Activators of Protein Kinase C Mimic Serotonin-Induced Modulation of a Voltage-Dependent Potassium Current in Pleural Sensory Neurons of Aplysia

SHUZO SUGITA, DOUGLAS A. BAXTER, AND JOHN H. BYRNE
Department of Neurobiology and Anatomy, University of Texas Medical School at Houston, Houston, Texas 77225

SUMMARY AND CONCLUSIONS

1. In the pleural mechanoafferent sensory neurons of Aplysia, serotonin (5-HT)-induced spike broadening consists of at least two components: a CAMP and protein kinase A (PKA)-dependent, rapidly developing component and a protein kinase C (PKC)-dependent, slowly developing component. Voltage-clamp experiments were conducted to identify currents that are modulated by PKC and thus may contribute to the slowly developing component of 5-HT-induced spike broadening.

2. We compared the effects of phorbol esters, activators of PKC, on membrane currents with those of 5-HT. Bath application of 5-HT had complex modulatory effects on currents elicited by voltage-clamp pulses to potentials >0 mV. The kinetics of both activation and inactivation of the membrane currents were slowed by 5-HT. This led to a decrease in an outward current at the beginning of the voltage-clamp pulse and an increase at the end of the pulse. Previous work has shown that these effects represent, in part, the modulation of a large, voltage-dependent K+ current (I_K,V) by 5-HT.

3. Active phorbol esters mimicked some of the actions of 5-HT on membrane currents in that they slowed activation and inactivation kinetics of current responses to voltage-clamp pulses more positive than 0 mV. This led to a decrease in an outward current at the beginning of the pulse and an increase at the end of the pulse. Because inactive phorbols did not mimic the actions of 5-HT, the effects of active phorbol esters appeared to be PKC specific. In addition, preexposure of the sensory neurons to active phorbol esters appeared to occlude the modulatory actions of 5-HT on I_K,V. Thus it is likely that modulation of I_K,V by 5-HT is mediated, at least in part, by PKC.

4. To further characterize which currents were modulated by PKC, low concentrations of tetraethylammonium (TEA, 2 mM) were used to block Ca²⁺-activated K⁺ current (I_K,CA). Low TEA partially blocked the phorbol ester-induced increase of the outward current at the end of voltage-clamp pulses. These results agree with previous reports that activation of PKC enhanced a fast component of I_K,CA in these sensory neurons. Such an enhancement would lead to an increase in outward current that should be blocked by low TEA. Low TEA, however, did not affect phorbol ester-induced decrease of the outward current at the beginning of pulse, where the predominant current is I_K,V, which is less sensitive to TEA. Nor did low TEA block phorbol ester-induced spike broadening. Thus modulation of I_K,V appears to contribute to the PKC-mediated component of 5-HT-induced spike broadening.

5. On the basis of our results and those of others, a scheme is proposed in which the modulatory actions of 5-HT are mediated via two protein kinase systems (the PKC- and PKA pathways) that converge and diverge to affect various ionic conductances.

