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CO-REGULATION OF cAMP-ACTIVATED Na^+ CURRENT BY Ca^{2+} IN NEURONES OF THE MOLLUSC *PLEUROBRANCHAEA*

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SUMMARY

1. The cAMP-gated Na^+ current ($I_{Na,cAMP}$) was studied in axotomized neurons of the pedal ganglion of the sea slug *Pleurobranchaea*. $I_{Na,cAMP}$ responses were elicited by iontophoretic injection of cAMP and recorded in voltage clamp.

2. The current–voltage relation for $I_{Na,cAMP}$ was flat between -90 and -50 mV, but declined steeply with depolarization from -50 to -30 mV. Depolarizing pulses also suppressed the $I_{Na,cAMP}$ response, which recovered slowly over tens of seconds.

3. The inactivating effects of depolarization on the current were abolished both by blockade of Ca^{2+} current and intracellular injection of Ca^{2+} chelator. Thus, Ca^{2+} influx through voltage-dependent Ca^{2+} channels probably mediates inactivation of $I_{Na,cAMP}$ within its normal physiological range of action.

4. Increasing intracellular cAMP levels antagonized the effects of Ca^{2+} influx on $I_{Na,cAMP}$. The mutual antagonism of the ions suggests that cAMP and Ca^{2+} act competitively in regulation of the $I_{Na,cAMP}$ channel.

5. Measures of fractional inactivation of $I_{Na,cAMP}$ provided evidence for the existence of an appreciable basal level of current, and hence cAMP, in the unstimulated neuron. Since $I_{Na,cAMP}$ is a direct function of cAMP activity, measures of fractional inactivation permit quantification of cAMP levels in the living neuron.

6. Calcium inactivation of $I_{Na,cAMP}$ completes a negative feedback loop that can contribute to endogenous burst activity. Over the burst cycle, depolarization and action potential activity driven by $I_{Na,cAMP}$ would lead to Ca^{2+} influx, consequent inactivation of the inward current, and hyperpolarization. This mechanism of endogenous bursting resembles others in which the burst cycle has been found to be regulated by kinetics of Ca^{2+} influx and removal. However, $I_{Na,cAMP}$ may vary in its Ca^{2+} sensitivity in different neurons and these variations may affect the functional expression of endogenous oscillatory activity.

INTRODUCTION

A slow Na^+ current activated by cAMP ($I_{Na,cAMP}$) is a prominent neuromodulatory feature in many molluscan neurons (Liberman, Minina & Golubtsov, 1975; Aldenhoff, Hofmeier, Lux & Swandulla, 1983; Green & Gillette, 1983; Kononenko, Kostyuk &

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Shcherbatko, 1983; Connor & Hockberger, 1984; Hara, Sawada & Maeno, 1985; Huang & Gillette, 1986; Swandulla, 1987; McCrohan & Gillette, 1988; Ichinose & McAdoo, 1989; Kehoe, 1990*a*). It contributes to endogenously rhythmic bursting mechanisms in single neurons (Green & Gillette, 1983), to aspects of behavioural arousal in neuronal networks (Gillette, 1988), and to synaptic mechanisms of neuromodulation (Huang & Gillette, 1986; Kehoe, 1990*b*). The prominence and importance of the current invite the further study of its regulation and roles in the nervous system.

The kinetics of the $I_{Na,cAMP}$ response to injected cAMP are determined largely by cAMP diffusion within the neuron and its degradation by phosphodiesterase activity (Huang & Gillette, 1991). Resistance of the current to kinase inhibitors and activation of the channel in the inside-out patch by cAMP in the absence of ATP argue against a role of phosphorylation in the activation of the current and suggest that the channel is directly gated by cAMP (Huang & Gillette, 1989), like that of the olfactory epithelium of vertebrates. Hill plots suggest that the stoichiometry of activation of $I_{Na,cAMP}$ is one molecule of cAMP to one $I_{Na,cAMP}$ channel (Huang & Gillette, 1991).

$I_{Na,cAMP}$ may comprise several subspecies of channel that are expressed differentially, since the voltage dependence of the current differs markedly among identified cells. In some molluscan neurons the $I_{Na,cAMP}$ response to injected cAMP is enhanced by depolarization within its normal functional range (−60 to −20 mV) (Green & Gillette, 1983; Connor & Hockerberg, 1984; Hara *et al.* 1985; Ichinose & McAdoo, 1989). In other neurons the size of the current does not change between −100 and −20 mV (Aldenhoff *et al.* 1983; Connor & Hockberger, 1984; McCrohan & Gillette, 1988). In still others, the current declines with depolarization from −50 to −30 mV (Kononenko *et al.* 1983; Huang & Gillette, 1986).

Previously, it was shown that the enhancement of $I_{Na,cAMP}$ by depolarization in buccal ganglion neurons of *Pleurobranchaea* was caused by a depolarization-sensitive blockade effect of extracellular Ca^{2+} (Gillette & Green, 1987). In the present study our goal was to describe the factors influencing the opposite voltage dependence of neurons in which $I_{Na,cAMP}$ decreases with depolarization, with particular attention to the role of Ca^{2+}. The experimental results suggest a novel interaction between cAMP and intracellular Ca^{2+}, whose actions are reciprocally antagonistic. The functional consequence is that $I_{Na,cAMP}$ is activated by intracellular Ca^{2+}. The resulting feedback loop could support endogenous bursting activity.

