation of free Ca^{2+} or to the residual binding of Ca^{2+} to synaptic proteins (Katz and Miledi, 1968; Stanley, 1986; Kamiya and Zucker, 1994). Thus, release during the second stimulus is enhanced by residual free or bound Ca^{2+} even if the Ca^{2+} influx is identical during each stimulus. Presumably, the extra facilitation produced by G protein agonists is due to partial disinhibition of the Ca^{2+} current by the first stimulus, so that the Ca^{2+} influx is greater during the second stimulus, compounding the effects of residual free or bound Ca^{2+}. (In some cases, some of the apparent extra facilitation may be due to a reduction in presynaptic depletion of the readily releasable vesicle pool (Dunwiddie and Haas, 1985).) In support of this mechanism, Williams et al. (1997) observed a 20% increase in whole-cell Ca^{2+} current during the second of a pair of short depolarizations. Because transmitter release has a superlinear dependence on Ca^{2+} current (Augustine and Charlton, 1986), it is likely that relief of inhibition will have a superlinear effect on facilitation.

In the present report we combine a mathematical model of a G-protein-regulated Ca^{2+} channel with a model of transmitter secretion to study the potential effects of G-protein-mediated channel inhibition and disinhibition on transmitter release and short-term synaptic plasticity. The aim of this study is to examine several scenarios so as to establish an intuition for the types of behavior that may occur in different synapses.

We first examine a scenario in which all of the presynaptic Ca^{2+} channels are susceptible to G-protein-mediated inhibition. This analysis suggests that facilitation produced during a short burst can be greatly enhanced by Ca^{2+} channel disinhibition, particularly if the G protein agonist concentration is high and if action potentials have a relatively long duration. Thus,

under certain conditions, G protein regulation of Ca^{2+} channels can play a major role in short-term synaptic plasticity.

We next examine a scenario in which only half of the Ca^{2+} channels are susceptible to G protein regulation. This is motivated by the findings that some Ca^{2+} channel types are more susceptible than others to regulation (see Wu and Saggau, 1997, for review) and by data from the calyx-type nerve terminal of the chick ciliary ganglion synapse showing that only Ca^{2+} channels that are coupled to release sites through the synaptic protein syntaxin are susceptible to regulation (Stanley and Mirotznik, 1997). We find that if both regulated and unregulated channels lie at equal distances from the release sites, then the enhanced facilitation of the regulated population produces only a minor enhancement of facilitation of the total release (the sum of the release from both subpopulations of release sites). However, if regulated channels are situated closer to release sites (as may be the case if they are bound to the sites via syntaxin), then the enhanced facilitation of this closer subpopulation can have a much larger effect on the overall facilitation. Thus, the effect of G protein regulation on synaptic facilitation depends critically on the relative locations of regulated and unregulated channels.

Finally, we examine a scenario in which the concentration of G protein agonist is determined by the amount of transmitter released. This is motivated by the presence of metabotropic transmitter autoreceptors in many presynaptic terminals (Langer, 1987; Starke et al., 1989; Chen and van den Pol, 1998). We find that the agonist released during the first of two bursts of impulses has little effect on release during this burst, due to depolarization-associated disinhibition of Ca^{2+} channels. However, activated G proteins bind to channels during the interburst interval when the membrane is hyperpolarized, so presynaptic inhibition can be profound during the subsequent burst. This is in contrast to presynaptic depression mediated by the depletion of releasable quanta, in which case release would be reduced during each of two successive bursts. In addition, if the unbinding rate of autoreceptors is relatively low, then the effects of G-protein-mediated channel inhibition become more pronounced as the duration of the interburst interval is increased up to a critical point, beyond which the inhibitory effects become less pronounced. In contrast, the residual inhibitory effects of vesicle depletion decrease monotonically with interburst duration.

Methods

The Ca^{2+} Channel Model

The Ca^{2+} channel model is based on one of several models for G protein regulation of N-type Ca^{2+} channels proposed by Boland and Bean (1993). In this model, the binding of a single G protein subunit puts the channel into a reluctant state. There are other models of G protein regulated channels that assume a higher stoichiometry of G protein binding (Boland and Bean, 1993; Golard and Siegelbaum, 1993); however, the present model is simpler and is consistent with recent evidence supporting a unitary stoichiometry (Zamponi and Snutch, 1998). The channel model used in the present investigation is described by the following kinetic scheme:

$$\begin{array}{ccccccccc} C_1 & \underset{\beta}{\overset{4\alpha}{\rightleftharpoons}} & C_2 & \underset{2\beta}{\overset{3\alpha}{\rightleftharpoons}} & C_3 & \underset{3\beta}{\overset{2\alpha}{\rightleftharpoons}} & C_4 & \underset{4\beta}{\overset{\alpha}{\rightleftharpoons}} & O \\ l \updownarrow k & & 64l \updownarrow k & & (64)^2 l \updownarrow k & & & & \\ C_{G1} & \underset{\beta'}{\overset{4\alpha'}{\rightleftharpoons}} & C_{G2} & \underset{2\beta'}{\overset{3\alpha'}{\rightleftharpoons}} & C_{G3} & & & & \end{array}$$

The bottom row represents G-protein-bound or reluctant states, while the top row represents unbound or willing states. There are four closed willing states (C_1–C_4), one open willing state (O), and three closed reluctant states (C_{G1}–C_{G3}). The transition rates α and α' are increasing functions of voltage, while β and β' are decreasing functions of voltage. As a result, voltage depolarization causes rightward movement in the diagram. For notational simplicity, we use C_j and C_{Gj} to denote both the closed state of a channel and the probability that the channel is in this state (or equivalently, the fraction of a large population of channels in this state). We use m to denote the probability that a channel is in the open state O. This model differs from the Boland and Bean model on which it was based, where each row of the kinetic scheme contains seven states: five closed, one open, and one inactivated. Simulations with both models (not shown) indicate that the additional states in the Boland and Bean model are important only during longer depolarizations than those considered in the present report.