INTRODUCTION

Plasticity at the connections between sensory neurons and their follower cells in Aplysia has been used extensively as a model system in which to study the cellular and molecular mechanisms of simple forms of learning such as sensitization (Byrne 1987; Carew and Sahley 1986; Kandel and Schwartz 1982). Sensitization is mediated, at least in part, by the transmitter serotonin (5-HT) and expressed in several forms including facilitation of sensorimotor connections. Serotonin-induced spike broadening is believed to be a key mechanism underlying facilitation of undepressed synapses (Gingrich et al. 1988; Hochner et al. 1986; Kandel and Schwartz 1987). Previously, this broadening was believed to be dependent primarily on CAMP-mediated reduction of a nonactivating, relatively voltage-independent K⁺ current termed the S-K⁺ current (I_K,S) (Kandel and Schwartz 1982; Klein et al. 1982). However, recent evidence suggests that 5-HT-induced somatic spike broadening is composed of, at least, two components: a CAMP-dependent, rapidly developing component of broadening and a CAMP-independent, slowly developing component of broadening (Baxter and Byrne 1990a; Hochner and Kandel 1992; Mercer et al. 1991; Sugita et al. 1992b). For example, application of CAMP analogues, such as bath-applied 8-bromo-cAMP and pept-cAMP, or intracellularly diffused Sp-CAMP, can mimic aspects of the rapidly developing component of 5-HT-induced broadening (within 3 min after 5-HT application), but do not mimic the slowly developing component (after 3 min) (Baxter and Byrne 1990a; Goldsmith and Abrams 1992; Hochner and Kandel 1992). In addition, CAMP antagonist, Rp-cAMP, and a peptide inhibitor of PKA inhibit ≤75% of the rapidly developing component of broadening (Goldsmith and Abrams 1992; Hochner and Kandel 1992), but the inhibition of a slowly developing component by these inhibitors has not been demonstrated. Based on the above considerations, other second messenger systems appear to be involved in the slowly developing component. One possibility is that the diacylglycerol/protein kinase C (PKC) second messenger system mediates some of the actions of 5-HT.

Biochemical evidence suggests that 5-HT activates PKC as well as protein kinase A (PKA) (Baeskai et al., 1993; Bernier et al. 1982; Greenberg et al. 1987; Iarrard et al. 1993; Ocorr and Byrne 1983; Pollock et al. 1985; Sacktor and Schwartz 1990; Sossin and Schwartz 1992). We have shown that phorbol esters, activators of PKC, can mimic
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1. Step to +20 mV

2. 5-HT Difference Current (a - b)

3. Step to 0 mV

4. +5-HT

5. Step to -20 mV

6. +5-HT

FIG. 1. Modulation of membrane currents by serotonin (5-HT). Membrane currents were elicited by voltage-clamp pulses from -70 to -20, 0, and 20 mV in artificial seawater (ASW, a) and after bath application of 5-HT (10 μΜ, b). Each panel represents averaged data obtained from 6 sensory neurons. The 5-HT difference currents were obtained by subtracting the current responses elicited in 5-HT from those elicited in ASW (i.e., a - b). At 20 mV, 5-HT slowed both the activation and inactivation kinetics of the membrane current. At -20 mV, 5-HT appeared to reduce an outward current with properties similar to $i_{k,s}$.

On the basis of our results as well as those of Hochner and Kandel (1992) and Abrams and Goldsmith (1992), a new scheme for 5-HT-induced somatic spike broadening is proposed in which the modulatory effects of 5-HT are mediated via two second messenger systems converging and diverging on multiple ionic conductances. A preliminary report of some of these results was presented in abstract form (Sugita et al. 1992a).

METHODS

Measurements of membrane current

Isolated clusters of sensory neuron somata from pleural ganglia were pinned to the floor of a recording chamber containing artificial seawater (ASW, Instant Ocean) with 10 mM tris(hydroxymethyl)aminomethane-HCl (pH 7.6), and the preparation was maintained at 15 ± 1°C (mean ± SD). Only a single neuron per cluster was used for any experiment, and, unless otherwise indicated, the cluster received only a single application of a given agent or concentration of an agent. The sensory neurons were voltage clamped at their resting potentials and conventional two-electrode voltage-clamp techniques were used to measure membrane currents. As in previous studies (Baxter and Byrne 1989, 1990a; Goldsmith and Abrams 1992; Hochner and Kandel 1992), membrane currents were activated by 200 ms voltage clamp pulses that were preceded by a 15-s, hyperpolarizing prepulse to -70 mV. This prepulse serves to remove inactivation of membrane currents and thus allows for a more complete examination of the characteristics and modulation of currents. Adherence to this protocol also