METHODS

Specimens of *Pleurobranchaea californica* were provided by Dr Rimmon C. Fay of Pacific BioMarine, Venice, CA, and Mr Michael Morris of Sea-life Supply, Sand City, CA, USA, and were maintained in artificial sea water at 13–15 °C. Pedal ganglia were dissected into saline where a group of eight to ten large and spheroidal (diameter 100–300 μm) neuron somata was isolated and stabilized on Sylgard with insect pins. While differing in axon paths and spontaneous activity *in situ*, the neurons in isolation possess in common a $I_{Na,cAMP}$ sensitive to low iontophoretic currents of cAMP. Extracellular saline composition was (mM): NaCl, 420; $MgSO_4$, 25; $MgCl_2$, 25; KCl, 10; $CaCl_2$, 10; and 3-*N*-(morpholino)propanesulphonic acid (MOPS), 10; adjusted to pH 7·5 with NaOH at 15 °C. For experiments requiring ion substitutions, equimolar arginine was substituted for Na^+, and Mg^{2+} or Co^{2+} was substituted for Ca^{2+}.

For voltage clamping, a pedal neuron was impaled with a single-barrelled voltage electrode and

a double-barrelled electrode. One of the double barrels, filled with a solution of 3 M KCl, served to pass current. The other barrel was filled with 0·2 M cAMP and 20 mM Tris buffer, adjusted to pH 7·3 with KOH, for iontophoretic injection of cAMP.

cAMP was iontophoretically injected at constant current. Injection pulses of cAMP were typically 5 s long. For experiments requiring a higher background of intracellular cAMP, the nucleotide was continuously iontophoresed to elicit a steady background $I_{Na,cAMP}$ (I_s). Most experiments were performed at holding potentials between −50 and −30 mV, where the $I_{Na,cAMP}$ response showed steepest voltage dependence.

The Ca^{2+} chelator, 1,2-bis(*O*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA; Sigma, USA) was buffered at pH 7·35 in 0·2 M KCl with a final concentration of 0·2 M and was pressure-injected intracellularly from a third microelectrode.

Quantifying depolarizing pulse suppression of $I_{Na,cAMP}$

Short depolarizing pulses from −50 mV to more positive potentials were applied to study suppressive effects of Ca^{2+} influx on the $I_{Na,cAMP}$ response. The extent of suppression was defined as: percentage suppression = $(1-I_{t,d}/I_t)\times 100$, where I_t was the test $I_{Na,cAMP}$ response amplitude recorded before a depolarizing pulse and $I_{t,d}$ was the $I_{Na,cAMP}$ response recorded after a depolarizing pulse. Since $I_{t,d}$ was recorded during a depolarizing pulse-induced tail current, its amplitude was determined by subtracting the tail current. The same procedure was used to determine the suppression at varying background levels of injected cAMP.

RESULTS

The current–voltage relation

The $I_{Na,cAMP}$ response of the pedal neurons showed a steep voltage dependence between −30 and −50 mV, increasing in amplitude with hyperpolarization, and reaching a steady level at voltages more negative than −50 mV, up to −90 mV (Fig. 1*A* and *B*). Previous work has shown that over this voltage range $I_{Na,cAMP}$ is almost exclusively carried by Na^+ (Gillette & Green, 1987; Huang, 1989). At −50 mV the $I_{Na,cAMP}$ response amplitude was about 2·3 times larger than at −30 mV, a magnitude too large to be fully explained by the estimated 1·3-fold increase in EMF for Na^+.

Effects of extracellular Ca^{2+} *and intracellular BAPTA injection on* $I_{Na,cAMP}$ *voltage dependence*

Substitution of extracellular Ca^{2+} by Mg^{2+} had two major effects. It increased the peak amplitude of $I_{Na,cAMP}$ at all voltages, and it also abolished the steep voltage sensitivity of the current between −30 and −50 mV (Fig. 2*A* and *B*). Intracellular injection of the Ca^{2+} chelator BAPTA also abolished the steep voltage dependence of the $I_{Na,cAMP}$ response (Fig. 2*C* and *D*). As described further below, BAPTA injection could also reduce the amplitude of the response.

The effect common to both extracellular Ca^{2+} substitution and chelation of intracellular Ca^{2+} was a blockade of the voltage sensitivity of the $I_{Na,cAMP}$, consistent with an origin of voltage dependence in intracellular Ca^{2+} concentrations, mediated by membrane Ca^{2+} channels.

Mixed effects of intracellular BAPTA injection on the holding current and the $I_{Na,cAMP}$ *response*

In each of thirty pedal neurons tested, intracellular injection of BAPTA induced a steady inward current. However, the effect of BAPTA injection on the $I_{Na,cAMP}$ response amplitude was variable: it could be either suppressed or enhanced (Fig. 3). The result apparently depended on the amount of chelator injected.

