Serotonergic Modulation of Two Potassium Currents in the Pleural Sensory Neurons of *Aplysia*

DOUGLAS A. BAXTER AND JOHN H. BYRNE

Department of Neurobiology and Anatomy, The University of Texas Medical School, Houston, Texas 77225

SUMMARY AND CONCLUSIONS

1. The properties of membrane currents that were modulated by serotonin (5-HT) were investigated with two-electrode voltage-clamp techniques in sensory neuron somata isolated from the pleural ganglion of *Aplysia californica*. The modulatory effects of 5-HT were revealed by computer subtraction of current responses elicited in the presence of 5-HT from current responses elicited prior to the application of 5-HT. The complexities of the resulting 5-HT difference currents (I_{5-HT}) suggested that 5-HT modulated more than one component of membrane current.

2. The 5-HT difference currents appeared to have at least two distinct components. One component was clearly evident at membrane potentials more negative than -10 mV and did not inactivate. A second component was activated at membrane potentials more positive than -10 mV, had complex kinetics, and was highly voltage dependent. In an attempt to identify the membrane currents that were modulated by 5-HT, we compared the pharmacologic sensitivity of I_{5-HT} to that of previously described K⁺ currents.

3. The two components of I_{5-HT} had different sensitivities to agents that block K⁺ currents. The relatively voltage-independent component of I_{5-HT} was not blocked by 2 mM 4-aminopyridine (4-AP) and was relatively insensitive to tetraethylammonium (TEA) (estimated Kᵦ of 92 mM). In contrast, the voltage-dependent component of I_{5-HT} was blocked by 4-AP (2 mM) and moderate concentrations of TEA (estimated Kᵦ of 5 mM).

4. The K⁺ current blockers that were used to examine I_{5-HT} were also used to examine voltage-activated membrane currents. Extracellularly applied TEA blocked the delayed or voltage-dependent K⁺ current (I_{Kd}) with an estimated dissociation constant (Kᵦ) of 8 mM and a membrane current similar to the Ca²⁺-activated K⁺ current (I_{KCa}) with an estimated Kᵦ of 0.4 mM. In addition, externally applied 4-AP (2 mM) blocked I_{Kd}. Thus TEA and 4-AP were equipotent in blocking both I_{Kd} and the voltage-dependent component of I_{5-HT}.

5. The suggestion that I_{5-HT} contained multiple components was supported further by examining the modulatory effects of adenine 3',5'-cyclic monophosphate (cAMP) that mediates some actions of 5-HT on membrane currents in these cells. cAMP difference currents (I_{cAMP}) were similar to the relatively voltage-independent component of I_{5-HT}. The subsequent addition of 5-HT to solutions already containing cAMP resulted in 5-HT difference currents similar to the voltage-dependent component of I_{5-HT}. Thus the voltage-dependent component of I_{5-HT} did not appear to be modulated by elevated levels of cAMP.

6. Similarly, the modulatory effects of small cardioactive peptide (SCP) that mimics 5-HT and elevates levels of cAMP revealed difference currents (I_{SCP}) that were similar to both I_{cAMP} and the relatively voltage-independent component of I_{5-HT}. The subsequent addition of 5-HT to solutions already containing SCP resulted in 5-HT difference currents similar to the voltage-dependent component of I_{5-HT}. Thus SCPs did not appear to modulate a current similar to the voltage-dependent component of I_{5-HT}.

7. Based on pharmacologic sensitivity and voltage dependence, we conclude that in somata of pleural sensory neurons brief depolarizing voltage-clamp pulses activate at least two currents that could be modulated by 5-HT. The relatively voltage-independent component had properties consistent with the previously described S current. The voltage-dependent component had properties similar to I_{Kd}.

INTRODUCTION

In *Aplysia californica*, cellular mechanisms underlying neuronal plasticity have been analyzed extensively in two populations of sensory neurons. One population is located in the abdominal ganglion and mediates the siphon-gill withdrawal reflex. The second population is located in the pleural ganglion and mediates the tail withdrawal reflex. Many analyses of the biophysical mechanisms of plasticity in these sensory neurons have focused on a serotonin (5-HT)-sensitive K⁺ current, which is termed the S current (Klein et al. 1982; Pollock et al. 1985). Extracellular application of 5-HT reduces the magnitude of the S current via cAMP-dependent protein phosphorylation that closes the channel (Klein et al. 1982; Siegelbaum et al. 1982, 1987; Camardo et al. 1983; Pollock et al. 1985, 1987; Shuster et al. 1985; Belardetti and Siegelbaum 1988). The S current contributes to the resting K⁺ conductance and to the repolarization of action potentials in these cells. The serotonergic modulation of the S current is postulated to be an important mechanism contributing to presynaptic facilitation of transmitter release from sensory neurons, which in turn is thought to be the cellular basis of several simple forms of learning (for reviews see Byrne 1985, 1987; Carew et al. 1986, 1987; Hawkins et al. 1986; Kandel and Schwartz 1982).

Several criteria are used to distinguish the S current from other K⁺ currents that are found in molluscan neurons (e.g., Thompson 1977; Adams et al. 1980a; Byrne 1980), including its voltage dependence, kinetics, and pharmacologic sensitivity. The S current is relatively voltage independent, active over a wide range of membrane potentials, noninactivating, increases in a time-dependent manner during depolarization, and is relatively insensitive to the K⁺ channel blockers 4-aminopyridine (4-AP) and tetraethylammonium (TEA) (Klein et al. 1980, 1982; Paupardin-Tritsch et al. 1981; Siegelbaum et al. 1982, 1987; Camardo et al. 1983; Walsh and Byrne 1984, 1989; Pollock et al. 1985, 1987; Shuster and Siegelbaum 1987; Scholz and Byrne 1987, 1988; Brezina et al. 1987, 1988). More recent
results indicate, however, that in addition to the S current other membrane currents are modulated by 5-HT. For example, Walsh and Byrne (1989) reported that in the pleural sensory neurons of *Aplysia* the modulatory effects of 5-HT on membrane currents were reduced by agents that block a Ca\(^{2+}\)-activated K\(^+\) current [e.g., low concentrations of extracellular TEA, replacement of extracellular Ca\(^{2+}\) with Ba\(^{2+}\), or intracellular injection of the Ca\(^{2+}\) chelator ethylene glycol-bis(\(\beta\)-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA)]. Furthermore, they reported that 5-HT reduced the outward currents that were activated by intracellular injections of Ca\(^{2+}\). Thus it appears that in addition to the S current, 5-HT also modulates a steady-state Ca\(^{2+}\)-sensitive K\(^+\) current that contributes to the resting conductance in these cells. This finding raises the possibility that 5-HT may modulate additional K\(^+\) currents in the pleural sensory neurons.

In the present study, we tested this hypothesis by examining the modulatory effects of 5-HT on membrane currents that were elicited by a series of depolarizing voltage-clamp pulses. The modulatory effects of 5-HT were revealed by computer subtraction of current responses that were elicited by brief voltage-clamp pulses in the presence of 5-HT from current responses that were elicited by identical voltage-clamp pulses prior to application of 5-HT. The resulting 5-HT difference currents (\(I_{5\text{-HT}}\)) were found to have at least two distinct components: a relatively voltage-independent component and a highly voltage-dependent component. By comparing the pharmacologic sensitivity, voltage dependence, and kinetics of these two components of \(I_{5\text{-HT}}\) to those of several other steady-state Ca\(^{2+}\)-sensitive K\(^+\) currents previously identified in the somata of these sensory neurons and other molluscan neurons (e.g., Connor and Stevens 1971; Thompson 1977; Byrne 1980; Adams et al. 1980b; Gorman and Thomas 1980; Klein et al. 1982; Hermann and Hartung 1983; Hille 1984; Latorre et al. 1985; Pollock et al. 1985; Deitmer and Eckert 1985; Walsh and Byrne 1985, 1989; Shuster and Siegelbaum 1987; Acosta-Urquidi 1988; Rudy 1988), we concluded that in addition to the S current and a steady-state Ca\(^{2+}\)-sensitive K\(^+\) current, 5-HT also modulates the delayed or voltage-dependent K\(^+\) current (\(I_{K,\text{d}}\)). Furthermore, a membrane-permeable analogue of cAMP and small cardioactive peptide (SCP) were found to modulate a component of membrane current identical to the relatively voltage-independent component of \(I_{5\text{-HT}}\), but they did not mimic the action of 5-HT on a voltage-dependent component of membrane current. Thus the modulation of \(I_{K,\text{d}}\) by 5-HT did not appear to be mediated via cAMP. The possible mechanism and functional significance of this novel action of 5-HT are discussed. Preliminary reports of these results have been presented (Baxter and Byrne 1986, 1987).