One possibility is that the slowly developing component of broadening is dependent on PKC-mediated modulation of a large, voltage-dependent K+ current ($i_{k,v}$). Baxter and Byrne (1989) found that 5-HT modulates $i_{k,v}$ as well as $i_{k,s}$. This modulation is complex and due to a reduction in the magnitude of $i_{k,v}$ as well as a slowing of both activation and inactivation kinetics of $i_{k,v}$ (White et al. 1992, 1994). In addition, this modulation did not appear to be mimicked by cAMP analogues (Baxter and Byrne 1989, 1990ab). In the present study, we have examined the effects of phorbol esters on membrane currents. We show that the activation of PKC mimics and partially occludes 5-HT-induced modulation of membrane currents at potentials >0 mV, where $i_{k,v}$ is significantly activated. The results support the hypothesis that PKC modulates $i_{k,v}$ and that this modulation contributes to the slowly developing component of 5-HT-induced broadening.
makes possible comparisons between the present results and previous work. At the end of the 200-ms depolarization, the membrane potential was stepped back to a value of -70 mV for 500 ms. In most cells, the series of three voltage-clamp pulses to membrane potentials of -20 (or -30), 0, and 20 mV were used. To avoid the cumulative inactivation of membrane conductances, the voltage-clamp pulses were separated by 90 s (see Baxter and Byrne 1989). Between the voltage-clamp pulses, the cell was returned to its resting potential.

Two stable membrane current responses were obtained at each voltage in the series of voltage-clamp pulses before and after addition of agents to the bathing solution. These two responses were averaged off-line to obtain a single trace for each potential in the series of clamp pulses. The effects on membrane currents of the agents added to the bath were revealed by computer subtraction of membrane currents elicited after drug treatment from membrane currents elicited before treatment.

Measurements of spike duration

Two-electrode current-clamp techniques were used. The membrane potential of the sensory neurons was adjusted to -45 mV by manually injecting the current ~30 s before the measurement of spike duration. Individual action potentials were elicited by passing 3-ms, 5- to 6-nA current pulses through the current passing electrode at an interstimulus interval of 3 min. The duration of an action potential was measured as the time between the peak of the spike and the point of the repolarizing phase at which the membrane potential was 10% of the peak amplitude of the spike (Sugita et al. 1992b). After three stable trials, concentrated aliquots of agents were applied directly to the static bath. In each preparation, data were normalized to the mean of the duration of the three baseline spikes before the application of agents.

Chemicals

Two active types of hydrophilic phorbol esters, 4b-phorbol 12,13-diacetate (PDAc, Sigma) and 4b-12-deoxyphorbol 13-isobutyrate (DPB, LC Services), were used. As a control, inactive 4a-phorphols (Sigma) also were used. Stock solutions of all phorbols (10 mM) were dissolved in dimethyl sulfoxide (DMSO) and stored at -20°C. The final concentration in the bath was 3 μM for PDAc and 4a-phorbol and 2 μM for DPB. The final concentration of DMSO used to dissolve the phorbols did not exceed 0.03% (v/v). The concentrations of phorbol esters used in the present study were the same as those used previously (Sugita et al. 1992b). Previous work (Critz and Byrne 1992) examined the dose-responsive relationship between concentrations of phorbol esters and the modulation of \( I_{\text{KCa}} \) and found that concentrations of ~1 μM or higher are necessary to ensure saturation of phorbol-mediated effects. In addition, Goldsmith and Abrams (1992) reported that 300 nM of 4b-phorbol 12,13-dibutyrate (PDBu) did
not reliably facilitate depressed synapses in intact sensorimotor connection and Sossin et al. (1993) reported that 200 nM of PDBu was not a saturating concentration for the translocation of PKC in intact neurons. Although lower concentrations of phorbol esters have been shown to modulate some properties of the sensory neurons in culture (Braha et al. 1990), higher concentrations appear to be necessary in intact ganglia and acutely isolated sensory neuron somata. We believed that the concentrations of phorbol esters used in the present study fully activate PKC-mediated processes.

Serotonin creatine sulfate (5HT, Sigma) was dissolved in ASW and prepared daily. Tetraethylammonium (TEA, Kodak, 100 mM) was dissolved in H2O and then diluted in ASW to the final concentrations of 2 mM. Small aliquots of concentrated agents were added directly into the recording chamber.