The differential equations for the fraction of channels in each of the eight channel states follow immediately from the law of mass action. They are given in the appendix, as are all parameter values. Consistent with data showing a "kinetic slowdown" in the presence of G protein agonists, the forward kinetic

rates are smaller for reluctant states than for willing states, while the backward rates are larger: $\alpha' = \alpha/8$, $\beta' = 8\beta$. For microscopic reversibility the G protein unbinding rate, l, is multiplied by 64 or 64^2. The G protein binding rate, k, is a sigmoidal function of the G protein agonist concentration $[A]$ (nanomolar): $k = 0.003[A]/(68 + [A])$ ms^{-1}. In the Boland and Bean study the agonist was luteinizing hormone-releasing hormone (LHRH), but the particular agonist and the functional dependence of k on the agonist concentration will be different for different systems. We therefore prefer to rewrite the expression for G protein binding in terms of the fraction of presynaptic receptors bound by agonist (B) rather than the agonist concentration. We assume a sigmoidal relation between agonist concentration and receptor binding and choose a dissociation constant so that approximately 90% of the receptors are bound when $[A] = 1000$ nM: $B = \frac{[A]}{100+[A]}$. Hence, $k = 0.3B/(68 + 32B)$ ms^{-1}.

Relief of inhibition during a short 100 Hz burst of action potentials (APs) is demonstrated in Fig. 1. In the presence of agonist, $B = 0.5$, most of the channels (88%) are initially in the first reluctant state, while the rest are in the first willing state. Therefore, few channels open during the first AP (peak m during the first AP is only about 12% of its value in the absence of agonist). However, during an AP a significant fraction of channels move first into the C_{G2} state and then into the willing C_2 state, since the vertical transition rate from C_{G2} to C_2 is relatively large. Hence, at the start of the second AP there is a larger pool of channels in a willing state, so more channels open. Disinhibition continues throughout the burst of APs (Fig. 1A), as the fraction of channels in a reluctant state declines with each AP (Fig. 1B). By the end of the burst approximately 60% of the channels are in a willing state, and the probability of channel opening is about 60% of that in the absence of agonist.

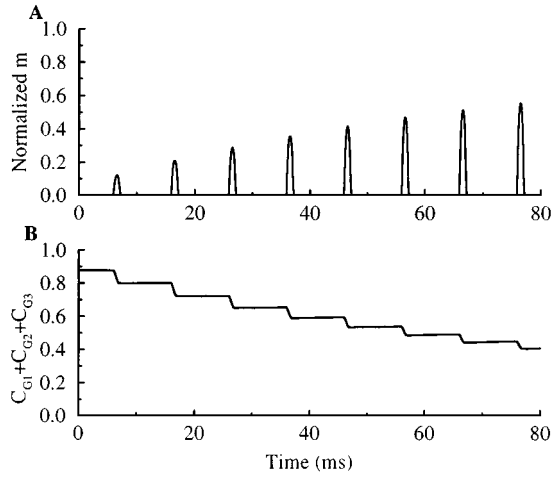

Fig. 1. Partial disinhibition during a 100 Hz burst of APs with $B = 0.5$. Most of the channels are initially in the first reluctant state, C_{G1}. **A**: The probability that a channel is in the open state m is normalized to the peak open state probability during an AP in the absence of agonist. The normalized open probability rises during the burst due to disinhibition. **B**: The probability that a channel is in a reluctant state declines with each AP as the channels become disinhibited. Equations for Ca^{2+} channel activity and for the generation of APs are given in the appendix.

The Transmitter Secretion Model

Exocytosis of neurotransmitters is evoked by microdomains of Ca^{2+} that are formed at open Ca^{2+} channels (Llinás et al., 1992). The Ca^{2+} concentration in a microdomain can be quite high, 100 μM or greater (Llinás et al., 1992; Simon and Llinás, 1985), as is necessary for activation of a low-affinity Ca^{2+} binding step in the vesicle fusion process (see Südhof, 1995, for review). A release site may be influenced by a single Ca^{2+} channel or by several nearby channels, depending on factors such as the geometry of channels and release sites, the concentration of stationary and mobile buffers, the types of Ca^{2+} channels present, and the external Ca^{2+} concentration. The transmitter secretion model used in the present study is based on an earlier model, where it was assumed that each transmitter release site is influenced by a single Ca^{2+} channel (Bertram, 1997; Bertram et al., 1996). This model is used primarily for its simplicity, but it should be noted that other models of transmitter secretion have been developed (Bennett et al., 1997; Bertram et al., 1999; Dudel et al., 1983; Tang et al., 1998; Worden et al., 1997; Yamada and Zucker, 1992; Zucker and Fogelson, 1986), and each may more accurately describe certain aspects of the secretion process.

We assume here that binding is sequential:

$$S_0 \underset{k_1^-}{\overset{4k_1^+ Ca_d}{\rightleftarrows}} S_1 \underset{2k_2^-}{\overset{3k_2^+ Ca_d}{\rightleftarrows}} S_2 \underset{3k_3^-}{\overset{2k_3^+ Ca_d}{\rightleftarrows}} S_3 \underset{4k_4^-}{\overset{k_4^+ Ca_d}{\rightleftarrows}} S_4,$$

where S_j represents a release site with j Ca^{2+} ions bound and Ca_d is the domain Ca^{2+} concentration at the release site. For notational simplicity, we also use S_j to denote the probability that a release site has j ions bound (or the fraction of release sites with j ions bound). We define release R as the probability that a site is in the completely bound state, $R = S_4$.

The opening and closing of Ca^{2+} channels is a stochastic process, so the domain Ca^{2+} concentration at a release site, Ca_d, is a random variable. However, use of the "average domain Ca^{2+}" concentration, Ca_d averaged over the entire population of Ca^{2+} channels, greatly simplifies calculations (Bertram and Sherman, 1998). Thus, we use the average domain Ca^{2+} concentration $\overline{Ca} = m\,Ca_{d,open}$, where $Ca_{d,open}$ is the domain Ca^{2+} concentration at an open channel (appendix). This is added to the constant background or bulk Ca^{2+} concentration, $Ca_{bk} = 0.1\ \mu$M, to yield $Ca = \overline{Ca} + Ca_{bk}$, the Ca^{2+} concentration used in the determination of transmitter release. As with the Ca^{2+} channel model, the differential equations describing the secretion model follow immediately from the law of mass action and are given in the appendix along with all parameter values.

We define facilitation of transmitter release during impulse n as $F_n = R_n/R_1$, where R_n is the peak release during impulse n. In the present model, facilitation depends on the backward transitions $S_3 \rightarrow S_2$, $S_2 \rightarrow S_1$, and $S_1 \rightarrow S_0$ being slow. That is, the unbinding rates k_1^-, k_2^-, k_3^- are small, so some of the Ca^{2+} bound during one AP remains bound at the start of the next AP. This "residual bound Ca^{2+}" mechanism is one of several possible mechanisms for facilitation (Stanley, 1986; Worden et al., 1997; Yamada and Zucker, 1992; Zucker and Fogelson, 1986; Wojtowica et al., 1994).

