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Analysis of the Modulation by Serotonin of a Voltage-Dependent Potassium Current in Sensory Neurons of *Aplysia*

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ABSTRACT Potassium currents in pleural sensory neurons of *Aplysia* were studied under control conditions and in the presence of serotonin (5-HT). Using pharmacological techniques we isolated a current that we refer to as $I_{K,V}$. Although it is not known whether $I_{K,V}$ represents a distinct type of membrane channel, we described its properties using a Hodgkin-Huxley type model. The effects of 5-HT on $I_{K,V}$ were complex. 5-HT decreased by 50% the steady-state magnitude (I_{ss}) of $I_{K,V}$ in response to a voltage-clamp pulse from −50 mV to +20 mV. In addition, 5-HT significantly slowed both activation kinetics (the time constant of activation was increased by 29% at +20 mV) and inactivation kinetics (the time constant of inactivation was increased by 518% at +20 mV). Mathematical descriptions of $I_{K,V}$ in control conditions and in the presence of 5-HT were used to estimate the relative contribution of serotonergic modulation of $I_{K,V}$ to the total 5-HT-induced modulation of membrane currents. Effects of 5-HT on $I_{K,V}$ account for more than 87% of the 5-HT-induced reduction in outward current during the first 20 ms of a voltage-clamp pulse to +20 mV. This result implies that 5-HT exerts many of its effects on spike width in sensory neurons via modulation of $I_{K,V}$. Effects of 5-HT on $I_{K,V}$ are consistent with a model in which the maximal conductance underlying the current is decreased by 50%, and the rate constants between open and closed states of both the activation and inactivation processes are diminished in magnitude across all membrane potentials.

INTRODUCTION

The neuronal circuits that mediate defensive withdrawal reflexes in the marine mollusc *Aplysia californica* are useful systems for studying the cellular and molecular mechanisms underlying several forms of neuronal plasticity that contribute to simple forms of learning and memory. Many of the studies of neuronal plasticity in *Aplysia* have focused on the modulation of K^+ currents in primary mechanoafferent neurons. The first-discovered current of this type was the S-current ($I_{K,S}$), a K^+ current that is modulated by serotonin (5-HT; Klein et al., 1982; Pollock et al., 1985). The S-current is characterized by its very weak voltage dependence, its lack of inactivation, and its relative insensitivity to the potassium channel blockers 4-aminopyridine (4-AP) and tetraethylammonium (TEA) (Baxter and Byrne, 1989; Camardo et al., 1983; Critz et al., 1991; Klein and Kandel, 1980; Klein et al., 1982; Pollock and Camardo, 1987; Pollock et al., 1985; Scholz and Byrne, 1987, 1988; Shuster and Siegelbaum, 1987; Shuster et al., 1991; Siegelbaum, 1987; Siegelbaum et al., 1982; Walsh and Byrne, 1989). Serotonin reduces the magnitude of $I_{K,S}$ (Baxter and Byrne, 1989; Camardo et al., 1983; Klein et al., 1982; Ocorr and Byrne, 1985; Pollock and Camardo, 1987; Siegelbaum, 1987; Siegelbaum et al., 1982). This reduction in $I_{K,S}$ is thought to contribute to presynaptic facilitation underlying several forms of learning (see Byrne, 1987; Byrne et al., 1993; Kandel and Schwartz, 1982).

Recently, evidence has accumulated indicating that other K^+ currents in the sensory neurons are modulated by 5-HT as well. Walsh and Byrne (1989) described a component of the response to 5-HT that is blocked by low (5 mM) concentrations of TEA, replacement of extracellular Ca^{2+} with Ba^{2+}, or intracellular injection of Ca^{2+} chelators. They concluded that a slow component of the Ca^{2+}-activated K^+ current is modulated by 5-HT (Walsh and Byrne, 1989). Baxter and Byrne (1989) described a third K^+ current, modulated by 5-HT, that is highly voltage-dependent, has complex kinetics, and is moderately sensitive to TEA ($K_d \approx 8$ mM). Although it is not known whether this current represents a single distinct K^+ channel type, the kinetics and TEA-dependence of this current are consistent with the criteria established by Rudy (1988) for delayed K^+ currents ($I_{K,V}$). Serotonergic modulation of this current has since been confirmed by Goldsmith and Abrams (1992) and by Hochner and Kandel (1992).