RESULTS

Membrane currents modulated by 5-HT

We first repeated the experiments of Baxter and Byrne (1989) and confirmed their results. Serotonin creatine sulfate (n = 6) slowed both the activation and inactivation kinetics of the current responses elicited by voltage-clamp pulses to membrane potentials >0 mV (Fig. 1A, 1). This resulted in a decrease in the net outward current at the beginning of the pulse and an increase at the end of the pulse (Fig. 1A, 1 and 2). This effect developed slowly and became stable 9–13.5 min after application of 5-HT. These effects represent, in part, a modulation of Ik,v by 5-HT (Baxter and Byrne 1989; White et al. 1992, 1994). Statistical analyses indicated that both the decrease near the beginning of the pulse (10 ms after the beginning) and the increase near the end of pulse (180 ms after the beginning) were significant (the average amplitude ± SE of an outward current at 10 ms: 90.6 ± 4.6 nA before 5-HT vs. 73.5 ± 2.6 after 5-HT, two-tailed paired t test, t5 = 4.50, P < 0.01; at 180 ms: 73.1 ± 5.9 nA before 5-HT vs. 81.6 ± 7.1 nA after 5-HT, t5 = 3.86, P < 0.02). At -20 mV, 5-HT appeared to reduce an outward current, which is similar to Ik,s (Fig. 1C, 1 and 2) (Baxter and Byrne 1989; Hochner and Kandel 1992; Klein et al. 1982; Pollock et al. 1985). At 0 mV, combined effects of 5-HT on Ik,v and Ik,s are apparent (Fig. 1B, 1 and 2).

Phorbol esters mimic 5-HT-induced modulation of membrane currents elicited at potentials more positive than 0 mV

Application of PDAc (3 μM, n = 9) appeared to slow both the activation and inactivation kinetics of membrane currents elicited at potentials above 0 mV (Fig. 2A, 1). Such effects were observed consistently in each of the nine cells examined. The PDAc difference current was very similar to 5-HT difference current (compare Fig. 2A, 2, with Fig. 1A, 2). The effect of PDAc developed slowly and became stable 9–22.5 min after application. This time course corresponds.
well to the effect of phorbol esters on increasing the duration of action potentials (Sugita et al. 1992b). DPB (2 μM) had very similar effects in 9 of 10 cells examined (Fig. 3). Because inactive α-phorbol (n = 4) did not mimic the effects of active phorbol esters (Fig. 4A), modulation of the current at 20 mV appeared PKC-specific. Statistical analyses indicated that both PDAc- and DPB-induced effects on outward currents at 20 mV are significant (PDAc: at 10 ms, 100.8 ± 7.9 nA before vs. 84.4 ± 7.2 nA after, t₈ = 13.42, P < 0.001; at 180 ms, 79.7 ± 4.4 nA before vs. 92.9 ± 4.5 nA after, t₈ = 5.81, P < 0.001. DPB: at 10 ms, 113.1 ± 4.7 nA before vs. 95.3 ± 5.2 nA after, t₈ = 7.33, P < 0.001; at 180 ms, 93.3 ± 6.0 nA vs. 102.0 ± 7.5 nA after, t₈ = 2.58, P < 0.05). Unlike 5-HT, neither PDAc or DPB reduced consistently the outward current at −20 mV (Figs. 2C and 3C). Therefore, phorbol esters do not appear to reduce Iᵥ (however, see below and Fig. 6C). Instead of reducing the outward current, PDAc and DPB slightly increased outward currents elicited at −20 mV. This effect, however, does not seem to be PKC-specific, because inactive α-phorbol had similar effects (Fig. 4C).