Figure 2 illustrates facilitation of transmitter release during a short 100 Hz burst of impulses in the absence of G-protein agonist. In this case $F_8 = 7.1$, so the rate of release during impulse number 8 is approximately sevenfold higher than during the first impulse of the burst. The figure is produced using Hodgkin-Huxley equations for voltage V and coupling the Ca^{2+} channel model to the secretion model. There is no Ca^{2+} channel inhibition or disinhibition since $B = 0$ (Fig. 2C), so facilitation is due solely to the slow accumulation of release sites in the S_2 and S_3 states (Fig. 2B).

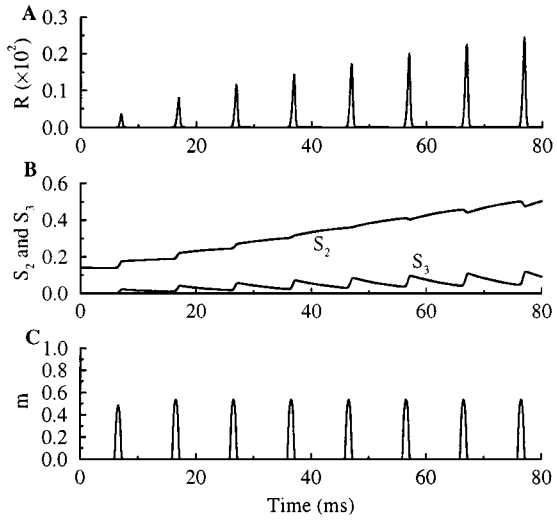

Fig. 2. (**A**) Facilitation of release during a 100 Hz burst of APs is due to (**B**) the accumulation of the fraction of release sites with two and three Ca^{2+}-bound sites, denoted by S_2 and S_3, respectively. (**C**) Ca^{2+} channels are uninhibited since G protein agonist is not present ($B = 0$), therefore there is no disinhibition of Ca^{2+} channels.

Results

Scenario 1: All Channels Regulated by Constant Agonist

In the majority of experiments on G-protein-mediated Ca^{2+} channel inhibition and the resulting presynaptic inhibition, the bathing solution contains a specified concentration of G protein agonist. Although this protocol is somewhat unphysiological, it simplifies the investigation of the dynamics of channel inhibition and disinhibition by keeping the agonist concentration relatively constant. Our first scenario employs this protocol in a computational setting, and examines the case in which all Ca^{2+} channels are subject to G protein inhibition. Thus, the agonist parameter B is set at 0, 0.1, or 0.5, depending on the degree of agonist binding desired. At the start of the simulations the model cell is polarized and all of the Ca^{2+} channels are either in state C_1 or state C_{G1}, determined by the C_1 and C_{G1} equilibrium equations:

$$C_1 = \frac{l}{l+k}, \quad C_{G1} = \frac{k}{l+k}. \tag{1}$$

It was demonstrated in Brody et al. (1997) that Ca^{2+} channel disinhibition is greater during bursts of long APs than during bursts of short APs. We therefore examine the implications of disinhibition for synaptic facilitation during bursts of both long and short APs. The AP duration is adjusted with the parameter λ in the Hodgkin-Huxley membrane potential equations (appendix).

Long Impulses. We first examine facilitation during a 100 Hz burst of long impulses ($\lambda = 1$). In this case, AP duration from initiation to maximum repolarization is 2.5 ms. Figures 1 and 2 were generated with APs of this duration. Transmitter release in the presence of agonist is shown in Fig. 3B, superimposed with release in the absence of agonist. Also shown is the fraction of Ca^{2+}

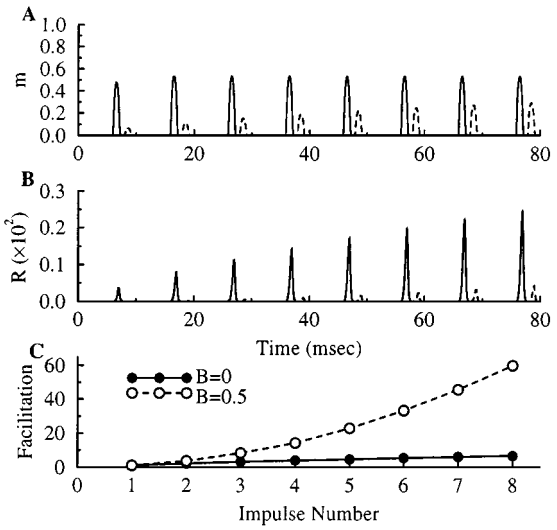

Fig. 3. Fraction of open Ca^{2+} channels (**A**) and transmitter release (**B**) during a 100 Hz burst of long APs, in the absence (solid) and presence (dashed) of a high concentration of agonist ($B = 0.5$). The agonist reduces the fraction of channels opened during the last AP by 45%, while release is reduced by 83%. This reflects the superlinear relation between transmitter release and Ca^{2+} current. (**C**) Although release is reduced by the agonist, facilitation is greatly increased. During this short train of eight impulses, the fivefold increase in Ca^{2+} current due to channel disinhibition acts superlinearly to produce an elevenfold increase in facilitation.

channels opened during each impulse (Fig. 3A). Even though the binding of G proteins reduces Ca^{2+} current by only 45% by the end of the burst, the subsequent reduction in transmitter release is 83%. That is, release is reduced much more than the Ca^{2+} current, reflecting the superlinear relation between transmitter release and Ca^{2+} current.

Figure 3A shows that the Ca^{2+} channels become partially disinhibited during the burst of impulses, and it is expected that this disinhibition will increase transmitter release superlinearly. Indeed, we see in Fig. 3C that facilitation is greatly amplified by the agonist. The enhancement increases with each impulse, so that by the end of the burst facilitation in the presence of agonist is larger by a factor of 11 than facilitation in the absence of agonist. Thus, a fivefold increase in Ca^{2+} current due to disinhibition leads to an elevenfold increase in faciliation. This illustrates the prediction that the increase in facilitation will be considerably larger than the increase in Ca^{2+} current as Ca^{2+} channels become disinhibited.