**METHODS**

*Apelis* californica (150–300 g) were obtained from Marine Specimens (Pacific Palisades, CA), Marinus Biomarine (Westchester, CA), Sea Life Supply (Sand City, CA) and Alacrite Marine Biological Services (Redondo Beach, CA). Animals were maintained at 15°C in aerated artificial seawater (ASW), which is commercially available (Instant Ocean, Aquarium Systems, Mentor, OH), and were fed a diet of dried seaweed. Dissections were performed after anesthetizing the animals by the injection of a volume of isotonic MgCl\(_2\) equal to about one-half of the animal’s volume.

The ventrocaudal (VC) clusters of the pleural ganglia contain the somata of ~200 mechanosensory neurons that appear to be homogeneous in their biochemical and electrophysiological properties (Walters et al. 1981a, b; Walsh and Byrne 1984, 1989; Occor and Byrne 1985, 1986; Pollock et al. 1985, 1987; Scholz and Byrne 1987, 1988). For these experiments, clusters of sensory neuron somata were surgically isolated by undercutting the VC cluster with indelicate scissors. The isolated clusters were pinned to the Sylgard (Dow-Corning) floor of an experimental chamber with a volume of 300 µl. The static ASW bathing solution was prepared at pH 7.6 with 10–30 mM Trizma (Sigma) and contained 150 µM tetrodotoxin (TTX; Sigma). During all experiments the bath temperature was regulated to 15 ± 1°C by a thermodielectric cooling unit placed under the experimental chamber.

Sensory neuron somata were impaled with two glass capillary microelectrodes that were filled with 3 M potassium acetate and that had resistances of 2–6 MΩ. The sensory neurons were voltage clamped at their resting membrane potential using an Axoclamp-2 amplifier (Axon Instruments) and conventional two-electrode voltage-clamp techniques. Membrane conductances were activated by 200 ms voltage-clamp pulses that were preceded by a 25 s, hyperpolarizing prepulse to either −50, −70, or −90 mV. After the 200 ms depolarization, the membrane potential was stepped back to a value of −50 mV for 500 ms. To avoid the cumulative inactivation of membrane conductances (e.g., Aldrich et al. 1979; Byrne 1980), the voltage-clamp pulses were separated by 90 s. During times between the standard voltage-clamp protocols (see above), the cells were returned to their resting membrane potential.

Membrane current responses and a calibration pulse were digitized (12 bit resolution) on-line with a laboratory microcomputer and stored for later display and analysis. During each membrane current response, 500 samples were taken at a sampling rate (1.67 kHz) that was sufficiently fast to retain the details of the original current waveform. A minimum of three stable membrane current responses were obtained at each voltage in the series of voltage-clamp pulses before and after addition of compounds to the bathing solution. These stable responses were averaged off-line into a single current trace for each potential in the series of voltage-clamp pulses. This procedure insured that these data represented stable responses to the applied compounds and not nonspecific run down, increased the signal-to-noise ratio, and minimized the contamination of the difference currents by small random fluctuations in the currents. Although the membrane voltages during the voltage-clamp pulses were not digitized, they were monitored constantly. Current records were accepted only if the step changes in membrane potential were completed in <1 ms and no voltage “sag” was detected. The series resistance was estimated under current-clamp conditions from the size of the initial step change in a potential recorded by one intracellular microelectrode when a 10 nA current step was injected via the other microelectrode (Finkel and Gage 1985). The average series resistance for this preparation was 4 kΩ (n = 17). No compensation for series resistance was used.

The effects on membrane currents of pharmacologic agents added to the bath were revealed by computer subtraction of membrane currents elicited after drug treatment from membrane currents elicited before drug treatment (see Byrne 1980 and Fig. 1). Small (30–50 µl) aliquots of ASW containing concentrated solutions of either serotonin creatinine sulfate (5-HT; Sigma), tetraethylammonium chloride (TEA; Kodak), 4-aminopyridine (4-AP; Sigma), 8-(4-parachlorophenylthio)cyclic AMP (8-ppct-cAMP; Sigma) or small cardioactive peptide (SCP; Peninsula Laboratories) were added directly into the experimental chamber. The effective range of concentrations for TEA, 4-AP, and 5-HT
were estimated from previously reported dose-response relationships (Thompson 1977; Hermann and Gorman 1981a,b; Stanfield 1983; Ocorr and Byrne 1985; Deitmer and Eckert 1985; Shuster and Siegelbaum 1987; Brezina et al. 1987, 1988; Rudy 1988) and independently confirmed during the course of these experiments. The concentrations and the method of bath application are similar to those used in other studies on the sensory neurons (e.g., Klein et al. 1980, 1982, 1986; Ocorr and Byrne 1985, 1986; Pollock et al. 1985; Hochner et al. 1986b; Shuster and Siegelbaum 1987; Walsh and Byrne 1989).

Recordings from cells with the following characteristics were included in the data set: 1) stable resting membrane conductances, 2) membrane currents that did not show progressive, non-specific alterations during repetitive voltage-clamp pulses, 3) resting membrane potentials greater than -40 mV [-43 \( \pm \) 3 mV (mean \( \pm \) SD)], and 4) input resistances greater than 15 M\( \Omega \) (32 \( \pm \) 8). The results of this study represent successful recordings from 140 preparations.

RESULTS

Modulatory effects of 5-HT on membrane currents

We examined the modulatory effects of 5-HT on membrane currents by isolating 5-HT difference currents (\( I_{5-HT} \)). An example of the method used to isolate \( I_{5-HT} \) is shown in Fig. 1. The membrane currents that were elicited in ASW by a voltage-clamp pulse from \(-90\) to \(-20\) mV are shown in Fig. 1A1 (trace a). The addition of 5-HT (30 \( \mu \)M) reduced the total membrane current (trace b). This reduction is particularly evident at the end of the voltage-clamp pulse. The difference between the current responses elicited in the presence of 5-HT (trace b) and the current responses elicited in ASW (trace a) represents the membrane current that was modulated by 5-HT. Computer subtraction of the 5-HT trace from the ASW trace results in a 5-HT difference current, \( I_{5-HT} \) (Fig. 1A2), in which the 5-HT-mediated reduction in outward current is represented as an upward deflection. Previous work indicates 5-HT reduces outward membrane current, at least in part, by initiating the closure of a distinct class of 5-HT sensitive K\(^+\) channels, the S channels (Klein et al. 1982; Siegelbaum et al. 1982, 1987; Camardo et al. 1983; Pollock et al. 1985, 1987). Thus the example of \( I_{5-HT} \) shown in Fig. 1A2 represents the S current and has properties consistent with those previously reported for the S current (see also below).

In contrast to the relatively simple modulation of membrane currents by 5-HT shown in Fig. 1A, a complex 5-HT difference current was observed at more depolarized mem-
brane potentials. The membrane current that was elicited in ASW by a voltage-clamp pulse from -90 to +20 mV are shown in Fig. 1B1 (trace a). Application of 5-HT (30 μM) appeared to slow the rate-of-rise of the total membrane current and thus markedly decreased the total current early during the voltage-clamp pulse. In addition, 5-HT appeared to slow the inactivation of the outward membrane current and thus increased the outward current later during the voltage-clamp pulse. The difference between the current responses elicited in the presence of 5-HT and the current responses elicited in ASW represents the modulatory effects of 5-HT at more depolarized levels and is shown in Fig. 1B2. The early upward peak of I_{5-HT} in Fig. 1B2 reflects the initial decrease in the total membrane current (Fig. 1B1), whereas, the downward shift in Fig. 1B2 represents the late increase in the total membrane current at the end of the voltage-clamp pulse after application of 5-HT (Fig. 1B1). A comparison of the 5-HT difference currents shown in Fig. 1, A2 and B2 indicates that the modulatory effects of 5-HT are more complex than simply initiating the closure of the S channel and that other membrane currents are involved.