**Phorbol esters partially occluded 5-HT-induced modulation of membrane current at 20 mV**

Because active phorbol mimics 5-HT-induced modulation of membrane current elicited at 20 mV, we examined the relationship between phorbol ester- and 5-HT-induced modulation of the current (Fig. 5). In these experiments (n = 5), the modulation by PDAc or DPB of the current was similar to that produced by 5-HT (compare Figs. 5B and 1A, 2). The subsequent application of 5-HT to the bath, which still contained PDAc or DPB, had some additional effects (Fig. 5, A and B). This 5-HT difference current (trace Iᵥ− in Fig. 5B), however, was significantly different from the 5-HT difference current in the absence of active phorbol esters (Fig. 4A, 2). Active phorbol esters completely occluded 5-HT-induced increase in the outward current at the end of the voltage-clamp pulse. Indeed, the 5-HT difference current in the presence of phorbol esters indicates the reduction at the end of the voltage clamp pulse (i.e., the upward deflection at the end of the Iᵥ+ trace in Fig. 5B). This slow component of the 5-HT difference current is likely to represent cAMP-dependent modulation of Iᵥ. In addition, preexposure to active phorbol esters significantly reduced the 5-HT-induced modulation of the membrane current at the beginning of the voltage-clamp pulse (compare the fast upward deflection at the Iᵥ+ trace in Fig. 5B with that in Fig. 1A, 2; at 10 ms after the beginning of the pulse: 7.3 ± 1.6% decrease by 5-HT in the presence of active phorbols vs. 18.4 ± 3.1% decrease by 5-HT in ASW, t₈ = 2.95, P < 0.02). This result suggests that phorbol esters partially occluded 5-HT-induced modulation of Iᵥ. Thus the modulation of Iᵥ by phorbol esters and 5-HT appears to share some common mechanisms. Alternatively, this occlusion might be explained by the phorbol ester-induced inhibition of the actions of 5-HT. Recently, prolonged acti-
STEP TO +20 mV

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DIFFERENCE CURRENTS

FIG. 5. Active phorbol esters (PDAc or DPB) partially occluded 5-HT-induced modulation of the membrane current. 

A: membrane currents were elicited by voltage-clamp pulses from -70 mV to 20 mV in ASW (a), 13.5 min after the addition of active phorbol esters (b) and 9 min after the addition of 5-HT to the bath already containing active phorbols (c). Traces represent averaged data from 5 sensory neurons. B: active phorbol ester difference current has characteristics similar to those of 5-HT difference current that is shown in Fig. 1A. 1. The 5-HT-difference current in the presence of phorbols was isolated by subtracting the current response elicited in the presence of 5-HT (and phorbols) from the current response elicited in ASW containing phorbols (i.e., b - c).

The occlusion of PKC by phorbol esters was shown to attenuate the ability of 5-HT to activate a cAMP-dependent pathway via possibly the phosphorylation of 5-HT receptors on the sensory neurons (Sugita et al. 1993).

PDAc- or DPB-induced modulation of membrane currents persist in the presence of low-TEA concentrations

Although I_{K_v} is the dominant current at 20 mV, other outward currents such as I_{K_{Ca}} also contribute to the net outward current and in turn are modulated by PKC. Critz and Byrne (1992) reported that active phorbol esters enhanced a fast component of I_{K_{Ca}} in these sensory neurons. Thus phorbol ester-induced increase in the outward current at the end of a pulse to 20 mV could be because of a direct modulation of I_{K_{Ca}} (Critz and Byrne 1992) or indirect increase in I_{K_{Ca}} via the phorbol ester-induced increase of a Ca²⁺ current (Braithwaite et al. 1993). To examine these possibilities, we used a low concentration of TEA (2 mM), which significantly blocks I_{K_{Ca}} but has little effect on I_{K_v} (Baxter and Byrne 1989; Critz and Byrne 1992; Walsh and Byrne 1989). Low TEA reduced an outward current at the end of pulse (compare a in Fig. 6A, 1, with a in Fig. 2A, 1). In addition, in the presence of TFA, a late component of phorbol ester-induced increase in the outward current was significantly reduced (compare Fig. 6A, 2, with 2A, 2; at 180 ms after the beginning of the pulse: 3.8 ± 7.9% increase in TFA vs. 12.9 ± 2.7% increase in ASW, t_{27} = 2.13, P < 0.05). These results suggest that phorbol ester-induced increase in the outward current is because of the modulation of both I_{K_v} and a fast component of I_{K_{Ca}}. In contrast to its effects toward the end of the voltage-clamp pulse, low TEA did not significantly reduce the modulation of the early component of the membrane current (Fig. 6A, 2; at 10 ms: 20.5 ± 1.8% decrease in TEA vs. 16.3 ± 1.2% decrease in ASW, t_{27} = 1.97). Thus phorbol esters appear to modulate a current with voltage-sensitivity and pharmacological properties similar to I_{K_v} (Baxter and Byrne 1989; White et al. 1992, 1994).