Even when agonist binding is low, $B = 0.1$, disinhibition significantly enhances facilitation. In this case, 61% of the channels are initially in the reluctant state

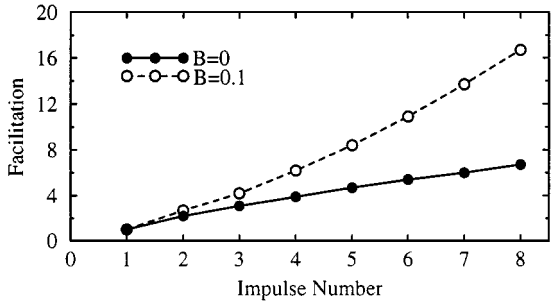

Fig. 4. Facilitation during a 100 Hz burst of long APs, in the absence (solid) and presence (dashed) of a low concentration of agonist ($B = 0.1$). Facilitation is significantly enhanced even though agonist binding is low.

C_{G1}, down from 88% in the last example. Thus, fewer channels can be converted to a willing state, and the enhancement of Ca^{2+} current and facilitation due to disinhibition will be less than before. In spite of this, facilitation is still amplified by close to a factor of three by the end of the burst of impulses (Fig. 4).

Short Impulses. In the absence of G protein agonists, it is expected that there will be less facilitation during bursts of short APs than during bursts of long APs. This is because there is less Ca^{2+} influx during short APs, and there will be less of an accumulation of partially bound release sites. This is demonstrated in Fig. 5 (solid curve), where facilitation is produced by a 100 Hz burst of short APs ($\lambda = 0.67$). Here facilitation at the end of the burst is about half of that produced by long APs (compare with Fig. 4). Since there are several possible physiological mechanisms for the modulation of AP duration (e.g., Ca^{2+} activated K^+ channels or G

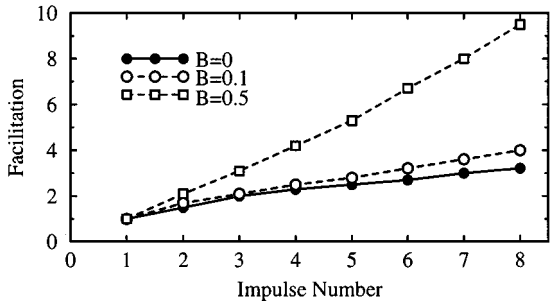

Fig. 5. Facilitation during a 100 Hz burst of short APs, in the absence (solid) and presence (dashed) of agonist. There is very little amplification of facilitation when agonist binding is low ($B = 0.1$), but the amplification is substantial with greater agonist binding ($B = 0.5$).

protein regulated K^+ channels), this suggests that the properties of facilitation may be dynamically altered even in the absence of G-protein-mediated inhibition of Ca^{2+} channels.

As with bursts of long APs, facilitation during bursts of short APs is amplified by a G protein agonist (Fig. 5), although the amplification is much less profound (compare with Figs. 3 and 4). By the end of the burst, facilitation is amplified by a factor of 1.3 with low agonist binding and 3.1 with high agonist binding, compared with factors of 2.6 and 11 during bursts of long APs.

In summary, bursts of short APs produce less facilitation than long APs in the absence of agonist. When a G protein agonist is present, this disparity is accentuated by the lower disinhibition induced by short APs.

Scenario 2: Half of the Channels Regulated by Constant Agonist

Both N and P type Ca^{2+} channels are regulated by G proteins (Hille, 1994), and many synaptic terminals contain both channel types (Mintz et al., 1995; Reid et al., 1998; Smith and Cunnane, 1997; Wu and Saggau, 1994b). However, G protein agonists are highly selective, and it is often the case that a specific agonist regulates only one channel type, and the channel type regulated is different for different synapses (see Wu and Saggau, 1997, for review). In addition, there is now evidence that only those channels physically linked to release sites by the protein syntaxin are susceptible to modulation by G proteins (Stanley and Mirotznik, 1997). In this section we consider the scenario in which half of the Ca^{2+} channels are regulated by G proteins, while the other half are insensitive. As before, each release site is associated with a single Ca^{2+} channel. However, there are now two separate populations of channels and associated release sites, one subject to regulation and the other insensitive to regulation. We first consider the case in which regulated and unregulated channels lie at equal distances from the release sites, and then we examine the effects on the enhancement of facilitation of locating the unregulated channels farther away.

We refer to the channel/release site complexes subject to regulation as population 1, and those insensitive to regulation as population 2. The distances between channels and release sites for the two populations are r_1 and r_2, respectively (Fig. 6). Assuming that the channel/release site complexes are divided equally between

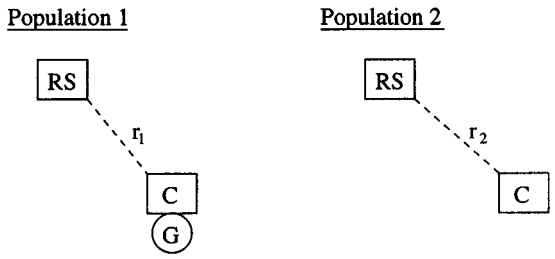

Fig. 6. Diagram of channel/release site complexes in Scenario 2. RS = release site, C = channel, G = G protein. Half of the channel/release site complexes in the model presynaptic terminal belong to population 1, and half belong to population 2.

the two populations, we compute the mean probability of release as

$$\bar{R} = (R_1 + R_2)/2, \tag{2}$$

where R_1 and R_2 are the release probabilities for the two populations. Both R_1 and R_2 are computed as before, with agonist present (population 1) or absent (population 2). Also, facilitation for each population is computed as before, while mean facilitation is computed as the facilitation of $\bar{R}$.

Facilitation of release produced during a 100 Hz burst of impulses is shown in Fig. 7. For the unregulated population, facilitation grows to a value close to 7 by the end of the burst. Because of Ca^{2+} channel disinhibition, facilitation is much greater for the regulated population, growing to nearly 17 by the end of the burst. In spite of this significant enhancement of facilitation for population 1, the mean probability of release, $\bar{R}$, facilitates only slightly more than the release of the unregulated population. This is because

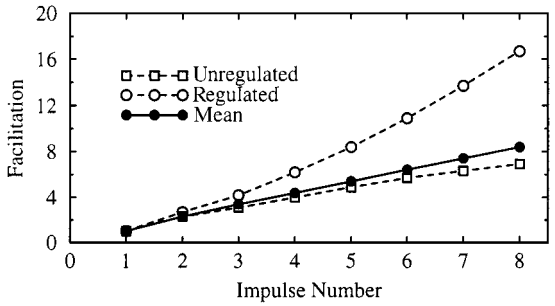

Fig. 7. Facilitation produced by a 100 Hz burst of long APs. One population of channel/release site complexes is regulated by a low concentration ($B = 0.1$) of G protein agonist. The other population is unregulated. The facilitation of mean release ($\bar{R}$) is only slightly more than that of the unregulated population. Channels lie at equal distances from release sites, $r_1 = r_2 = 10$ nm.

the probability of release for population 1 is smaller than that for population 2 due to the presence of reluctant channels. Consequently, the unregulated population contributes more to the mean release than does the regulated population, and facilitation of $\bar{R}$ will be similar to facilitation of R_2.