In general, a relatively small BAPTA injection induced a small inward current and caused an increase in $I_{Na,cAMP}$ amplitude (Fig. 3*A*). For a larger BAPTA injection, the BAPTA-induced inward current was larger and the $I_{Na,cAMP}$ amplitude was reduced (Fig. 3*B*). In three experiments in which BAPTA injection induced a

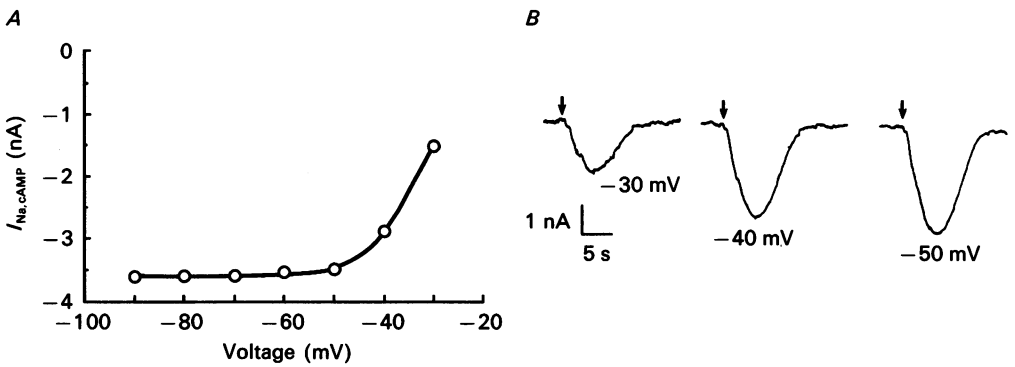

Fig. 1. The current–voltage relation of the $I_{Na,cAMP}$ response to a 5 s injection of cAMP. *A*, the peak amplitude of $I_{Na,cAMP}$ increases with more negative potentials from −30 to −50 mV, and is stable at more hyperpolarized potentials. *B*, $I_{Na,cAMP}$ response records obtained at −30, −40 and −50 mV. Arrows mark the onset of cAMP injection.

relatively large inward current of several nA, the activation of $I_{Na,cAMP}$ was completely suppressed. These results are interpretable in terms of removal of Ca^{2+} inactivation of $I_{Na,cAMP}$. Thus, small injections of BAPTA would open a larger population of channels to activation by injected cAMP, thereby increasing the current response. However, larger BAPTA injections could open the channel population to saturating binding of endogenous cAMP by more extreme lowering of intracellular Ca^{2+}, causing the observed suppression of $I_{Na,cAMP}$ responses to cAMP injection.

Depolarizing pulse inactivation of $I_{Na,cAMP}$ and its antagonism by cAMP

Short depolarizing pulses preceding cAMP injection suppressed $I_{Na,cAMP}$ response amplitude (Fig. 4*A*), an effect that recovered only over some tens of seconds. However, this effect was antagonized by continuous iontophoresis of cAMP, tonically increasing the background level of $I_{Na,cAMP}$ upon which the test pulse of cAMP was superimposed (Fig. 4*B*). In such a case, the amplitude of the $I_{Na,cAMP}$ response following a depolarizing pulse could even be augmented over the prepulse response amplitude. Under these conditions also, a slowly decaying outward tail current followed the depolarizing pulse.

The extent of suppression of the $I_{Na,cAMP}$ response was found to depend on the prepulse duration, the prepulse potential, and the latency from prepulse to injection of cAMP. Increasing prepulse duration from 10 to 100 ms monotonically decreased the response recorded 5 s later by up to 80% (Fig. 5*A*). Varying prepulse magnitude from −30 to +130 mV yielded a biphasic curve for suppression with a maximum

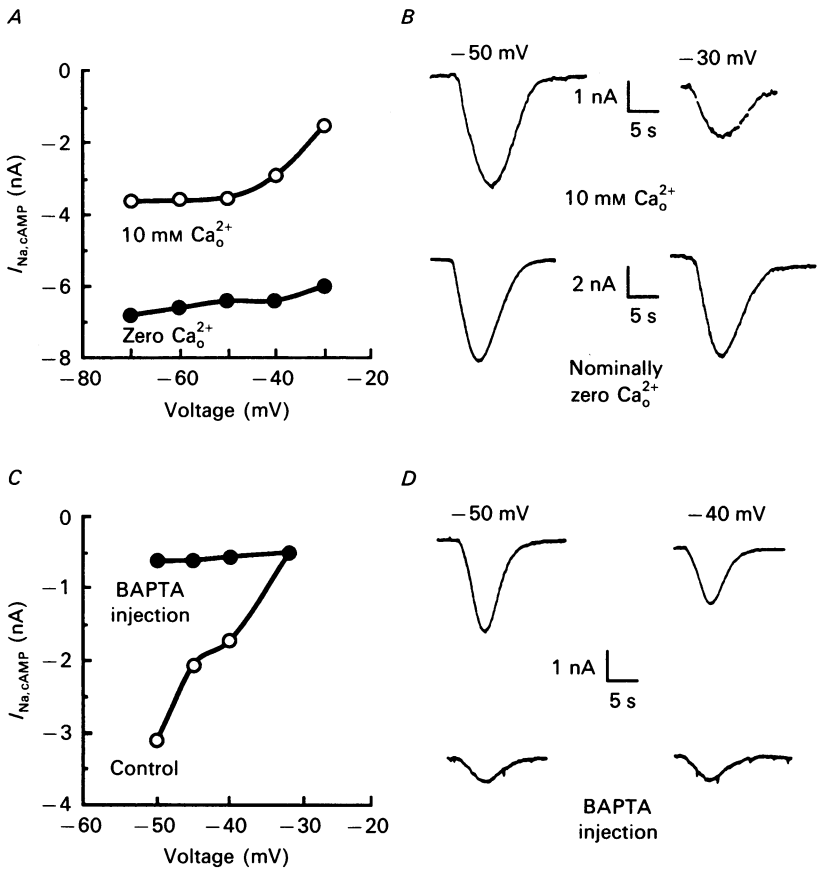

Fig. 2. Ca^{2+} dependence of voltage sensitivity. *A*, the steep voltage dependence of $I_{Na,cAMP}$ amplitude between −50 and −30 mV was abolished in zero Ca^{2+} saline. *B*, $I_{Na,cAMP}$ responses recorded in 10 mM and nominally zero Ca^{2+} salines at holding potentials of −50 and −30 mV. *C*, intracellular BAPTA injection caused the current–voltage relation to become virtually flat. Larger BAPTA injections also decreased $I_{Na,cAMP}$ amplitude (cf. Fig. 3). *D*, $I_{Na,cAMP}$ responses before and after BAPTA injection.