To examine the possible effect of PKC-induced increase in the fast component of I_{K_{Ca}} on spike duration, we also examined the effects of PDAc on spike duration in the presence of 2 mM TEA. PDAc increased the spike duration by 69 ± 11% above baseline (Fig. 7). This PKC-induced spike broadening in 2 mM TEA is more than twice that observed in ASW (compare Fig. 7B and Fig. 1E in Sugita et al. 1994). Thus the PKC-induced increase in I_{K_{Ca}} appears to partially counteract the broadening derived from the modulation of I_{K_v}. These results support our hypothesis that PKC-induced spike broadening is due to the modulation of I_{K_v}.

Unexpectedly, we observed that active phorbol esters sometimes reduced an outward current with slow kinetics at -20 mV in the presence of 2 mM TEA (Fig. 6C). In contrast, the reduction of the outward current by PKC was not observed in ASW (Figs. 2C and 3C). This discrepancy might be reconciled by assuming that PKC-induced increase in I_{K_{Ca}} counteracted PKC-induced modest reduction of the other outward current, such as I_{K_S} in ASW. Thus we do not exclude the possibility that activation of PKC may modestly reduce I_{K_S}. It was recently reported that PKC induced a small increase in excitability of the sensory neurons (Sugita et al. 1992b).

DISCUSSION

PKC mimics 5-HT-induced modulation of I_{K_v}

Although previous work indicated that activation of PKC plays a key role in the slowly developing component
of 5-HT-induced broadening (Sugita et al. 1992b), the particular membrane currents that were modulated by PKC were not identified. The present study suggests that activation of PKC modulates $I_{K, V}$ in a similar manner to that of 5-HT. The fact that PKC modulates $I_{K, V}$ and produces spike broadening, together with previous simulation studies (Baxter and Byrne 1990c; Belkin et al. 1992; Byrne et al. 1990; Canavier et al. 1991), also supports the hypothesis that 5-HT-induced modulation of $I_{K, V}$ plays a major role in 5-HT-induced broadening.

In addition to the modulation of $I_{K, V}$, our results confirmed the previous work by Critz and Byrne (1992) in that activation of PKC increases a fast component of $I_{K, Ca}$. Walsh and Byrne (1989) suggested that there may be two types of $I_{K, Ca}$ in the sensory neurons. One type has slow kinetics, contributes to the resting membrane conductance, and is decreased by 5-HT. The second type of $I_{K, Ca}$ has fast kinetics, is activated by relatively brief voltage-clamp pulses at membrane potentials above $-10$ mV and is not decreased by 5-HT. The $I_{K, Ca}$ increased by PKC appears to be the fast type.

Identity of $I_{K, V}$

Given the role of $I_{K, V}$ in 5-HT-induced spike broadening, it will be important to identify the channels underlying this current and determine whether the pharmacologically identified current is composed of a single or multiple types of K+ channels. Efforts have been made to identify K+ channels in *Aplysia* (Pfaffinger et al. 1991; Quattrocki et al. 1991).
FIG. 7. PDAc produced spike broadening in the presence of 2 mM TEA. A: 2 mM of TEA broadened the action potentials, as compared with those in ASW (see action potentials in ASW in Figs. 1 and 2 of Sugita et al. 1994). PDAc (3 μM, n = 6) induced additional broadening in the presence of TEA. Action potentials shown are before and 15 min after the application of PDAc. Arrow indicates the application of the PDAc. Error bars represent means ± SE.