The present study extended the previous analyses of Baxter and Byrne (1989) by characterizing quantitatively $I_{K,V}$, both in the absence and presence of 5-HT. Moreover, we examined, over a restricted range of potentials, the specific properties of $I_{K,V}$ that are altered and determined the quantitative contribution of $I_{K,V}$ to 5-HT-induced changes in total membrane current. We constructed a simple model, based on rate theory, in which 5-HT acts to reduce the maximal conductance underlying $I_{K,V}$ by 50% and to scale the rate constants underlying transitions between open and closed states. This quantitative description of $I_{K,V}$ will permit an assessment of the contributions of the modulation of $I_{K,V}$ to alterations in spike width and excitability induced by 5-HT (Baxter and Byrne, 1990a, b; Belkin et al., 1992; Byrne et al.,

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1990; Canavier et al., 1991). Portions of this work have appeared in preliminary form (White et al., 1992).

METHODS

Experimental methods

Experiments were performed on isolated somata of tail mechanoafferent neurons from *Aplysia californica*. Methods for isolating and voltage-clamping these sensory neurons have been described in detail previously (Baxter and Byrne, 1989; Walsh and Byrne, 1989). Briefly, animals were anesthetized by injecting a volume of isotonic $MgCl_2$ equal to one-half the volume of the animal. The somata of the tail sensory neurons were surgically isolated from the ventrocaudal cluster of the pleural ganglion. The isolated clusters were pinned to the floor of a recording chamber (volume $\approx$ 200 μl) lined with Sylgard (Dow-Corning, Midland, MI). The isolated clusters were bathed in artificial sea water (ASW), buffered with 10 mM Trizma (Sigma Chemical Co., St. Louis, MO; pH ~ 7.6), and maintained at 15 ± 1°C with a piezoelectric cooling unit. The ASW contained 150 μM tetrodotoxin (TTX; Calbiochem) to block the fast Na^+ conductance and 2 mM TEA (Eastman Kodak Co, Rochester, NY) to reduce the Ca^{2+}-activated K^+ conductance ($K_d \approx 0.4$ mM; Baxter and Byrne, 1989). The isolated somata were allowed to sit in the cooled chamber for at least 45 min before recordings were begun.

Individual somata were penetrated with two glass micropipette electrodes (resistances of 2–6 MΩ), filled with 3 M potassium acetate. Conventional two-electrode voltage-clamp techniques were used. Cells were held at resting potential (–38 to –50 mV) during each 90-s interstimulus interval. After a 5-s prepulse to a potential of –50 mV, current responses were elicited by voltage-clamp pulses of 200–500-ms duration to membrane potentials of 0, +10, and +20 mV. Each test pulse was preceded by a 10-ms calibration pulse and a 10-ms voltage-clamp pulse to –60 mV, used for leak correction (see below). Data were digitized using a microcomputer (1500 points, sampling interval = 600 μs, 12-bit resolution) and stored for subsequent analysis. Recorded membrane potentials during voltage-clamp steps were monitored using a storage oscilloscope (Tektronix 5111A). Data were accepted if step changes in membrane potential settled at the command voltage in less than 3 ms and if no "sags" in membrane potential were noted.

The protocol used to isolate $I_{K,V}$ under control conditions had two phases. First, responses were elicited in 2 mM TEA by a series of voltage-clamp pulses to membrane potentials of 0, +10, and +20 mV. In these and all other experiments, responses were used only if they were stable over two to three stimulus presentations at each voltage level examined. Second, the bath concentration of TEA was increased to 50 mM, and the voltage-clamp protocol was repeated once the responses stabilized in the new concentration of TEA (~10 min). All traces were corrected for leakage resistance, using the current response to a voltage-clamp step to –60 mV as an indicator of the leakage resistance of the cell. Difference currents were obtained by subtracting the current responses elicited in 50 mM TEA from those elicited in 2 mM TEA (e.g., Fig. 2). This TEA-difference current represents those membrane currents blocked by 50 mM TEA but not by 2 mM TEA. According to previously published dose-response curves for TEA (Baxter and Byrne, 1989), this TEA-subtraction method should isolate $I_{K,V}$.