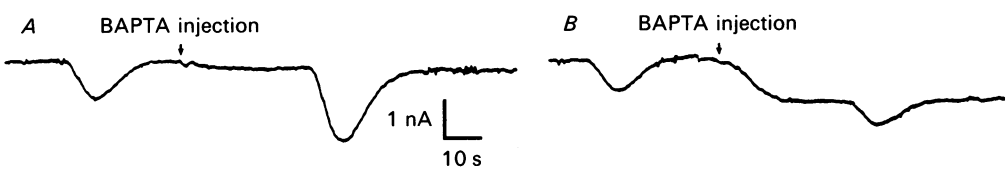

Fig. 3. Biphasic effects of BAPTA injection on $I_{Na,cAMP}$ response amplitude. *A*, an initial BAPTA injection induced a small persistent inward current, and enhanced the peak amplitude of $I_{Na,cAMP}$. *B*, a subsequent, larger BAPTA injection induced a larger inward current, but reduced the peak amplitude of $I_{Na,cAMP}$.

near +40 mV (Fig. 5*B*). The response recovered over tens of seconds following the depolarizing pulse (Fig. 5*C*). These results are consistent with the kinetics of intracellular Ca^{2+} accumulation and clearance following activation of voltage-

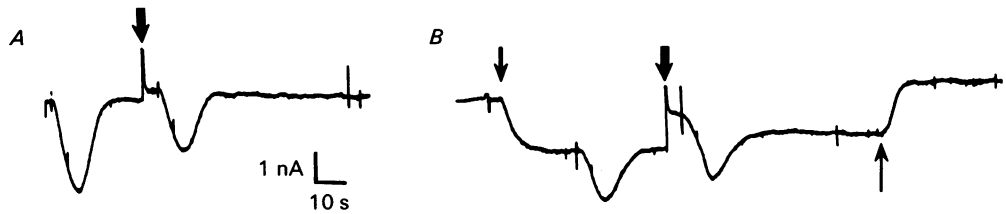

Fig. 4. Depolarizing voltage pulses suppress $I_{Na,cAMP}$ amplitude, an effect reversed by increasing cAMP levels. *A*, a 100 ms command pulse from −50 to +10 mV (arrow) suppressed peak amplitude of the $I_{Na,cAMP}$ response by 36%. *B*, continuous iontophoresis of cAMP induced a steady background current (onset at the small downward arrow, off at the upward arrow); the superimposed $I_{Na,cAMP}$ response was reduced in amplitude. The same depolarizing prepulse as in *A* (large arrow) generated a larger tail current and actually enhanced the $I_{Na,cAMP}$ response.

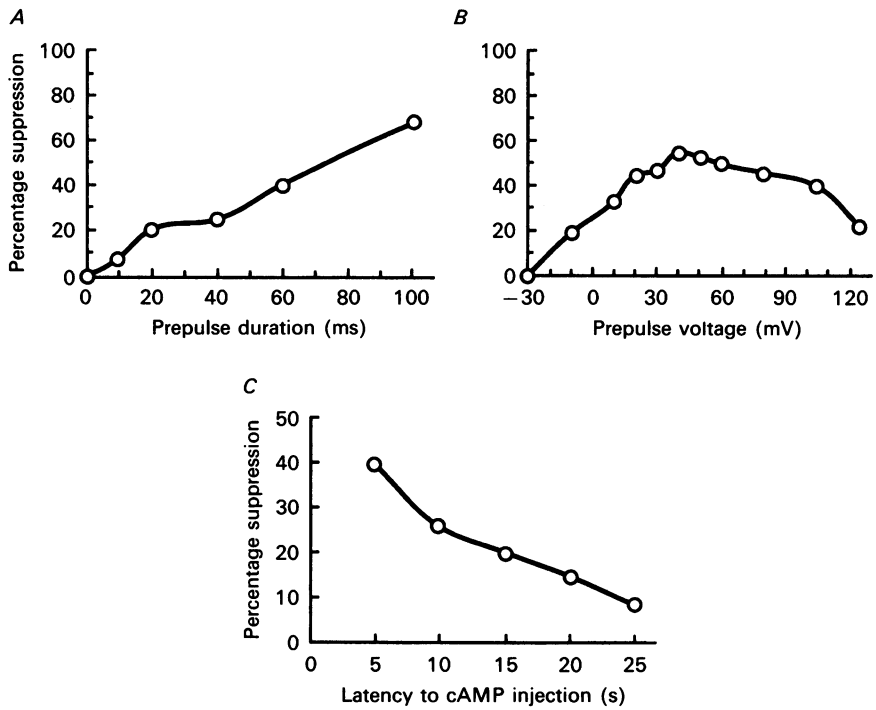

Fig. 5. Dependence of the $I_{Na,cAMP}$ suppression on prepulse amplitude and duration. *A*, increasing depolarizing pulse duration from 10 to 100 ms increased extent of suppression. The prepulse was stepped from −50 to +10 mV. *B*, the plot of suppression against the depolarizing strength. Prepulse duration, 100 ms. *C*, increasing the latency from pulse to cAMP injection decreased suppression.

dependent Ca^{2+} current; they suggested a Ca^{2+} mechanism for inactivation of $I_{Na,cAMP}$ like that of the voltage dependence.