Figure 7 demonstrates that if regulated and unregulated channels lie at equal distances from release sites then there will be little G-protein-induced amplification of facilitation. However, the contribution to mean release of regulated channels should be increased if they are located closer to release sites than unregulated channels. In this case, there should be greater amplification of facilitation. We investigate this next, setting $r_1 = 10$ nm and $r_2 = 10, 20, 30, 40,$ or 50 nm. For simplicity, we examine facilitation only at the eighth impulse, the last in the burst, and we define the *mean amplification* E_8 as

$$E_8 = \frac{F_8(\bar{R})}{F_8(R_2)}, \tag{3}$$

the ratio of facilitation of mean release to facilitation of unregulated release at the eighth impulse. Thus, a mean amplification of two means that facilitation of mean release is twice as large in the presence of agonist than in its absence.

With a 100 Hz burst of short APs the mean amplification is small. When agonist binding is high ($B = 0.5$), the mean amplification reaches a value of 2 when $r_2 = 50$ nm (Fig. 8). When agonist binding is low ($B = 0.1$), the mean amplification is negligible for r_2 up to 50 nm (not shown). When the APs have long duration, the mean amplification is again small if $r_2 = 10$ or 20 nm. However, it grows rapidly with distance, particularly when agonist binding is high (Fig. 8).

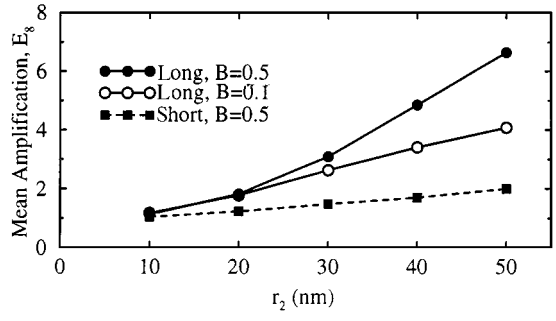

Fig. 8. Amplification of the facilitation of mean release (Eq. (3)) at the eighth impulse in a 100 Hz burst for a range of unregulated channel distances. Regulated channel distance is $r_1 = 10$ nm. Action potentials are long or short and agonist binding is high or low, as indicated.

In summary, G-protein-mediated amplification of facilitation is small when both regulated and unregulated channels are at equal distances from their release sites, in which case regulated channels contribute little to the mean release. Amplification is also small if action potentials are short, since in this case there is little disinhibition. However, if action potentials have a longer duration and if unregulated channels are located farther from the release sites, then facilitation can be greatly amplified by G protein agonists.

Scenario 3: Homosynaptic Inhibition

In the previous two scenarios it was assumed that the concentration of G protein agonist is constant in time. This assumption is reasonable for the majority of experiments on the presynaptic effects of G protein agonists, where the agonist is added to the bathing solution at a specified concentration. However, in physiological situations the agonist concentration affecting a synapse is determined largely by the amount of transmitter released from neighboring synapses (leading to *heterosynaptic inhibition*) and from the target synapse itself (leading to *homosynaptic inhibition*). For example, GABA released from a presynaptic terminal can diffuse to neighboring terminals and bind to presynaptic $GABA_B$ receptors (Dittman and Regehr, 1997) and can bind to autoreceptors in the releasing terminal itself (Chen and van den Pol, 1998). In this section we investigate the potential effects of an agonist binding to autoreceptors, assuming that all channels are subject to regulation as in Scenario 1.

We describe the binding of a transmitter molecule, T, to an autoreceptor by the following first-order kinetic scheme:

$$U + T \underset{k_a^-}{\overset{k_a^+}{\rightleftharpoons}} B,$$

where U and B are unbound and bound autoreceptors, respectively. For notational simplicity we also use U and B to denote the fraction of autoreceptors in the unbound and bound states, and we use T to denote the concentration of neurotransmitter in the synaptic cleft. Assuming that transmitter molecules are uniformly distributed in the cleft and are rapidly removed following the impulse by uptake or degradation, the concentration will be proportional to the release probability, R. Using a proportionality constant of 200 mM, the transmitter

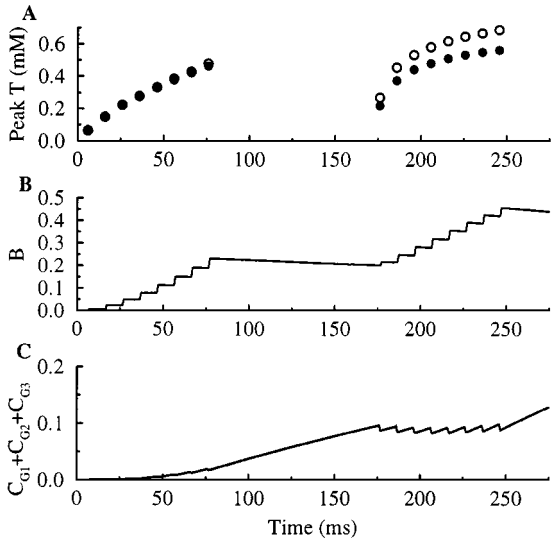

Fig. 9. Transmitter release in the absence (open circles) and presence (filled circles) of homosynaptic inhibition. Release is evoked by two 100 Hz bursts of long impulses, with an interburst interval of 100 ms. **A:** Peak concentration of transmitter released by each impulse. Transmitter concentration is computed as $T = 200R$. **B:** Fraction of bound autoreceptors. **C:** Fraction of channels in one of the three reluctant closed states.

concentration reaches values of several hundred micromolar (Fig. 9), consistent with the affinity of $GABA_A$ receptors (Destexhe et al., 1994). The values of agonist binding and unbinding rates, $k_a^+ = 0.2\,\mathrm{mM}^{-1}\,\mathrm{ms}^{-1}$ and $k_a^- = 0.0015\,\mathrm{ms}^{-1}$, are based on data on the binding kinetics of GABA to $GABA_B$ receptors (Dittman and Regehr, 1997). The fraction of bound autoreceptors, B, feeds directly into the forward kinetic rate k for the binding of G proteins to Ca^{2+} channels, as before.