A complete series of 5-HT difference currents that were isolated from a wide range of voltage-clamp potentials is shown in Fig. 2. The membrane currents that were elicited in ASW by voltage-clamp pulses from -90 to -30, 20, and -10 mV are shown in A2 and the membrane currents that were elicited by voltage-clamp pulses to 0, +10, and +20 mV are shown in A1 (note the change in gain). The membrane currents that were elicited by identical voltage-clamp pulses following application of 5-HT are shown in Fig. 2B. The 5-HT difference currents that were isolated by subtracting the current responses elicited in the presence of 5-HT from the current responses elicited in ASW are shown in Fig. 2C. At membrane potentials more negative than 0 mV (Fig. 2C2), I_{5-HT} increased slowly during the voltage-clamp pulses, and the peak amplitudes increased nearly linearly with voltage (also see Fig. 8). This relatively

![Image of Figure 2](image-url)

**FIG. 2.** Voltage sensitivity of currents modulated by 5-HT. Membrane currents were elicited by voltage-clamp pulses from -90 mV to membrane potentials ranging between -30 and +20 mV. A: current responses were elicited in ASW. Small currents elicited with small depolarizations are shown in A2, whereas large currents elicited by larger depolarizations are shown in A1. B: current responses to potentials corresponding to those shown in A1 and A2 were elicited in the same cell following bath application of 5-HT (30 μM). C: 5-HT difference currents were isolated by subtracting the current responses elicited in the presence of 5-HT from the corresponding current responses elicited in ASW (A - B). Results similar to these were obtained in all preparations (n = 33) in which I_{5-HT} was isolated from neurons bathed in ASW. Note changes in ordinate scales. The component of I_{5-HT} expressed at small depolarizations (C2) exhibits little voltage-dependence and has simple kinetics, whereas the component expressed at larger depolarizations (C1) is highly voltage dependent and has complex kinetics.
The voltage-independent component of $I_{\text{S-HT}}$ did not inactivate during the 200 ms depolarizing voltage-clamp pulses. Moreover, it did not inactivate during long-duration (1 min) voltage-clamp pulses to $-20 \text{ mV}$ ($n = 9$; data not shown). These properties are consistent with the previous descriptions of S current (Klein et al. 1980, 1982; Siegelbaum et al. 1982, 1987; Camardo et al. 1983; Walsh and Byrne 1984, 1989; Ocorr and Byrne 1985: Pollock et al. 1985, 1987; Scholz and Byrne 1987, 1988). In contrast, the magnitude, rate of activation, and rate of inactivation of 5-HT difference currents that were isolated from voltage-clamp pulses to membrane potentials more positive than $-10 \text{ mV}$ were highly voltage dependent (Fig. 2C1). The characteristics of $I_{\text{S-HT}}$ at depolarized membrane potentials (Fig. 2C1) are inconsistent with the those of the S current. These results suggest that 5-HT modulates an additional voltage-dependent membrane conductance that is activated at membrane potentials more positive than $-10 \text{ mV}$.

**Separation of K' currents**

While the relatively voltage-independent component of the $I_{\text{S-HT}}$ has properties similar to the S current, the voltage-dependent component of $I_{\text{S-HT}}$ has not been described previously. We were interested, therefore, in determining whether this second component represented the modulation by 5-HT of either the voltage-dependent K' current ($I_{K,\text{v}}$), Ca$^{2+}$-activated K' current ($I_{K,\text{Ca}}$), or transient K' current ($I_{K,t}$), which are found in these sensory neurons (Klein et al. 1982; Pollock et al. 1985; Baxter and Byrne 1988; Walsh and Byrne 1989) and other molluscan neurons (e.g., Connor and Stevens 1971; Thompson 1977, Aldrich et al. 1979; Adams et al. 1980a,b; Byrne 1980; Gorman and Thomas 1980; Hermann et al. 1981a,b, 1983; Hockberger and Connor 1984; Strong et al. 1984, 1986; Thomas 1984; Deitmer and Eckert 1985; Junge 1985; Kehoe 1985; Bezanilla et al. 1986; Acosta-Urquidi 1988; also see, Hille 1984; Latorre et al. 1984; Rogawski 1985; Rudy 1988). The method that we chose to investigate this possibility was to compare the pharmacologic sensitivity, voltage-dependence, and kinetics of the voltage-dependent component of $I_{\text{S-HT}}$ to those of $I_{K,\text{v}}, I_{K,\text{Ca}},$ and $I_{K,t}$. An extensive body of literature indicates that $I_{K,\text{v}}$ and $I_{K,\text{Ca}}$ can be blocked by the use of appropriate concentrations of TEA and 4-AP and that $I_{K,t}$ can be selectively inactivated at rather hyperpolarized membrane potentials (e.g., Connor and Stevens 1971; Thompson 1977; Byrne 1980; Hermann et al. 1981a,b, 1983; Klein et al. 1982; Stanfield 1983; Hille 1984; Latorre et al. 1984; Rogawski 1985; Blatz and Magleby 1987; Shuster and Siegelbaum 1987; Cook 1988; Rudy 1988; Walsh and Byrne 1989). Therefore, we used these methods to isolate examples of these three classes of K' currents and compare their characteristics with those of $I_{\text{S-HT}}$.

$I_{K,\text{v}}$. In previously studied neurons of *Aplysia*, TEA has been reported to block $I_{K,\text{v}}$ with an apparent dissociation constant ($K_{d}$) of 6–8 mM and some examples of $I_{K,\text{Ca}}$ with a $K_{d}$ near 0.5 mM (Thompson 1977; Byrne 1980; Hermann and Gorman 1981b; Klein et al. 1982; Deitmer and Eckert 1985; Walsh and Byrne 1989). Thus low concentrations of TEA should block $I_{K,\text{Ca}}$ significantly but have little effect on $I_{K,\text{v}}$. Subsequent increases in the bath concentration of TEA should block the remaining $I_{K,\text{v}}$. Consequently, we examined the membrane currents that are sensitive to low and high concentrations of TEA. The membrane currents that were elicited by voltage-clamp pulses to $+20 \text{ mV}$ first in ASW, following addition of 2 mM TEA, and following the increase of the concentration of TEA to 50 mM are shown in Fig. 3A1. Although the low concentration of TEA reduced the membrane current at the end of the voltage-clamp pulse, the low concentration of TEA had little effect on the peak amplitude of the current response. Increasing the concentration of TEA to 50 mM markedly reduced the peak amplitude of the current response. The membrane currents that were blocked by low concentrations of TEA were isolated by subtracting current responses elicited by voltage-clamp pulses to 0, $+10$, and $+20 \text{ mV}$ in the presence of 2 mM TEA from corresponding current responses elicited in ASW (Fig. 3A2). The current affected by low concentrations of TEA was slow to activate, showed little inactivation, and had a mild voltage dependence. In these respects, it bears some resemblance to the S current and the 5-HT difference currents illustrated in Fig. 2C2. It differs from $I_{\text{S-HT}}$ observed at small depolarizations, however, in its threshold for activation and sensitivity to TEA (see below). The sensitivity to low concentrations of TEA suggests that the difference currents in Fig. 3A2 may represent a TEA-sensitive form of $I_{K,\text{Ca}}$. The membrane currents that were blocked by high concentrations of TEA were isolated by subtracting the current responses elicited by voltage-clamp pulses to 0, $+10$, and $+20 \text{ mV}$ in high concentrations of TEA from corresponding current responses elicited in low concentrations of TEA (Fig. 3A3). The current affected by high concentrations of TEA began to be activated at potentials more positive than $-10 \text{ mV}$, and both its activation and inactivation kinetics were voltage dependent. The large magnitude of the high TEA difference current, the marked voltage dependence of its peak amplitude and the voltage dependence of its kinetics of activation and inactivation are similar to those reported for $I_{K,\text{v}}$ in these sensory neurons and other *Aplysia* neurons (Thompson 1977; Aldrich et al. 1979; Adams and Gage 1980a; Byrne 1980; Hermann and Gorman 1981b; Klein et al. 1982; Pollock et al. 1985; Strong and Kaczmarek 1986).