1994; Zhao et al. 1992), but K+ channels with properties identical to $I_{K,V}$ have not been identified. One type of K+ channel ($AKO_{1a}$) isolated from *Aplysia* appears to induce a current with some similarities to $I_{K,V}$, however (Pfaffinger et al. 1991). Both $I_{K,V}$ and $AKO_{1a}$ show rather fast activation and inactivation kinetics and are more sensitive to 4-AP than TEA. These two currents also have some characteristics that differ, however. One critical difference is that $I_{K,V}$ shows steeper voltage sensitivity than does $AKO_{1a}$ (Furukawa et al. 1992; White et al. 1994). In addition, $AKO_{1a}$ shows less sensitivity to TEA than does $I_{K,V}$. Despite these differences, these two K+ currents may represent similar subfamilies of K+ channels. Interestingly, $AKO_{1a}$ is not modulated by analogues of cAMP (Furukawa et al. 1992; Pfaffinger et al. 1991). $AKO_{1a}$ is modulated by phorbol esters, however, and the modulation is similar to that of $I_{K,V}$ in

FIG. 8. A scheme for time-dependent spike broadening by 5-HT. A: at early times (e.g., 3 min), PKA plays a primary role in 5-HT-induced spike broadening via the closure of $I_{K,s}$ and modulation of the activation kinetics of $I_{K,v}$; 5-HT also reduces a slow component of $I_{K,ca}$, but it is unlikely to contribute to spike broadening due to its slow kinetics. Dashed lines indicate relatively weaker interactions between protein kinases and membrane currents than solid lines. B: at later times (e.g., 12 min), PKC plays a dominant role in 5-HT-induced spike broadening via the modulation of $I_{K,v}$ (and possibly $I_{K,s}$). PKC is known to increase a fast component of $I_{K,ca}$ (not shown), but it is not clear whether 5-HT also modulates this current.
that there is a slowing of both activation and inactivation kinetics (Kubo and Furukawa 1992). Kubo and Furukawa (1992) identified a specific amino acid, which when phosphorylated, slows the inactivation kinetics. Although an amino acid whose phosphorylation is involved in the slowing of activation kinetics has not been identified, these results suggest that molecular sites involved in the modulation of activation and inactivation kinetics are independent.

Another K+ channel (Aplysia Shab) also induces a current with some similarities to \( I_{k,v} \) (Quattrocchi et al. 1994). \( I_{Aplysia Shab} \) is similar to \( I_{k,v} \) in that it shows rather fast activation and inactivation kinetics, but differs in that it is not sensitive to 4-AP. Although \( Aplysia Shab \) has consensus sequences that may be phosphorylated by PKA and PKC, the effects of phosphorylation on the current have not been examined. At present, it is not known whether \( I_{k,v} \) is mediated by a distinct type of membrane channel or whether it may represent a heterogeneous assembly of \( I_{Aplysia Shab} \) and \( I_{Aplysia Shab} \).

**Hypothesis for 5-HT-induced somatic spike broadening**

Sugita et al. (1992b) reported that 5-HT-induced spike broadening appeared to have two distinct time courses, which suggests that more than one mechanism underlies the broadening (see also Sugita et al. 1994). The multiple time courses also have been observed in other laboratories (Hochner and Kandel 1992; Stark et al. 1992). For example, 5-HT increased spike duration by 15% of baseline after 3 min exposure and by 40% of baseline after 12 min exposure (Sugita et al. 1994). The slowly developing component of 5-HT-induced broadening was mimicked by phorbol esters and blocked by staurosporine (Sugita et al. 1992b). In addition, the slowly developing component was not mimicked by cAMP analogues (Baxter and Byrne 1989, 1990a; Hochner and Kandel 1992). Therefore the slowly developing component appears to be primarily dependent on PKC. The present study indicates that the slow component is due primarily to the modulation of \( I_{k,v} \) by PKC. Recently, it was also shown that 5-HT increases a nifedipine-sensitive Ca\(^{2+}\) current via the activation of PKC (Brahà et al. 1993). Although modulation of this current is relatively small compared with the modulation of \( I_{k,v} \), it is plausible that an increase of Ca\(^{2+}\) current also contributes to the spike broadening.