Figure 9A shows the peak concentration of transmitter released during each impulse in two successive bursts. This is computed in the absence ($k_a^+ = 0$, open circles) and presence (filled circles) of autoreceptors. The stimulation protocol is a 100 Hz burst of eight impulses followed 100 ms later by an identical burst. In the absence of autoreceptors the release facilitates during the first burst and facilitates to a higher level during the second burst. The greater release during the second burst is due to Ca^{2+} bound to release sites during the first burst that remains bound at the start of the second. When autoreceptors are present, transmitter molecules released with each impulse bind to the receptors during both bursts and unbind slowly between bursts (Fig. 9B). This results in an accumulation of bound autoreceptors, which can potentially inhibit presynaptic Ca^{2+} channels. However, during the first burst there is very little channel inhibition (Fig. 9C), and transmitter release is only slightly reduced (Fig. 9A). This weak effect is due to the channel disinhibition that accompanies membrane depolarization. After the first burst, when the membrane is hyperpolarized, the fraction of channels in the reluctant state increases rapidly (Fig. 9C), so at the start of the second burst there is considerable channel inhibition and a significant reduction in transmitter release compared to the control. This reduction in release is maintained throughout the second burst, as the fraction of channels in a reluctant state remains relatively constant due to the competing effects of depolarization-induced disinhibition and the accumulation of bound autoreceptors.

Since channels enter a reluctant state primarily between bursts, it appears that the inhibitory effect of G protein binding on release will be greater with longer interburst intervals, provided that the autoreceptor unbinding rate is low. This is demonstrated in Fig. 10. The reduction in release compared to the control during the second burst is considerably greater with an 200 ms interburst interval (Fig. 10B) than with a shorter 30 ms interburst interval (Fig. 10A). Since transmitter molecules unbind from autoreceptors during the interburst, there will be an optimal interburst duration at which the competing effects of G protein binding to Ca^{2+} channels and agonist unbinding from

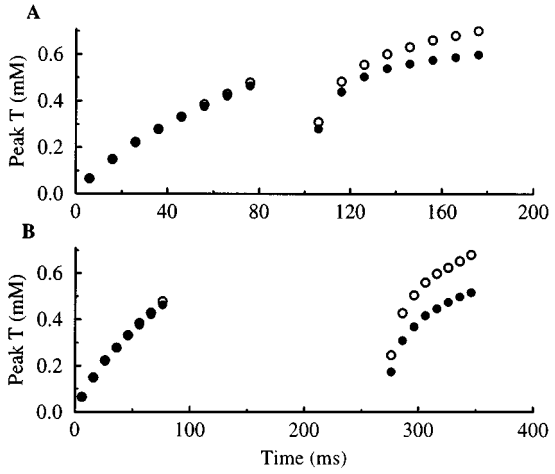

Fig. 10. Peak transmitter release evoked by 100 Hz bursts of impulses with an 30 ms interburst interval (**A**) and an 200 ms interburst interval (**B**), in the absence (open circles) and presence (filled circles) of autoreceptors. The inhibitory effect of G protein binding is more pronounced with the longer interburst interval, during which G proteins bind to Ca^{2+} channels and put them into a reluctant state.

autoreceptors will yield a maximum reduction in release during the second train. In our model this optimal interburst duration is 1600 ms for the 8-impulse, 100 Hz burst protocol used.

Figures 9 and 10 illustrate a striking difference between G-protein-mediated presynaptic inhibition and inhibition due to depletion of releasable vesicles, a phenomenon observed in many synapses. In the first case, the effects of the inhibitory factor (Ca^{2+} channel inhibition) become apparent only *after* the initiating burst, and these effects are *more pronounced* with longer interburst intervals (up to a certain point). In the case of vesicle depletion, the inhibitory factor (a reduction in the number of releasable vesicles) has an immediate effect on release *during* the initiating burst. In addition, between bursts the readily releasable pool of vesicles is replenished, so the depletion-mediated synaptic inhibition exhibited during the second burst will be *less pronounced* with longer interburst intervals.

Discussion

In this report we have examined some of the possible effects of G-protein-mediated Ca^{2+} channel inhibition on transmitter release and facilitation. Using a model of a G-protein-regulated Ca^{2+} channel coupled to a secretion model, we observed that channel inhibition leads to a reduction in transmitter release and an *increase* in facilitation of release during a short 100 Hz burst of impulses (Figs. 3–5). This amplification of facilitation is greater for larger agonist concentrations, in which case more Ca^{2+} channels are initially in a reluctant state. In addition, long action potentials produce a greater amplification of facilitation than do short ones, since channel disinhibition is greater. We also observed that the amplification of facilitation is significantly reduced when half of the channels are insensitive to inhibition. If the regulated and unregulated channels are located at equal distances from release sites, then there is very little amplification (Fig. 7). However, if unregulated channels are located at a greater distance from release sites, then facilitation can still be greatly amplified, particularly if impulses have a relatively long duration (Fig. 8). Finally, in simulations of G-protein-mediated homosynaptic inhibition we found that transmitter release is reduced primarily during the second of two successive bursts of impulses, highlighting the effects of depolarization-induced disinhibition of Ca^{2+} channels (Fig. 9). In addition, the degree of inhibition during the second burst is greater for longer interburst intervals up to a certain point (Fig. 10), beyond which the inhibitory effects become less pronounced. These effects are in contrast to the effects of depletion of the releasable vesicle pool, where release would be reduced during both bursts and where the degree of reduction during the second burst is less for longer interburst intervals. Also, facilitation is reduced, not amplified, by vesicle depletion.

There is a large body of data on G-protein-mediated inhibition and voltage-dependent disinhibition of Ca^{2+} current, and a growing body of data on the consequences of channel inhibition on transmitter release. These data reveal an important role for G protein agonists in the regulation of transmitter release in many synapses. However, there is little data on the effects of G protein agonists on short-term synaptic plasticity. Several studies have shown that G protein agonists enhance paired-pulse facilitation (Dittman and Regehr, 1997; Dunwiddie and Haas, 1985; Isaacson et al., 1993; Shen and Johnson, 1997), but this enhancement is small due to the limited disinhibition that can occur during a single conditioning impulse. Our modeling study predicts that the amplification of facilitation can be much greater during longer bursts, so G proteins can have a large effect not only on the amount of neurotransmitter released during a single impulse but also on the way in which the synapse facilitates during the burst. This latter effect is an example of *metaplasticity*, a higher-order form of short-term plasticity (Abraham and Bear, 1996).