In certain neurons of *Aplysia*, low concentrations of 4-AP have been reported to block $I_{K,\text{v}}$ with a $K_{d} \approx 0.8 \text{ mM}$, while having no effect on $I_{K,\text{Ca}}$ (Hermann and Gorman 1981a; also see Rudy 1988). Thus we examined the membrane currents that are sensitive to low concentrations of 4-AP. An example of the membrane currents that were elicited first by a voltage-clamp pulse to $+20 \text{ mV}$ in ASW and then with an identical depolarizing step after applying 1.5 mM 4-AP are shown in Fig. 3B1. The 4-AP markedly reduced the peak amplitude of the current response but had little effect on the current at the end of the voltage-clamp pulse. The membrane currents that were blocked by low concentrations of 4-AP were isolated by subtracting current responses elicited by voltage-clamp pulses to 0, $+10$, and $+20 \text{ mV}$ in the presence of 4-AP from corresponding current responses elicited in ASW (Fig. 3B2). These 4-AP difference currents are similar to the high TEA difference current (Fig. 3A1) and have characteristics consistent with those of $I_{K,\text{v}}$. 

**Conclusion**

The results of this investigation suggest that 5-HT modulates an additional voltage-dependent membrane conductance that is activated at membrane potentials more positive than $-10 \text{ mV}$. This conductance is sensitive to both low and high concentrations of TEA and low concentrations of 4-AP. Further studies are needed to determine the nature of this conductance and its role in the modulation of neuronal activity.
FIG. 3. Pharmacologic sensitivity of $I_{K,1}$. A1: currents were elicited by a voltage-clamp pulse from $-50$ to $+20$ mV in ASW (trace a), following the addition of 2 mM TEA (trace b), and following the increase of the concentration of TEA to 50 mM (trace c). A2: TEA difference currents in this cell were isolated from voltage-clamp pulses to 0, +10, and +20 mV. The component of membrane current that was blocked by the low concentration of TEA was isolated by subtracting current responses elicited in the presence of 2 mM TEA from corresponding current responses elicited in ASW (e.g., a – b in A1). A3: the component of membrane current that was blocked by higher concentrations of TEA was isolated by subtracting current responses elicited in the presence of 50 mM TEA from corresponding current responses elicited in the presence of 2 mM TEA (e.g., b – c in A1). These TEA difference currents are representative of results in 14 different preparations. B1: in a different cell, membrane currents were elicited by voltage-clamp pulses from $-50$ mV (a holding potential that inactivated $I_a$, e.g., see Fig. 5A) to $+20$ mV in ASW (trace a) and following the addition of 1.5 mM 4-AP to the bath (trace b). B2: 4-AP difference currents were isolated from voltage-clamp pulses to 0, +10, and +20 mV by subtracting current responses elicited in the presence of 4-AP from corresponding current responses elicited in ASW (e.g., a – b in B1). These difference currents are representative of results obtained in 12 different preparations. The currents blocked by high concentrations of TEA (50 mM, A3) and low concentrations of 4-AP (1.5 mM, B2) are nearly identical in their voltage sensitivity and kinetics of activation and inactivation. These features are characteristic of $I_{K,1}$.

$I_{K,1,\text{Ca}}$. Walsh and Byrne (1989) have shown that in these sensory neurons low concentrations of TEA (5 mM) block a $K^+$ current that is activated by intracellular injection of Ca$^{2+}$ (also see, Hermann et al. 1981b, 1983; Klein et al. 1982; Stanfield 1983; Hille 1984; Thomas 1984; Deitmer and Eckert 1985; Kehoe 1985; Sawada et al. 1987, 1989). As shown in Fig. 3, externally applied 4-AP (2 mM) blocks $I_{K,1,\text{Ca}}$, but apparently not the low TEA difference current, which resembles $I_{K,1,\text{Ca}}$ (Thompson 1977; Hermann et al. 1981a, 1983). Thus we examined the membrane currents that were insensitive to 4-AP but were sensitive to TEA. Membrane currents were elicited by a voltage-clamp pulse to $+20$ mV first in ASW containing 4-AP, following the addition of 2 mM TEA, and following the increase of the concentration of TEA to 50 mM (Fig. 4A). Application of a low concentration of TEA markedly reduced the membrane current. Increasing the concentration of TEA to 50 mM had little additional effect on the membrane currents. This result indicates that an additional component of membrane current can be blocked by low concentrations of TEA even after a significant component of membrane current has been blocked already by 4-AP. This pharmacologic sensitivity is consistent with TEA-sensitive forms of $I_{K,1,\text{Ca}}$. The membrane current that is blocked by low TEA (but not by 4-AP) was isolated by subtracting current responses elicited by voltage-clamp pulses to 0, +10, and +20 mV in the presence of low concentrations of TEA (plus 4-AP) from corresponding current responses elicited in ASW that contained 4-AP (Fig. 4B). The low TEA difference currents became significantly activated at membrane potentials more positive than $-10$ mV and were slow to develop. With voltage-clamp pulses above $+30$ mV, the amplitude of the low TEA difference currents began to decrease (data not shown). These low TEA difference currents and those in Fig. 3A2 appear to be $I_{K,1,\text{Ca}}$, based on the voltage dependence and kinetics of this current and its
A Step to +20 mV

a ASW + 1.5 mM 4-AP
b + 2 mM TEA
c + 50 mM TEA

20 nA

20 msec

B Low TEA Difference Currents (a - b)

20 mV

15 nA

20 msec

FIG. 4. Effects of TEA on membrane currents after preexposure to 4-AP. A: membrane currents were elicited by a voltage-clamp pulse from -70 to +20 mV in ASW containing 1.5 mM 4-AP (trace a) to block IK1 (see Fig. 3B), in the presence of 2 mM TEA (trace b) to block TEA-sensitive IKCa, and in the presence of 50 mM TEA (trace c) to block any residual IK1 or TEA-sensitive IKCa. Application of a low concentration of TEA markedly reduced the membrane current. Increasing the concentration of TEA to 50 mM had little additional effect on the membrane currents. B: low TEA difference currents were isolated from voltage-clamp pulses to 0, +10, and +20 mV by subtracting the current responses elicited in the presence of 2 mM TEA (and 1.5 mM 4-AP) from the corresponding current responses elicited in ASW containing 1.5 mM 4-AP (e.g., a - b in A). These difference currents are representative of results obtained in 13 different preparations. The currents blocked by low concentrations of TEA (2 mM) are slow to activate, do not inactivate, and are mildly voltage-dependent. These features are characteristic of IKCa.

FIG. 5. Isolation of IA. A: examples of membrane currents elicited by voltage-clamp pulses to -20 mV from holding potentials of -50 and -90 mV. B: component of outward current that was highly sensitive to the holding potential was isolated by subtracting current responses elicited by voltage-clamp pulses to -30, -20, and -10 mV from a holding potential -30 mV from the corresponding current responses elicited by depolarizations to the same levels but from a holding potential -90 mV. This current is relatively voltage independent with respect to both its kinetics of activation and its kinetics of inactivation. The kinetics of activation and inactivation are rapid compared to both IK1 (e.g., Fig. 3, A1 and B2) and IKCa (Fig. 4B). These features are characteristic of IA. These results indicate that the properties of IA are different from those of the voltage-dependent component of IKHT, and thus, it is unlikely that modulation of IA by 5-HT could account for the voltage-dependent component of IKHT.
Pharmacologic sensitivity of 5-HT difference currents

While other interpretations are possible, the similarity between the voltage dependence and the complex kinetics of $I_{K,v}$ and those of the voltage-dependent component of $I_{SHT}$ suggested to us that this component of $I_{SHT}$ may be due to a modulation of $I_{K,v}$ by 5-HT. It appeared that 5-HT slowed the activation and inactivation of $I_{K,v}$ (e.g., Fig. 1B1). Such a modulation would explain both the initial decrease and the later increase in the outward membrane current induced by 5-HT. Alternatively, the late increase in membrane current may represent an indirect modulation of $I_{K,CA}$ by 5-HT. Boyle et al. (1984) and Spira et al. (1987) found that application of 5-HT increased the concentration of intracellular free Ca$^{2+}$ as measured by the Ca$^{2+}$ indicators arsenazo III and fura-2. These results raise the possibility that 5-HT could lead to increased activation of $I_{K,CA}$ by increasing the levels of intracellular Ca$^{2+}$ (see also Sacktor et al. 1986; Braha et al. 1988). Thus the late increase in outward current induced by 5-HT (Fig. 1B1) could be due to an increase in $I_{K,CA}$. To distinguish between these two possibilities, we examined the sensitivity of $I_{SHT}$ to agents that differentially affect $I_{K,v}$ and $I_{K,CA}$.