The dependence of depolarizing pulse suppression of the $I_{Na,cAMP}$ response on Ca^{2+} influx was tested in three ways: by lowering extracellular Ca^{2+} (Mg^{2+}

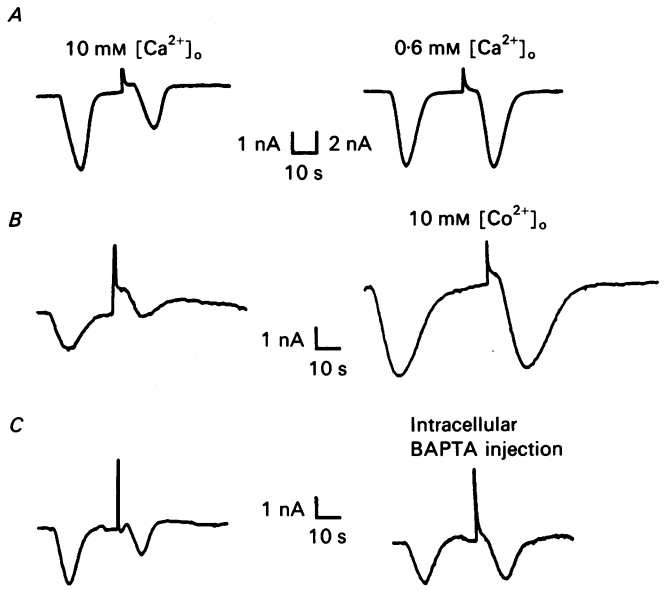

Fig. 6. Intracellular Ca^{2+} origin of depolarizing pulse suppression. A depolarizing pulse from -50 to $+10$ mV for 100 ms suppressed $I_{Na,cAMP}$ as much as 45%, but had no effect in 0·6 mM Ca^{2+} (*A*), or Co^{2+}-substituted saline (*B*), or after intracellular BAPTA injection (*C*).

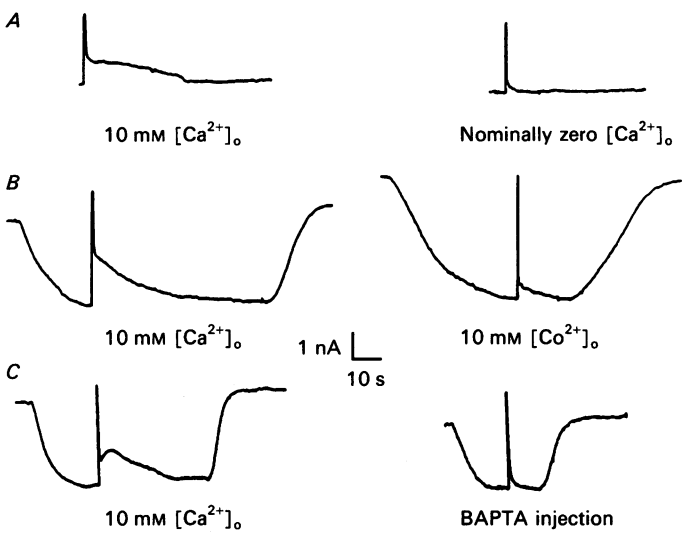

Fig. 7. Ca^{2+} dependence of the depolarizing pulse-induced decaying outward tail current. *A*, the tail current, activated by a depolarizing pulse in the absence of cAMP injection (left), was abolished in nominally zero Ca^{2+}, Mg^{2+}-substituted saline (right). In this particular experiment the tail current was prominent without injection of exogenous cAMP. *B*, tail currents superimposed on steady background cAMP currents were also abolished in Co^{2+}-substituted saline. *C*, suppression of tail current by intracellular BAPTA injection.

substitution), by substitution of Ca^{2+} by the Ca^{2+} current blocker Co^{2+}, and by intracellular injection of the Ca^{2+} chelator BAPTA. Each of these treatments abolished the depolarizing pulse suppression of $I_{Na,cAMP}$ (Fig. 6*A–C*), presumably through the blockade of intracellular Ca^{2+} accumulation.