Some synapses may be subject to tonic G-protein-mediated inhibition due to ambient levels of G protein agonists, in which case disinhibition can contribute to facilitation even in the absence of exogenous agonists. Several brain slice studies show evidence for tonic inhibition (Chen and van den Pol, 1998; Isaacson et al., 1993; Shen and Johnson, 1997; Wu and Saggau, 1994a); however, it is likely that the degree of tonic inhibition will vary from preparation to preparation. The possibility of tonic inhibition, and the subsequent enhancement of facilitation by disinhibition, suggests that one must be careful in the interpretation of facilitation data from brain slices.

This study suggests several predictions that may be tested experimentally. First, facilitation should be greater in the presence of G protein agonists than in their absence. As a corollary, ambient levels of agonists will enhance facilitation. Second, the increase in facilitation during a burst will be greater than the increase

in Ca^{2+} current as Ca^{2+} channels become disinhibited, due to the superlinear relation between transmitter release and Ca^{2+} current. Third, action potentials of a longer duration will produce greater facilitation. This is due both to the resulting increase in residual bound or free $Ca^{2+}$and to an increase in Ca^{2+} channel disinhibition. Fourth, when autoreceptors linked to activation of G proteins are present in the presynaptic terminal, homosynaptic (and presumably heterosynaptic) inhibition will be more pronounced in the second of two bursts of impulses, and this inhibition will be greater with longer interburst intervals (up to a point). Finally, since G protein Ca^{2+} channel inhibition occurs only in specific channel types in different synapses, Ca^{2+} blocking agents such as ω-conotoxins, ω-agatoxins, or dihydropyridines (Dunlap et al., 1995) could be used to separate the native facilitating properties of the release mechanism from the enhancement due to Ca^{2+} channel disinhibition.

The goal of this report is to illustrate some of the implications of G protein inhibition on transmitter release and facilitation. We have intentionally used simple models in this study, since simple models are more amenable to analysis and interpretation than more complex models, and since we are interested in generic properties of transmitter release. We stress that the results obtained in this report are intended only to illustrate some of the possible consequences of G protein inhibition and disinhibition and serve as predictions that should be tested experimentally.

The secretion model is based on several simplifying assumptions. It is assumed that each release site is colocalized with a single Ca^{2+} channel and is activated by the Ca^{2+} microdomain that forms when the channel opens. Although single-channel release can occur (Stanley, 1993; Yoshikami et al., 1989), it is more likely that a release site is influenced by Ca^{2+} from several colocalized channels (Borst and Sakman, 1996; Regehr and Mintz, 1994; Wu et al., 1999). However, extending the model to account for the effects of overlapping Ca^{2+} domains involves either greatly increasing the number of equations used to describe release (Bertram et al., 1999) or solving the Ca^{2+} diffusion equation, and for our purposes we prefer a simpler approach. The secretion model also assumes that the releasable vesicle pool is not depleted during a short burst of impulses. We make this assumption since we are interested in the effects of channel inhibition on synaptic facilitation, and including a mechanism for depletion could confound this analysis. It is also assumed that there are no mobile Ca^{2+} buffers and that all Ca^{2+} channels have an identical unitary conductance.

The final assumption is that synaptic facilitation is due to the slow unbinding of Ca^{2+} from the release sites, the "residual bound Ca^{2+}" hypothesis. An alternate hypothesis is that the probability of release is facilitated by free Ca^{2+} ions that accumulate in the synaptic terminal as a result of prior stimulation. Mathematical models have been developed for this "residual free Ca^{2+}" mechanism of facilitation (Fogelson and Zucker, 1985; Zucker and Fogelson, 1986) and for a hybrid of the free and bound Ca^{2+} mechanisms (Yamada and Zucker, 1992). At present there is evidence for and against the bound Ca^{2+} and free Ca^{2+} mechanisms (Blundon et al., 1995; Kamiya and Zucker, 1994; Winslow et al., 1994), and it is possible that facilitation is due to a combination of these mechanisms or to some other mechanism. For example, it has been suggested that synaptic enhancement is due largely to an activity-dependent increase in the size of the readily releasable vesicle pool (Worden et al., 1997) or to an activity-dependent increase in the number of active synapses contributing to release (Wojtowicz et al., 1994). Of course, facilitation may be produced by different mechanisms or combinations of mechanisms in different synapses.

The scenarios that we examine in this report are but a few of the many ways in which G protein agonists can regulate synaptic transmission. Rather than inactivating Ca^{2+} channels, agonists can activate presynaptic K^+ channels (Scholfield and Steel, 1988; Thompson and Gähwiler, 1992; Vaughan et al., 1997) or can regulate release at a step downstream of Ca^{2+} entry (Dittman and Regehr, 1996; Boehm and Betz, 1997). Agonists released at one synapse can inhibit neighboring synapses (Dittman and Regehr, 1997). Also, agonists can act on postsynaptic sites (Thompson and Gähwiler, 1992). Thus, a picture emerges in which the properties of synaptic transmission and plasticity are regulated by a variety of agents and through a variety of mechanisms, greatly enhancing the information processing capacity of neural systems.

Appendix

Equations for Membrane Potential

Action potentials are generated with the Hodgkin-Huxley (1952) equations by applying periodic 1 ms

current pulses of magnitude $I_{ap} = 30\ \mu\text{Acm}^{-2}$. The equations describing membrane potential and Na^+ and K^+ gating variables are

$$C_m \frac{dV}{dt} = -[\bar{g}_{Na} x^3 h(V - V_{Na}) + \bar{g}_K n^4 (V - V_K) + \bar{g}_l (V - V_l) - I_{ap}] \quad (4)$$

$$\frac{dx}{dt} = \lambda(x_\infty(V) - x)/\tau_x(V) \quad (5)$$

$$\frac{dn}{dt} = \lambda(n_\infty(V) - n)/\tau_n(V) \quad (6)$$

$$\frac{dh}{dt} = \lambda(h_\infty(V) - h)/\tau_h(V), \quad (7)$$

where $x_\infty(V) = \alpha_x/(\alpha_x + \beta_x)$, $\tau_x(V) = 1/(\alpha_x + \beta_x)$ (similarly for n_∞, h_∞, τ_n, and τ_h) and $\alpha_x = 0.2(V + 40)/[1 - e^{-(V+40)/10}]$, $\beta_x = 8\,e^{-(V+65)/18}$, $\alpha_n = 0.02(V+55)/[1-e^{-(V+55)/10}]$, $\beta_n = 0.25\,e^{-(V+65)/80}$, $\alpha_h = 0.14\,e^{-(V+65)/20}$, and $\beta_h = 2/[1 + e^{-(V+35)/10}]$. Here $C_m = 1\ \mu\text{Fcm}^{-2}$ is the membrane capacitance; $\bar{g}_{Na} = 120$, $\bar{g}_K = 36$, $\bar{g}_l = 0.3$ are the maximum conductances for the sodium, potassium, and leakage currents (in mScm^{-2}); and $V_{Na} = 50$, $V_K = -77$, and $V_l = -54$ are the corresponding reversal potentials (in mV). We assume that Ca^{2+} current is affected by, but does not affect, the duration of action potentials. This is consistent with findings in several synapses (Sabatini and Regehr, 1997; Takahashi et al., 1998). Action potential duration is adjusted with the parameter λ. Long and short APs are generated with $\lambda = 1$ and $\lambda = 0.67$, respectively.