The mechanisms of a rapidly developing component appear more complex. Goldsmith and Abrams (1992) found that a PKA inhibitor blocked 75% of this rapidly developing component, and a PKC inhibitor blocked 25% of this component. Similar results were obtained by Hochner and Kandel (1992) and Sugita et al. (1992b). In addition, intracellularly injected Sp-cAMP or extracellularly applied 8-bromo-cAMP produced broadening that is comparable with 5-HT-induced broadening at 3 min (Baxter and Byrne 1989; Hochner and Kandel 1992; Sugita et al. 1994). Therefore a major part of a rapidly developing component appears to be dependent on PKA. The remaining question is how PKA produces this rapidly developing broadening. One possibility is that this broadening is due totally to the reduction of \( I_{k,s} \) by PKA. Another possibility is that PKA modulates \( I_{k,v} \) as well as \( I_{k,s} \). Support for this latter possibility comes from the experiments using cAMP antagonist, Rp-cAMP (Hochner and Kandel 1992). This antagonist inhibited 50% of 5-HT-induced modulation of activation kinetics of \( I_{k,v} \) although it did not affect the modulation of inactivation kinetics. Because PKC activators did not completely occlude the modulation of \( I_{k,v} \) by 5-HT, our results also suggest that PKA not only modulates \( I_{k,s} \) but also may have an effect on the modulation of the activation kinetics of \( I_{k,v} \). Therefore, PKA appears to be involved in some aspects of the modulation of \( I_{k,v} \) by 5-HT at early times.

On the basis of our results as well as those of Hochner and Kandel (1992) and Goldsmith and Abrams (1992), we propose a time-dependent hypothesis for 5-HT-induced broadening (Fig. 8). In the rapid component of broadening (3 min after application), PKA plays a major role because of the reduction of \( I_{k,s} \) and slowing of the activation kinetics of \( I_{k,v} \). For example, Bacsakai et al. (1993) found that the concentrations of cAMP reached a peak in 2 min after the application of 5-HT and that they gradually declined with time even in the continuous presence of 5-HT. In the slow component of broadening, PKC plays a major role because of the additional modulation of \( I_{k,v} \) and the increase in \( I_{ca} \). 5-HT also appears to reduce a slow component of \( I_{k,ca} \) via a CAMP pathway (Walsh and Byrne 1989). Because of its slow kinetics, the reduction of the slow component of \( I_{k,ca} \) may not contribute to the spike broadening. Although PKC was shown to increase a fast component of \( I_{k,ca} \) (Critz and Byrne 1992), it is not known whether 5-HT also increases this component.

Interestingly, recent biochemical work supports the time-dependent hypothesis we proposed here. Using 2-D polyacrylamide gel electrophoresis, Homayouni et al. (1992) examined the effects of different durations of 5-HT application (2 min, 25 min, or 1.5 h) on the phosphorylation of proteins. Then they compared the results of 5-HT with those of PDAc. They found that different sets of proteins are phosphorylated by different durations of 5-HT. Some proteins affected by a 25 min or 1.5 h exposure to 5-HT are also affected by PDAc, but none of the proteins affected by a 2 min exposure of 5-HT are affected by PDAc.

The data clearly indicate that the modulatory actions of 5-HT on pleural sensory neurons are mediated via at least two second messenger systems, the cAMP/PKA and DAG/PKC systems. Moreover, these data suggest that these two systems operate in different time domains. The cAMP/PKA system appears to mediate rapidly developing effects of 5-HT; whereas the DAG/PKC system appears to mediate more slowly developing effects of 5-HT. The possibility for cross-talk between these two systems also is suggested (Sugita et al. 1993). Thus neuronal plasticity that may contribute to learning and memory appears to be expressed in several overlapping time domains and transitions between short-term and longer term memory may involve several complex and interactive processes.

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