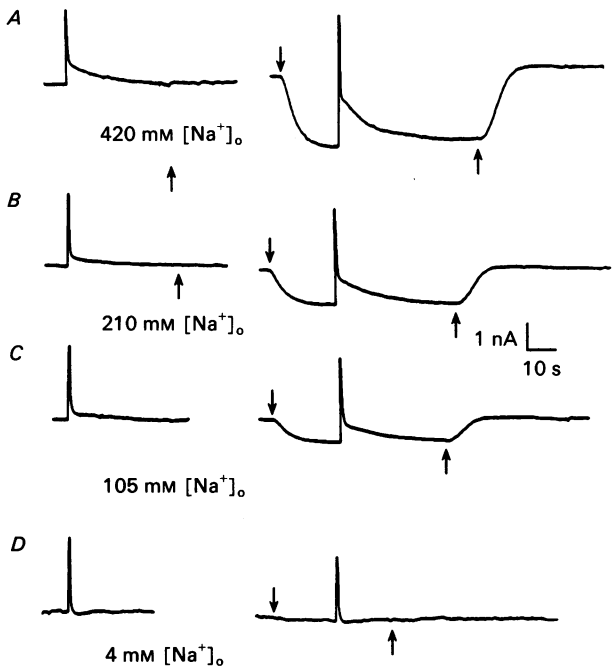

Fig. 8. Na^+ dependence of Ca^{2+}-induced outward tail current. Tail currents induced by depolarizing pulses (left-hand records) were increased in amplitude when superimposed on steady background $I_{Na,cAMP}$ induced by cAMP iontophoresis (right-hand records). Tail current amplitudes decreased in both conditions as Na^+ was substituted by arginine. *A*, normal Na^+ (420 mM); the peak amplitude of the tail currents decreased by half. *B*, 210 mM Na^+; tail current amplitude measured 2 s following the pulse was reduced by one-half. *C*, 105 mM Na^+; tail current amplitude reduced to one-quarter. *D*, 4 mM Na^+; no measurable tail current. Measurements were made at −50 mV, following a 50 ms depolarizing pulse from −50 to +10 mV. Arrows mark the onset and end of steady cAMP injections.

Divalent ion replacement and BAPTA injection also blocked the appearance of depolarizing tail currents following depolarizing pulses (Fig. 7*A–C*). The tail current itself proved to be Na^+ dependent. With decreasing extracellular Na^+ concentration it decreased in parallel with $I_{Na,cAMP}$ (Fig. 8). The tail current was thus a likely manifestation of the Ca^{2+} inactivation of $I_{Na,cAMP}$.

Competition of cAMP and Ca^{2+}

The data just discussed imply that the experimental results shown in Fig. 4 are a result of a mechanism for regulation of $I_{Na,cAMP}$ by cAMP and Ca^{2+} wherein the two ligands have mutually antagonistic actions. We examined the relationship between

cAMP and Ca^{2+} more closely in experiments where both variables were systematically changed. The extent of inactivation was measured following pulses of varying duration; the pulses were superimposed on different tonic levels of $I_{Na,cAMP}$ induced by cAMP iontophoresis. The resulting reduction of depolarizing pulse suppression of

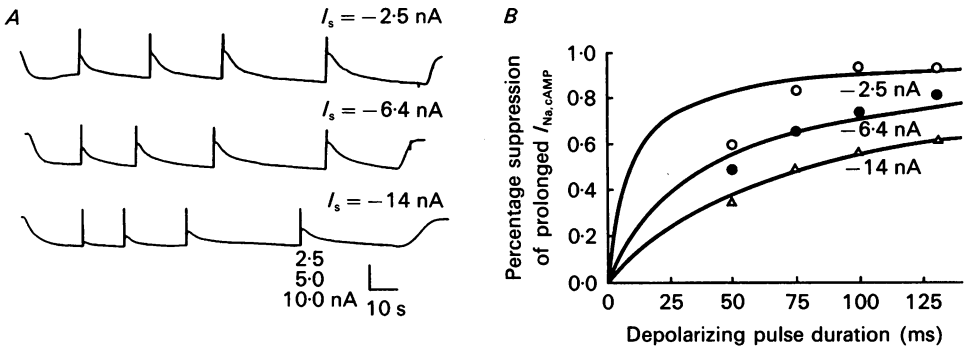

Fig. 9. Intracellular cAMP effects on the extent of depolarizing pulse suppression. *A*, four depolarizing pulses with sequentially increasing durations were stepped from −50 to +8 mV at three different steady backgrounds of iontophoretic cAMP injection. Top, −2·5; middle, −6·4; and bottom, −14 nA. *B*, values determined for suppression of $I_{Na,cAMP}$ by depolarizing pulses are graphed against pulse duration. Curve fits are based on a hypothetical model of competitive binding for cyclic AMP and Ca^{2+} (Huang, 1989).

the test $I_{Na,cAMP}$ is shown in Fig. 9*A* and *B*. Increasing the duration of the depolarizing pulse enhanced the amplitude of the tail current caused by $I_{Na,cAMP}$ inactivation; conversely, increasing the background level of $I_{Na,cAMP}$ antagonized the inactivating effect of the pulse. The extent of $I_{Na,cAMP}$ inactivation with increasing pulse duration was significantly decreased with increasing cAMP injection current, consistent with a competitive model for regulation of $I_{Na,cAMP}$.

The relation of outward tail current to basal $I_{Na,cAMP}$

Finally, in the light of the above results, we investigated whether in the unstimulated neuron there might be a basal level of $I_{Na,cAMP}$ that could be affected by depolarization. We measured the basal current as it expressed itself in its inactivation. When superimposed on relatively low levels of persistent, induced $I_{Na,cAMP}$, I_s, the outward current amplitude was increased in direct proportion to I_s (Fig. 10*A*). The plot of I_{tail} amplitude against I_s was linear (Fig. 10*B*); from the slope of this relation the fractional inactivation, m, could be determined. In this case, the tail current approximates a function of the fractional inactivation (m) of $I_{Na,cAMP}$ such that:

$$I_{tail} = mI_{Na,cAMP}$$
$$= m(I_s + I_b),$$

where I_b is the basal $I_{Na,cAMP}$. For the experiment shown in Fig. 10, the slope of the relation had a value of 0·7, suggesting that 70% of the cAMP-opened channels were inactivated by accumulated Ca^{2+} at the time of measurement.