Equations for the Ca^{2+} Channel and Secretion Models

The differential equations for the Ca^{2+} channel and transmitter secretion models follow directly from the kinetic schemes described in the text by application of the law of mass action. The Ca^{2+} channel equations are

$$\frac{dC_1}{dt} = \beta C_2 + l C_{G1} - (4\alpha + k) C_1 \quad (8)$$

$$\frac{dC_2}{dt} = 4\alpha C_1 + 2\beta C_3 + 64 l C_{G2} - (\beta + 3\alpha + k) C_2 \quad (9)$$

$$\frac{dC_3}{dt} = 3\alpha C_2 + 3\beta C_4 + (64)^2 l C_{G3} - (2\beta + 2\alpha + k) C_3 \quad (10)$$

$$\frac{dC_4}{dt} = 2\alpha C_3 + 4\beta m - (3\beta + \alpha) C_4 \quad (11)$$

$$\frac{dC_{G1}}{dt} = \beta' C_{G2} + k C_1 - (4\alpha' + l) C_{G1} \quad (12)$$

$$\frac{dC_{G2}}{dt} = 4\alpha' C_{G1} + 2\beta' C_{G3} + k C_2 - (\beta' + 3\alpha' + 64 l) C_{G2} \quad (13)$$

$$\frac{dC_{G3}}{dt} = 3\alpha' C_{G2} + k C_3 - (2\beta' + (64)^2 l) C_{G3}, \quad (14)$$

and the open channel probability is given by the conservation relation $m = 1 - C_1 - C_2 - C_3 - C_4 - C_{G1} - C_{G2} - C_{G3}$.

The transmitter secretion equations are

$$\frac{dS_0}{dt} = k_1^- S_1 - 4k_1^+ Ca_d S_0 \quad (15)$$

$$\frac{dS_1}{dt} = 4k_1^+ Ca_d S_0 + 2k_2^- S_2 - (3k_2^+ Ca_d + k_1^-) S_1 \quad (16)$$

$$\frac{dS_2}{dt} = 3k_2^+ Ca_d S_1 + 3k_3^- S_3 - (2k_3^+ Ca_d + 2k_2^-) S_2 \quad (17)$$

$$\frac{dS_3}{dt} = 2k_3^+ Ca_d S_2 + 4k_4^- S_4 - (k_4^+ Ca_d + 3k_3^-) S_3, \quad (18)$$

and the probability that the site is in the completely bound state is given by the conservation relation $S_4 = 1 - S_0 - S_1 - S_2 - S_3$.

Parameters for the Ca^{2+} Channel and Secretion Models

The forward and backward kinetic rates for the Ca^{2+} channel model are from Boland and Bean (1993). They are (in ms^{-1})

$$\alpha = 0.9e^{V/22}, \quad \beta = 0.03e^{-V/14} \quad (19)$$

$$\alpha' = \alpha/8, \quad \beta' = 8\beta. \quad (20)$$

The G protein unbinding and binding rates are (in ms^{-1}) $l = 0.00025$ and:

$$k = \frac{0.3B}{68 + 32B}. \quad (21)$$

The Ca^{2+} binding and unbinding kinetic rates for the secretion model are from Bertram et al. (1999) and were

chosen according to data from the squid giant synapse (Stanley, 1986). These rates would likely be different for different synapses. The binding rates used are (in $ms^{-1}\mu M^{-1}$) $k_1^+ = 9.375 \times 10^{-4}$, $k_2^+ = 1.25 \times 10^{-3}$, $k_3^+ = 1.875 \times 10^{-3}$, and $k_4^+ = 3.75 \times 10^{-3}$. The unbinding rates are (in ms^{-1}) $k_1^- = 4 \times 10^{-4}$, $k_2^- = 5 \times 10^{-4}$, $k_3^- = 3.33 \times 10^{-2}$, $k_4^- = 2.5$. Initial conditions for the release and membrane potential state variables are determined by allowing the system to relax to equilibrium in the absence of applied current.

Domain Calcium Concentration

A Ca^{2+} microdomain forms and equilibrates within microseconds of the opening of a Ca^{2+} channel (Simon and Llinás, 1985). A steady-state formula for domain Ca^{2+} concentration at an open channel is therefore appropriate for use with the present model, which assumes that each release site is affected by a single colocalized Ca^{2+} channel. The steady-state formula used here is from Neher (1986), and it is assumed that mobile Ca^{2+} buffers are not present:

$$Ca_{d,open} = \sigma/(2\pi D_c r), \tag{22}$$

where $D_c = 220\ \mu m^2\,sec^{-1}$ is the Ca^{2+} diffusion coefficient (Allbritton et al., 1992), $r = 10$ nm is the assumed distance from the channel to the release site (unless stated otherwise), and $\sigma = -5.182 \cdot i(V)$ is the Ca^{2+} flux through the channel (Bertram et al., 1999), where $i(V)$ is the single-channel current determined by the Goldman-Hodgkin-Katz formula (Goldman, 1943):

$$i(V) = \hat{g}_{Ca} P \frac{2FV}{RT}\left[\frac{Ca_{ex}}{1 - \exp(2FV/RT)}\right]. \tag{23}$$

For the single-channel conductance we used $\hat{g}_{Ca} = 12$ pS; for the concentration to membrane potential conversion factor we used $P = 6\ mVmM^{-1}$; for the thermal voltage we used $RT/F = 26.7$ mV; and for the external Ca^{2+} concentration we used $Ca_{ex} = 2$ mM. For a discussion on single and overlapping Ca^{2+} microdomains see Bertram et al. (1999).

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