As mentioned above (Figs. 3A and 4), $I_{K,v}$ and some forms of $I_{K,CA}$ can be distinguished by their different sensitivities to TEA; $I_{K,CA}$ can be blocked by low concentrations of TEA that have little effect on $I_{K,v}$. As shown in Fig. 6A, we examined the sensitivity of the voltage-dependent component of $I_{SHT}$ to low concentrations of TEA by isolating $I_{SHT}$ in solutions containing 2 mM of TEA. Membrane currents were elicited by voltage-clamp pulses to +20 mV; first in ASW, following the addition of 2 mM TEA and following addition of 5-HT to the solution already containing TEA (Fig. 6A1). Despite the presence of a low concentration of TEA, $I_{SHT}$ still displayed the characteristic voltage-dependent component (Fig. 6A2). Thus a concentration of TEA that blocks an $I_{K,CA}$-like current (but not $I_{K,v}$) does not block the voltage-dependent component of $I_{SHT}$. (A more complete analysis of the sensitivity of $I_{SHT}$ to TEA is presented in Figs. 7 and 9.)

$I_{K,v}$ and $I_{K,CA}$ also can be distinguished on the basis of their sensitivity to 4-AP; $I_{K,v}$ is blocked by low concentra-

![Diagram](image_url)
tions of 4-AP, whereas $I_{K,cs}$ is not (see Figs. 3B and 4). Therefore, we examined the sensitivity of the voltage-dependent component of $I_{S-HT}$ to 4-AP by isolating $I_{S-HT}$ in solutions that contained 4-AP (Fig. 6B). Membrane currents were elicited by voltage-clamp pulses to +20 mV; first in ASW, following addition of 1.5 mM 4-AP and following addition of 5-HT to the solution already containing 4-AP (Fig. 6B1). As shown in Fig. 6B2, 4-AP blocked the voltage-dependent component of $I_{S-HT}$ (also see Fig. 8). The 5-HT difference current that was isolated in the presence of 4-AP revealed only the residual voltage-independent component that is identical to the S current. [Previous work has shown that the S current is relatively insensitive to 4 AP and has a $K_v$ for 4-AP of 10 mM (Shuster and Siegelbaum 1987; Brezina 1988).] Thus an agent (4-AP) that blocks $I_{K, L}$ (but not $J_{K, Ca}$) also blocks the voltage-dependent component of $I_{S-HT}$.

To more completely compare the sensitivities to TEA of $I_{K, V}$ and the voltage-dependent component of $I_{S-HT}$, we examined $I_{S-HT}$ from sensory neurons that were bathed in ASW (Fig. 7A) and from neurons bathed in ASW that contained various concentrations of TEA (Fig. 7, B–E). In each case, the voltage-clamp pulse was from −70 to +20 mV. Thus, in the experiments of Fig. 7, different preparations were first exposed to a specific concentration of TEA and then the ability of 5-HT to modulate the membrane currents was examined. At concentrations of 2 and 5 mM TEA, the voltage-dependent component of $I_{S-HT}$ was still present (Fig. 7, B and C). These concentrations of TEA can be effective in blocking $I_{K, Ca}$, but have less of an effect on $I_{K, V}$ (Hermann and Gorman 1981b; Klein et al. 1982; Walsh and Byrne 1989) (also see Figs. 3, 4, and 9). Therefore, it is unlikely that the complex kinetics of $I_{S-HT}$ can be accounted for by an increase in the amplitude of $I_{K, Ca}$. The voltage-dependent component of $I_{S-HT}$ was blocked only at the high concentrations of TEA that are also effective in blocking $I_{K, V}$ (Fig. 7, D and E; note the change in the value of the calibration bar). 5-HT difference currents that were isolated in ASW containing 100 mM TEA reveal only the residual voltage-independent component that is identical to the S current obtained at less depolarized membrane potentials (compare Figs. 7E and 2C2).

Figure 8 compares the current-voltage (I-V) relationships of 5-HT difference currents that were isolated from sensory neurons under three conditions: 1) from sensory neurons bathed first in ASW and then in 5-HT, 2) from sensory neurons first bathed in ASW that contained 100 mM TEA (to reduce $I_{K, Ca}$ and $I_{K, V}$) and then in 5-HT, and 3) from sensory neurons bathed first in 2 mM 4-AP (to block $I_{K, V}$) and then 5-HT. In all three cases, the membrane currents were elicited by voltage-clamp pulses from −70 mV to membrane potentials ranging between −40 and +30 mV. The amplitudes of 5-HT difference currents were measured at the end of the voltage-clamp pulses and were plotted such that a positive value represented a decrease and a negative value represented an increase in the outward membrane currents. In cells exposed to ASW and then to 5-HT, the relatively voltage-independent component of $I_{S-HT}$ was evident during voltage-clamp pulses to −40 mV through −0 mV (also see Fig. 2C2). The downward shift in the I-V relationship of $I_{S-HT}$ at 0 mV reflects the development of the voltage-dependent component of $I_{S-HT}$ (also see Fig. 2C1). Of particular significance is the observation that the concentrations of 4-AP and TEA that block $I_{K, V}$ also block this downward shift in the I-V relationship for $I_{S-HT}$. Since 4-AP does not block $I_{K, Ca}$, the absence of the down-
two components of the effects of TEA on an \( I_{K,Ca} \) like current, (Klein et al. 1982; Brezina et al. 1987, 1988; Shuster and Siegelbaum 1987; Walsh and Byrne 1989).

The relatively voltage-independent component of \( I_{K,HT} \) is identical to that of the S current (e.g., Fig. 1). The voltage-dependent component of \( I_{K,H} \) was isolated from voltage-clamp pulses at membrane potentials more positive than 0 mV, the highly voltage-dependent component of \( I_{K,H} \) is observed. Pretreatment with 2 mM 4-AP does not affect \( I_{K,H} \) in the range of -40 to 0 mV but completely blocks the highly voltage-dependent component of \( I_{K,H} \) normally observed at potentials more depolarized than 0 mV. TEA (100 mM) also blocks the highly voltage-dependent component of \( I_{K,H} \), and has a slight effect on the relatively voltage-independent component of \( I_{K,H} \). These results indicate that pharmacologic agents that block \( I_{K,H} \) also block the voltage-dependent component of \( I_{K,H} \).

ward shift in the \( I-V \) relationship for \( I_{K,H} \) in the presence of 4-AP indicates again that modulation of \( I_{K,CA} \) cannot account for the complex kinetics and voltage dependence of \( I_{K,H} \). It is also interesting to note that this concentration of 4-AP had absolutely no effect on the component of \( I_{K,H} \) elicited by voltage-clamp pulses to membrane potentials more negative than 0 mV. In contrast, high concentrations of TEA reduced the amplitude of \( I_{K,H} \) elicited at these same membrane potentials. Therefore, it appears that the relatively voltage-independent component of \( I_{K,H} \) is slightly sensitive to TEA and insensitive to 4-AP. This pharmacologic sensitivity of the relatively voltage-independent component of \( I_{K,H} \) is identical to that of the S current (Klein et al. 1982; Brezina et al. 1987, 1988; Shuster and Siegelbaum 1987; Walsh and Byrne 1989).