The plot of I_{tail} *vs.* I_s also yielded a straightforward method of estimating the basal $I_{Na,cAMP}$ prevailing in the absence of exogenous cAMP injection. The y-axis intersect of the fitted straight line was extrapolated with precision to the value of tail current measured in the absence of cAMP injection. Thus, in this case $I_b = I_{tail}/m = 0{\cdot}5$ nA$/-0{\cdot}7 = -0{\cdot}71$ nA.

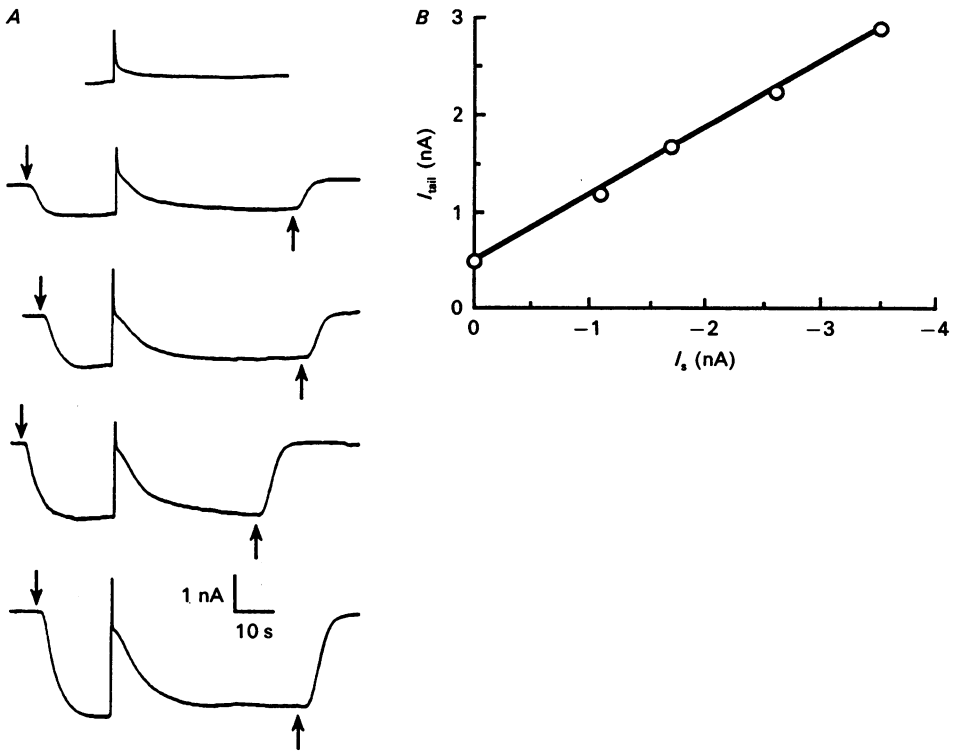

Fig. 10. Ca^{2+} inactivation of $I_{Na,cAMP}$ at varying levels of induced $I_{Na,cAMP}$. *A*, the amplitude of the tail current following a depolarizing pulse increased with larger iontophoretic injections of cAMP (between arrows). *B*, plot of tail current amplitude against the induced $I_{Na,cAMP}$. The slope of the linear relation was 0·7, which is taken as fractional inactivation. See pp. 317–318 for discussion. Depolarizing pulses were stepped from −50 to +10 mV for 50 ms. Tail current amplitude was measured at 2 s following the pulse.

DISCUSSION

Intracellular Ca^{2+} effects on the $I_{Na,cAMP}$ response

These results suggest a common mechanism underlying the steep voltage sensitivity and the depression of $I_{Na,cAMP}$ caused by depolarizing prepulses. Both phenomena are likely to result from the inactivation of $I_{Na,cAMP}$, by increasing levels of intracellular Ca^{2+}, mediated by a voltage-sensitive Ca^{2+} channel. The voltage sensitivity of $I_{Na,cAMP}$, and the relationship of the step voltage of the depolarizing prepulse to the extent of $I_{Na,cAMP}$ inactivation are both consistent with the current–voltage relation of Ca^{2+} current in molluscan neurons (Gorman & Thomas, 1978; Ahmed & Connor, 1979). Both Ca^{2+} current and the extent of $I_{Na,cAMP}$ inactivation peak near +50 mV, and decline with depolarization. Moreover, the time

course of the decay of intracellular Ca^{2+} activity following a depolarizing pulse is similar to the recovery of the $I_{Na,cAMP}$ from depolarizing pulse suppression (*ibid.*). The evidence for the causal role of voltage-dependent Ca^{2+} current is strengthened by the effects of blockade of Ca^{2+} influx, and of chelating intracellular Ca^{2+}. These treatments abolish both the voltage sensitivity of $I_{Na,cAMP}$ and the suppression of $I_{Na,cAMP}$ by depolarizing pulses.

Antagonism of Ca^{2+} suppression of the $I_{Na,cAMP}$ response by high levels of cAMP

The relationship between Ca^{2+} and cAMP was reciprocally antagonistic. High levels of cAMP reduced the degree of Ca^{2+} inactivation of $I_{Na,cAMP}$. These results suggest two possible explanations: (1) Ca^{2+} may be suppressing the action of cAMP through a mechanism of competitive inhibition, or (2) cAMP could be reducing the influx of Ca^{2+} through voltage-sensitive channels. The probability that cAMP-induced suppression of Ca^{2+} influx is important seems small, since it does not explain the enhancement of the $I_{Na,cAMP}$ response when it is imposed on the declining tail current (Fig. 4).