Figure 9 illustrates the dose-response relationships for the effects of TEA on an \( I_{K,CA} \) like current, \( I_{K,V} \), and the two components of \( I_{K,H} \). The relatively voltage-independent component of \( I_{K,H} \) was isolated from voltage-clamp pulses to -20 mV (\( I_{K,H,-20} \)), which did not activate the voltage-dependent component (e.g., Fig. 1). The voltage-dependent component of \( I_{K,H} \) was isolated from voltage-clamp steps to +20 mV (\( I_{K,H,+20} \)). External TEA produced an outward membrane current. Each point represents the average of between 4 and 15 experiments. For each experiment, the coefficient of variation was no greater than 7% of the mean value. In ASW, \( I_{K,H} \) is present at potentials as negative as -40 mV and increases with depolarization in a relatively voltage-independent manner up to a potential of ~0 mV. At potentials more positive than 0 mV, the highly voltage-dependent component of \( I_{K,H} \) was isolated from voltage-clamp pulses to membrane potentials more depolarized than 0 mV. TEA (100 mM) also blocks the highly voltage-dependent component of \( I_{K,H} \), and has a slight effect on the relatively voltage-independent component of \( I_{K,H} \). These results indicate that pharmacologic agents that block \( I_{K,H} \) also block the voltage-dependent component of \( I_{K,H} \).

ward shift in the \( I-V \) relationship for \( I_{K,H} \) in the presence of 4-AP indicates again that modulation of \( I_{K,CA} \) cannot account for the complex kinetics and voltage dependence of \( I_{K,H} \). It is also interesting to note that this concentration of 4-AP had absolutely no effect on the component of \( I_{K,H} \) elicited by voltage-clamp pulses to membrane potentials more negative than 0 mV. In contrast, high concentrations of TEA reduced the amplitude of \( I_{K,H} \) elicited at these same membrane potentials. Therefore, it appears that the relatively voltage-independent component of \( I_{K,H} \) is slightly sensitive to TEA and insensitive to 4-AP. This pharmacologic sensitivity of the relatively voltage-independent component of \( I_{K,H} \) is identical to that of the S current (Klein et al. 1982; Brezina et al. 1987, 1988; Shuster and Siegelbaum 1987; Walsh and Byrne 1989).

Figure 9 illustrates the dose-response relationships for the effects of TEA on an \( I_{K,CA} \) like current, \( I_{K,V} \), and the two components of \( I_{K,H} \). The relatively voltage-independent component of \( I_{K,H} \) was isolated from voltage-clamp pulses to -20 mV (\( I_{K,H,-20} \)), which did not activate the voltage-dependent component (e.g., Fig. 1). The voltage-dependent component of \( I_{K,H} \) was isolated from voltage-clamp steps to +20 mV (\( I_{K,H,+20} \)). External TEA produced an outward membrane current. Each point represents the average of between 4 and 15 experiments. For each experiment, the coefficient of variation was no greater than 7% of the mean value. In ASW, \( I_{K,H} \) is present at potentials as negative as -40 mV and increases with depolarization in a relatively voltage-independent manner up to a potential of ~0 mV. At potentials more positive than 0 mV, the highly voltage-dependent component of \( I_{K,H} \) was isolated from voltage-clamp pulses to membrane potentials more depolarized than 0 mV. TEA (100 mM) also blocks the highly voltage-dependent component of \( I_{K,H} \), and has a slight effect on the relatively voltage-independent component of \( I_{K,H} \). These results indicate that pharmacologic agents that block \( I_{K,H} \) also block the voltage-dependent component of \( I_{K,H} \).

ward shift in the \( I-V \) relationship for \( I_{K,H} \) in the presence of 4-AP indicates again that modulation of \( I_{K,CA} \) cannot account for the complex kinetics and voltage dependence of \( I_{K,H} \). It is also interesting to note that this concentration of 4-AP had absolutely no effect on the component of \( I_{K,H} \) elicited by voltage-clamp pulses to membrane potentials more negative than 0 mV. In contrast, high concentrations of TEA reduced the amplitude of \( I_{K,H} \) elicited at these same membrane potentials. Therefore, it appears that the relatively voltage-independent component of \( I_{K,H} \) is slightly sensitive to TEA and insensitive to 4-AP. This pharmacologic sensitivity of the relatively voltage-independent component of \( I_{K,H} \) is identical to that of the S current (Klein et al. 1982; Brezina et al. 1987, 1988; Shuster and Siegelbaum 1987; Walsh and Byrne 1989).

Figure 9 illustrates the dose-response relationships for the effects of TEA on an \( I_{K,CA} \) like current, \( I_{K,V} \), and the two components of \( I_{K,H} \). The relatively voltage-independent component of \( I_{K,H} \) was isolated from voltage-clamp pulses to -20 mV (\( I_{K,H,-20} \)), which did not activate the voltage-dependent component (e.g., Fig. 1). The voltage-dependent component of \( I_{K,H} \) was isolated from voltage-clamp steps to +20 mV (\( I_{K,H,+20} \)). External TEA produced an outward membrane current. Each point represents the average of between 4 and 15 experiments. For each experiment, the coefficient of variation was no greater than 7% of the mean value. In ASW, \( I_{K,H} \) is present at potentials as negative as -40 mV and increases with depolarization in a relatively voltage-independent manner up to a potential of ~0 mV. At potentials more positive than 0 mV, the highly voltage-dependent component of \( I_{K,H} \) was isolated from voltage-clamp pulses to membrane potentials more depolarized than 0 mV. TEA (100 mM) also blocks the highly voltage-dependent component of \( I_{K,H} \), and has a slight effect on the relatively voltage-independent component of \( I_{K,H} \). These results indicate that pharmacologic agents that block \( I_{K,H} \) also block the voltage-dependent component of \( I_{K,H} \).

ward shift in the \( I-V \) relationship for \( I_{K,H} \) in the presence of 4-AP indicates again that modulation of \( I_{K,CA} \) cannot account for the complex kinetics and voltage dependence of \( I_{K,H} \). It is also interesting to note that this concentration of 4-AP had absolutely no effect on the component of \( I_{K,H} \) elicited by voltage-clamp pulses to membrane potentials more negative than 0 mV. In contrast, high concentrations of TEA reduced the amplitude of \( I_{K,H} \) elicited at these same membrane potentials. Therefore, it appears that the relatively voltage-independent component of \( I_{K,H} \) is slightly sensitive to TEA and insensitive to 4-AP. This pharmacologic sensitivity of the relatively voltage-independent component of \( I_{K,H} \) is identical to that of the S current (Klein et al. 1982; Brezina et al. 1987, 1988; Shuster and Siegelbaum 1987; Walsh and Byrne 1989).

Figure 9 illustrates the dose-response relationships for the effects of TEA on an \( I_{K,CA} \) like current, \( I_{K,V} \), and the two components of \( I_{K,H} \). The relatively voltage-independent component of \( I_{K,H} \) was isolated from voltage-clamp pulses to -20 mV (\( I_{K,H,-20} \)), which did not activate the voltage-dependent component (e.g., Fig. 1). The voltage-dependent component of \( I_{K,H} \) was isolated from voltage-clamp steps to +20 mV (\( I_{K,H,+20} \)). External TEA produced an outward membrane current. Each point represents the average of between 4 and 15 experiments. For each experiment, the coefficient of variation was no greater than 7% of the mean value. In ASW, \( I_{K,H} \) is present at potentials as negative as -40 mV and increases with depolarization in a relatively voltage-independent manner up to a potential of ~0 mV. At potentials more positive than 0 mV, the highly voltage-dependent component of \( I_{K,H} \) was isolated from voltage-clamp pulses to membrane potentials more depolarized than 0 mV. TEA (100 mM) also blocks the highly voltage-dependent component of \( I_{K,H} \), and has a slight effect on the relatively voltage-independent component of \( I_{K,H} \). These results indicate that pharmacologic agents that block \( I_{K,H} \) also block the voltage-dependent component of \( I_{K,H} \).
MODULATION OF K⁺ CURRENTS

see Stanfield 1983; Hille 1984; Rudy 1988) indicate that each K⁺ channel is blocked by a single molecule of TEA. Thus the block of K⁺ currents by TEA can be fit by a one-to-one binding reaction, which assumes that the percentage of K⁺ current remaining is equal to the number of K⁺ channels not occupied by a molecule of TEA: % control = 100 - 100x ([TEA]/(K_d + [TEA])), where K_d is the apparent dissociation constant. The I_k,L-like current was the most sensitive to TEA with an estimated K_d of 0.4 mM. I_k,C and I_k,L+H had very similar sensitivities to TEA with estimated K_d of 8 and 5 mM, respectively. Therefore, it appears that I_k,L+H represents the serotonergic modulation of a membrane current that has properties closely resembling I_k,L. Finally, I_k,T,-20 had an estimated K_d of ~92 mM. These values for K_d are close to those previously reported by others (Thompson 1977; Hermann and Gorman 1981b; Brezina et al. 1987, 1988; Shuster and Siegelbaum 1987).

Modulation of membrane currents by cAMP

Previous work on sensory neurons has shown that serotonergic modulation of the S current is mediated via cyclic AMP (cAMP) (Siegelbaum et al. 1982, 1987; Camardo et al. 1983; Walsh and Byrne 1984, 1989; Ocorr and Byrne 1985; Pollock et al. 1985; Shuster et al. 1985; Belardetti and Siegelbaum 1988). We were interested, therefore, in determining whether cAMP also mediates the serotonergic modulation of I_k,L. If cAMP does mediate both actions of 5-HT, then the application of a membrane-permeable analogue of cAMP should produce the same modulation of membrane currents as 5-HT (e.g., Fig. 1), and preexposure to a saturating concentration of cAMP should occlude any further modulation of membrane currents by 5-HT. Thus we compared the modulatory actions of 8-pcpt-cAMP and 5-HT on membrane currents (Fig. 10). Membrane currents were elicited by voltage-clamp pulses to +20 mV; first in ASW, following addition of 8-pcpt-CAMP (50 μM) and following the addition of 5-HT (30 μM) to the bath already containing 8-pcpt-CAMP (Fig. 10A). Application of 8-pcpt-cAMP reduced the total membrane current at the end of the voltage-clamp pulse but had little effect on total membrane current early during the voltage-clamp pulse. The membrane current that was modulated by 8-pcpt-cAMP was isolated by subtracting the current response elicited in the presence of 8-pcpt-cAMP from the current response elicited in ASW (Fig. 10B). The resulting cAMP difference current (I_cAMP) was similar to the relatively voltage-independent component of I_k,L that can be isolated in ASW at hyperpolarized membrane potentials (e.g., Fig. 2C), and at depolarized membrane potentials when I_k,V has been blocked by 4-AP or high concentrations of TEA (e.g., Figs. 6B2 and 7E). Increasing the bath concentration of 8-pcpt-cAMP by an order of magnitude did not produce any further modulation of membrane currents, and the preexposure to 50 μM of 8-pcpt-cAMP occluded additional modulation of the relatively voltage-independent component of I_k,L by subsequent application of 5-HT (Baxter and Byrne 1987). Thus 50 μM 8-pcpt-cAMP appeared to produce a maximal effect on membrane currents and appeared to modulate only the relatively voltage-independent component of I_k,L.

Although 8-pcpt-cAMP mimicked the actions of 5-HT on the S current, 8-pcpt-cAMP failed to modulate I_k,L. The addition of 5-HT to the bath, which still contains the 8-pcpt-cAMP, produced additional modulation of membrane currents (Fig. 10A, trace c). Application of 5-HT reduced the membrane current early during the voltage-clamp pulse and increased the membrane current at the end of the voltage-clamp pulse. The modulatory effect of 5-HT was isolated by subtracting the current response elicited in the presence of 5-HT (plus 8-pcpt-cAMP) from the...
current elicited in ASW and 8-pcpt-cAMP (Fig. 10B). This $I_{\text{K,HT}}$ waveform displayed the characteristic voltage-dependent component. These results indicate that the modulation of $I_{\text{K,HT}}$ by 5-HT is not mediated by elevated levels of cAMP. Furthermore, these results further support the finding that there are at least two distinct components to $I_{\text{K,HT}}$.

**Modulation of membrane currents by SCP$_b$**

To provide further support for the suggestion that the modulation of $I_{\text{K,HT}}$ by 5-HT is not mediated via cAMP, we compared the modulatory effects of small cardioactive peptide b (SCP$_b$) and 5-HT. In sensory neurons, the actions of SCP$_b$ parallel the actions of 5-HT by elevating intracellular levels of cAMP, which in turn results in the closure of the S channel (Abrams et al. 1984; Ocorr and Byrne 1985, 1986). Thus we compared the modulatory actions of SCP$_b$ and 5-HT on membrane currents. Membrane currents were elicited by voltage-clamp pulses to $+20$ mV first in ASW, following addition of SCP$_b$ (20 PM) and following the addition of 5-HT (20 PM) to the bath already containing SCP$_b$ (Fig. 11A, trace c). The action of SCP$_b$ paralleled that of cAMP and reduced the total membrane current at the end of the voltage-clamp pulse while having little effect on total membrane current early during the voltage-clamp pulse. The application of 5-HT to the bath, which still contained the SCP$_b$, produced additional modulation of membrane currents (Fig. 11A). 5-HT reduced the membrane current early during the voltage-clamp pulse and increased the membrane current at the end of the pulse. The membrane current that was modulated by SCP$_b$ was isolated by subtracting the current response elicited in the presence of SCP$_b$ from the current elicited in ASW (a - b). This difference current is representative of results obtained in 4 different preparations. The 5-HT difference current was isolated by subtracting the current response elicited in the presence of 5-HT (and SCP$_b$) from the current response elicited in ASW containing SCP$_b$ (b - c). These results indicate that SCP$_b$, mimics the actions of 5-HT on the S current, but does not modulate an additional voltage-dependent component as does 5-HT.

**FIG. 11.** Comparison of the modulatory effects of SCP$_b$ and 5-HT on membrane currents. A: membrane currents were elicited by voltage-clamp pulses from $-70$ to $+20$ mV in ASW (trace a), following the addition of SCP$_b$ (20 PM) (trace b), and following the addition of 5-HT (20 PM) to the bath already containing SCP$_b$ (trace c). The action of SCP$_b$ paralleled that of cAMP and reduced the total membrane current at the end of the voltage-clamp pulse while having little effect on total membrane current early during the voltage-clamp pulse. The application of 5-HT to the bath, which still contained the SCP$_b$, produced additional modulation of membrane currents (Fig. 11A). 5-HT reduced the membrane current early during the voltage-clamp pulse and increased the membrane current at the end of the pulse. The membrane current that was modulated by SCP$_b$ was isolated by subtracting the current response elicited in the presence of SCP$_b$ from the current response elicited in ASW (a - b). This difference current is representative of results obtained in 4 different preparations. The 5-HT difference current was isolated by subtracting the current response elicited in the presence of 5-HT (and SCP$_b$) from the current response elicited in ASW containing SCP$_b$ (b - c). These results indicate that SCP$_b$, mimics the actions of 5-HT on the S current, but does not modulate an additional voltage-dependent component as does 5-HT.

**FIG. 12.** The current-voltage ($I-V$) relationships are shown for cAMP and SCP$_b$ difference currents that were isolated from neurons bathed in ASW. [The data for 5-HT difference currents isolated in ASW (Fig. 8) are replotted in this figure for the purpose of comparison.] The amplitudes of the cAMP and SCP$_b$ difference currents were measured at the end of the voltage-clamp pulses, and the data are plotted such that positive values represent a decrease in the outward membrane current (e.g., see Figs. 10 and 11). Each point represents the average of between 4 and 15 experiments. For each average, the coefficient of variation was no greater than 13% of the mean value.
The observation that the I-V relationships for $I_{\text{AMP}}$ and $I_{\text{SCP}_h}$ for membrane potentials above 0 mV do not show the downward shift (Fig. 12) further supports the finding that there are two distinct components to $I_{5-HT}$, only one of which is modulated via elevated levels of cAMP.

**DISCUSSION**

We conclude that in somata of sensory neurons isolated from pleural ganglia 5 HT modulates at least two K⁺ currents that are elicited by brief voltage-clamp pulses. One component has properties consistent with the previously described S current (Klein et al. 1980, 1982; Siegelbaum et al. 1982, 1987; Camardo et al. 1983; Walsh and Byrne 1984, 1989; Ocorr and Byrne 1985; Pollock et al. 1985, 1987; Scholz and Byrne 1987, 1988). It is relatively voltage independent, activates relatively slowly, does not inactivate, is not blocked by 2 mM 4-AP, is relatively insensitive to TEA, and under proper pharmacologic conditions can be isolated and observed at membrane potentials ranging between -40 and +30 mV. The other component has properties similar to $I_{K_s}$. It is voltage dependent with complex kinetics, and it is blocked by 4-AP and TEA. Therefore, we propose that in addition to the S current, 5-HT also modulates $I_{K_s}$, possibly by slowing its activation and inactivation kinetics.