The mechanisms underlying the hypothesis of competitive inhibition have several possible forms. One is that cAMP activation through binding would put the channel into a state inaccessible to the effects of Ca^{2+}. Reciprocally, the action of Ca^{2+} acting at some regulatory site, possibly binding to the channel itself, could alter the equilibrium so as to render the channel inaccessible to cAMP activation. Thus, at high basal levels of cAMP a depolarizing Ca^{2+} prepulse could enhance the $I_{Na,cAMP}$ response by freeing channels for activation by subsequently injected cAMP.

The competitive effects of Ca^{2+} could be exerted through a variety of intermediary mechanisms, such as kinase reactions that might act on the channel to inhibit cAMP binding. However, our unpublished observations are that Ca^{2+} inactivation is resistant to trifluoperazine, W-7, and H-7, wide spectrum blockers of Ca^{2+} action and phosphorylation. It is simplest to suggest a model where Ca^{2+} may actually bind to or nearby to the intracellular side of the channel and mediate its actions directly. The simplest possibility in terms of channel state equilibria is:

$$Ca^{2+}\text{–}R_c \overset{Ca^{2+}}{\leftrightarrow} R_c \overset{cAMP}{\leftrightarrow} cAMP\text{–}R_c \leftrightarrow cAMP\text{–}R_o,$$

where R_c is the closed channel and R_o is the open channel. In this interpretation, raising intracellular Ca^{2+} activity would reduce the apparent binding affinity of the channel receptor for cAMP, and vice versa.

Basal $I_{Na,cAMP}$

We have introduced a method for calculating the basal $I_{Na,cAMP}$ existing in the neuron through simple and rapid measurements of tail current inactivation (Fig. 10). The demonstration of a small basal $I_{Na,cAMP}$ in these measurements is in accord with an expected basal level of cAMP in unstimulated molluscan neurons (Hockberger & Yamane, 1987). The results also agree with the interpretation that the inward current stimulated by BAPTA injection was due in large part to removal of Ca^{2+} inactivating from basal $I_{Na,cAMP}$.

An interesting and potentially useful consequence of the ability to calculate the

basal level of $I_{Na,cAMP}$ arises from observations that $I_{Na,cAMP}$ amplitude is a direct expression of the submembrane cAMP activity (Huang & Gillette, 1991). Therefore, the measurement of $I_{Na,cAMP}$ may provide a rapid and quantitative assay of cAMP equivalents in terms of nanoamperes in the pedal neurons of *Pleurobranchaea* and in other neurons expressing Ca^{2+}-inactivated $I_{Na,cAMP}$.

Ca^{2+} sensitivity of $I_{Na,cAMP}$ as an oscillatory mechanism

Calcium inactivation of inward Ca^{2+} current has been well known for some years (for review, see Eckert & Chad, 1984). Functionally, the consequence of this inactivation has been elucidated in mechanisms of spike train adaptation and rhythmic oscillation; depolarization leading to spike activity admits Ca^{2+} into the cell which leads to hyperpolarization through Ca^{2+} current inactivation (Adams & Levitan, 1985; Kramer & Zucker, 1985). In an analogous fashion Ca^{2+} inactivation of $I_{Na,cAMP}$ forms a negative feedback loop between electrical activity of the cell and the cAMP-activated channel; this loop could support endogenous oscillatory activity in the pedal neurons. In fact, tonic stimulation of the pedal neurons with inhibitors of cAMP-phosphodiesterase or cAMP analogues does induce rhythmical bursting activity. We postulate a model where neuromodulator-elevated levels of cAMP stimulate $I_{Na,cAMP}$ resulting in electrical activity; the resulting Ca^{2+} influx suppresses the current, terminating activity and thus causing the interburst interval. Restoration of spike activity would result from Ca^{2+} buffering processes within the cell, thereby repeating the bursting cycle as seen in other neurons where Ca^{2+} inactivation of Ca^{2+} currents are a major burst mechanism (Adams & Levitan, 1985; Kramer & Zucker, 1985).

The regulation of $I_{Na,cAMP}$ by Ca^{2+} may vary among molluscan neurons. The Ca^{2+} sensitivity of $I_{Na,cAMP}$ shown here for the pedal neurons of *Pleurobranchaea* is very high relative to that previously shown for other identified neurons of the buccal ganglion, the ventral white cells. In order to achieve the extent of $I_{Na,cAMP}$ suppression caused by depolarizations lasting only fractions of a second in the pedal neurons, many seconds of depolarization were necessary in the ventral white cells (Green & Gillette, 1988). This difference could be due to differences in Ca^{2+} channel density, intracellular Ca^{2+} buffering, or in species of channel (in fact $I_{Na,cAMP}$ of the buccal neurons shows an opposite voltage sensitivity in the range -50 to -30 mV, as discussed in the Introduction). Functionally, the difference is correlated with a marked difference in the duration of burst episodes in spontaneously bursting cells. Burst episodes in the buccal neurons last for up to 5 min, a physiological character in keeping with the role of the neurons in driving intense feeding episodes (Gillette, Gillette & Davis, 1980). In contrast, bursts in the pedal neurons last only 10–20 s (authors' unpublished observations). Potentially, Ca^{2+} inactivation of cAMP activation of $I_{Na,cAMP}$ is a major contributor to the endogenous bursting mechanisms of many molluscan neurons, and neuron-specific differences in the sensitivity to Ca^{2+} inactivation of the current shape the functional expression of oscillatory activity.

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