There are several possible reasons why previous investigations have not observed the voltage-dependent component of $I_{5-HT}$ in the sensory neurons of *Aplysia*. First, this component, like $I_{K_s}$, is susceptible to cumulative inactivation during repetitive depolarizations. For example, during voltage clamp pulses to +20 mV that were separated by 90 s and preceded by hyperpolarizations, we observed outward membrane currents that reached an average peak amplitude of 148 nA within 20 ms (e.g., Fig. 24). Eliminating the hyperpolarizing prepulse and shortening the interpulse interval to 10 s reduced the outward current by an order of magnitude and significantly altered the actions of 5-HT on these attenuated membrane currents (data not shown). In addition, the cumulative inactivation is temperature sensitive, and inactivation is more pronounced at room temperature (22° to 25°C) than at the normal physiological temperature of 15°C used in this study (data not shown). Second, 5-HT often does not significantly reduce the maximum conductance of this voltage-dependent component (e.g., Figs. 1, 2, 6, and 11). Therefore, examining the effects of 5-HT on the peak amplitudes of membrane currents would not detect the changes in the kinetics that were revealed by difference currents. Finally, the possibility that abdominal and pleural sensory neurons respond differently to 5-HT cannot yet be ruled out.

Previous patch-clamp studies have shown that in abdominal sensory neurons 5-HT closes the S channel by a cAMP-dependent phosphorylation of a protein closely associated with this K⁺ channel (Siegelbaum et al. 1982; Shuster et al. 1985). Of the two currents modulated by 5-IIT that are described in this paper, only the relatively voltage-independent component appears to be sensitive to elevated levels of intracellular cAMP (see also Walsh and Byrne 1984, 1989; Pollock et al. 1985). Bath applications of cAMP analogues mimic the action of 5-HT on the relatively voltage-independent component of $I_{5-HT}$, but do not mimic the action of 5-HT on the voltage-dependent component of $I_{5-HT}$ (Baxter and Byrne 1987). Furthermore, SCF₃, which elevates levels of cAMP, modulates only the relatively voltage-independent component of $I_{5-HT}$. These results indicate not only that the modulation of $I_{K_s}$ by 5-HT may require another second messenger system, but also provide additional evidence that there are two separate currents modulated by 5-HT. Although the mechanism by which 5-HT modulates $I_{K_s}$ is not known, a tentative hypothesis can be advanced. We have observed that the relationship between the time-to-peak for $I_{K_s}$ and membrane voltage is shifted to more positive membrane potentials by 5-IIT (data not shown). Recent experiments in squid axon have indicated that protein phosphorylation can result in a shift in the voltage dependence of both the activation and inactivation of $I_{K_s}$ (Bezanilla et al. 1986; see also Lagrutta et al. 1989). A similar phosphorylation could account for the modulation of $I_{K_s}$ in the sensory neurons.

While it is not yet clear whether both components of $I_{5-HT}$ are modulated during endogenous nervous activity, it is interesting to note that the somata of these sensory neurons are enveloped by serotonergic varicosities (Lo et al. 1987; Zhang et al. 1988). Physiological stimuli that cause release of 5-HT from these varicosities would have profound effects on the electrophysiological activity of the sensory neurons. Since the relatively voltage-independent component (S current) does not inactivate and is a prominent current at the resting membrane potential, it reduces the excitability of sensory neurons by shunting depolarizing currents. Inhibiting the S channel by bath application of cAMP analogues doubles the number of action potentials stimulated during 1 s depolarizing current pulses, and modestly broadens the action potential (Klein et al. 1986; Baxter and Byrne 1987). Although $I_{K_s}$ is not activated at the resting membrane potential, it increases dramatically with large depolarizations, becoming the predominant outward current. Thus activation of $I_{K_s}$ contributes significantly to the repolarization phase of the action potential. The modulation of $I_{K_s}$ by 5-HT causes a significant decrease in this outward current and a threefold increase in spike duration (Baxter and Byrne 1987 and manuscript in preparation). Thus closure of S channels may account primarily for the enhanced excitability (Klein et al. 1986; Baxter and Byrne 1987), and modulation of $I_{K_s}$ may account primarily for the broadening of the action potential that is observed in the pleural sensory neurons during application of 5-HT.

The pharmacologic methods and the parameters for the voltage-clamp protocol that were used in this study do not rule out the possibility that other currents are modulated by 5-HT. Indeed, Walsh and Byrne (1989) found that 5-HT reduces a Ca²⁺-sensitive K⁺ current that is blocked by low concentrations of TEA but that does not appear to be activated by brief voltage-clamp pulses. Although a possibility exists for a T-A-like species of $I_{K_v}$, in these cells (e.g., Hermann and Hartung 1983; Deitmer and Eckert 1985; Kchoc 1985), it is unlikely that the modulation by 5-HT of a T-E insensitive $I_{K_v}$ could account for the voltage-dependent component of $I_{5-HT}$. To account for the block of the downward shift in the I-V relationship of $I_{5-HT}$ by 4-AP (Fig. 6), such a current would have to be also sensitive to 4-AP. At present, none of the Ca²⁺-activated
K⁺ currents so far described have been shown to be blocked by aminopyridines (Thompson 1977; Adams et al. 1980b; Hermann et al. 1981a, 1983; Stanfeld 1983; Hille 1984; Latorre et al. 1984; Thomas 1984; Deitmer and Eckert 1985; Kehoe 1985; Mallart 1985; Blatz and Magleby 1987; Ritchie 1987; Smart 1987; Cook 1988; Rudy 1988). Furthermore, it is unlikely that the kinetics of IₖCa could account for both the initial upward peak and the late downward shift in the voltage-dependent component of Iₛₛ(MT) (e.g., Fig. 1B2). There also remains the possibility that 5-IIT may modulate inward currents that are not affected by 4-AP or TEA (e.g., Hockberger and Connor 1984; Pellmar 1984; Paupardin-Tritsch et al. 1986; Edmonds et al. 1987, Tsien 1987, Braha et al. 1988).

The results presented in this paper and by others indicate that the modulatory effects of 5-HT on the cellular properties of sensory neurons in Aplysia involve several components. At least three different K⁺ currents are altered: the S current (Klein et al. 1980, 1982; Pollock et al. 1985), a slowly activating Ca²⁺-sensitive K⁺ current (Walsh and Byrne 1989), and Iₛₛ. There is also an increase in the levels of free intracellular Ca²⁺ following application of 5-HT that is not merely secondary to the changes in K⁺ currents (Boyle et al. 1984). In addition, 5-IIT has other actions; including, translocation of the phospholipid-dependent C kinase (Hochner et al. 1986a; Säktor et al. 1986), stimulation of protein synthesis that is required for the development of long-term presynaptic facilitation and increased excitability (Montarolo et al. 1986; Dale et al. 1987), and mobilization of releasable neurotransmitter (Gingrich et al. 1985, 1987, 1988; Hochner et al. 1986a). Thus there are multiple sites for plasticity within a single neuron, and while complex, all of the effects of 5-HT may act synergistically to facilitate synaptic transmission at the sensory to motor neuron synapse.

We thank Drs. L. Cleary, S. Citri, E. Kandel, M. Klein, and K. Scholz for their helpful comments on an earlier draft of this manuscript. This research was sponsored by the Air Force Office of Scientific Research, Air Force Command, USAF, under Grants AFSOR 87-0274 and National Institute of Mental Health Award K02 MH-00649. Address for reprint requests: D. A. Baxter, Dept. of Neurobiology and Anatomy, The University of Texas Medical School, P.O. Box 20708, Houston, TX 77225.

Received 24 August 1987; accepted in final form 10 April 1989.

REFERENCES


HERMANN, A. AND GORMAN, A. L. F. Effects of 4-aminopyridine on...