Experiments designed to measure $I_{K,V}$ in the presence of 5-HT involved a slightly longer protocol with three phases. First, a stable response at +20 mV was obtained in 2 mM TEA. (This initial response was used for normalization purposes; see below.) Second, 5-HT (final concentration, 50 μM; creatinine sulfate compound, Sigma) was added to the recording chamber and, after the responses stabilized (~8 min), recordings were obtained over the range of voltage-clamp pulse potentials. Third, the concentration of TEA was increased to 50 mM and the recording protocol repeated once the responses had stabilized in the new TEA concentration (~10 min). Subtraction of the leakage-corrected responses elicited in 50 mM TEA + 50 μM 5-HT from those elicited in 2 mM TEA + 50 μM 5-HT should isolate $I_{K,V}$ under conditions of serotonergic modulation.

Data analysis

To correct for differences in the sizes of sensory neurons among preparations and to allow for between-group comparisons, it was necessary to normalize the $I_{K,V}$ traces from individual neurons. The normalization procedure involved dividing each $I_{K,V}$ trace by the peak total membrane current (I_p) at +20 mV in 2 mM TEA from that particular neuron. For both control and 5-HT-modulated data, I_p was derived from the first phase of the recording protocol. These normalized traces were adequate for estimating activation and inactivation time constants, but to assess the quantitative contributions of serotonergic modulation of $I_{K,V}$ and to make comparisons with results from other studies, we wished to transform the data into units of nA. To perform this transformation, we calculated the mean value of I_p across all experiments ($\bar{I}_p$) and multiplied each normalized $I_{K,V}$ trace by $\bar{I}_p$ = 72.2 nA; ±SE = 6.0 nA; n = 16). The results in Table 1 and Figs. 3–6 are in this form.

$I_{K,V}$ was described using a Hodgkin-Huxley type model. Although such models are not accurate in all details (Hille, 1992), they are relatively simple and adequate for describing membrane currents quantitatively. In these models, the current was assumed to be described by the equation:

$$I_{K,V}(V, t) = g_{max}A(V, t)^p B(V, t)(V - E_K) \quad (1)$$

where t is time, V is transmembrane voltage, g_{max} is the maximum value of the conductance, $A(V, t)$ is the activation function, $B(V, t)$ is the inactivation function, p is a positive integer, and E_K is the reversal potential of K^+ (Hodgkin and Huxley, 1952; Hille, 1992). $A(V, t)$ and $B(V, t)$ were described by the following differential equations:

$$\frac{dA}{dt} = \frac{A_\infty - A}{\tau_A} \qquad \frac{dB}{dt} = \frac{B_\infty - B}{\tau_B}$$

where A_∞ and B_∞ are the voltage-dependent functions describing the steady-state values of activation and inactivation, respectively, and τ_A and τ_B are the voltage-dependent time constants of activation and inactivation, respectively.

Using a nonlinear least-squares curve-fitting routine (SAAM; Resource Facility for Kinetic Analysis, Seattle, WA), we fit the following equation to the normalized experimentally measured responses:

$$I_{K,V}(V, t) = g_{max}[A_\infty(1 - e^{-t/\tau_A})]^p[e^{-t/\tau_B} + B_\infty(1 - e^{-t/\tau_B})][V - E_K]. \quad (2)$$

Equation 2 represents the response to voltage clamp of a Hodgkin-Huxley type current under the assumptions that the initial value of $A = 0$ and that $B = 1$. (Results indicate that these assumptions are valid; see below.)

RESULTS

Effects of 5-HT on total membrane current

Fig. 1 demonstrates the effects of 5-HT on total membrane current in the presence of 2 mM TEA. Fig. 1 *A* shows the outward current induced in a pleural sensory neuron by a voltage-clamp step from –50 mV to +20 mV. Fig. 1 *B* shows the response of the same neuron to an identical voltage-clamp step 10 min after application of 50 μM 5-HT. Fig. 1 *C* shows the 5-HT difference current obtained by subtracting the trace in panel B from that in panel A. The upward deflection at the beginning of the 5-HT difference current indicates that the net outward current was reduced in magnitude. The downward deflection later in the 5-HT difference current indicates that net outward current was increased in magnitude. We have speculated that this complex modulation of total membrane current by 5-HT results largely from the modulation of $I_{K,V}$ by 5-HT (Baxter and Byrne, 1989). To test this hypothesis more directly, and to examine the details of the putative

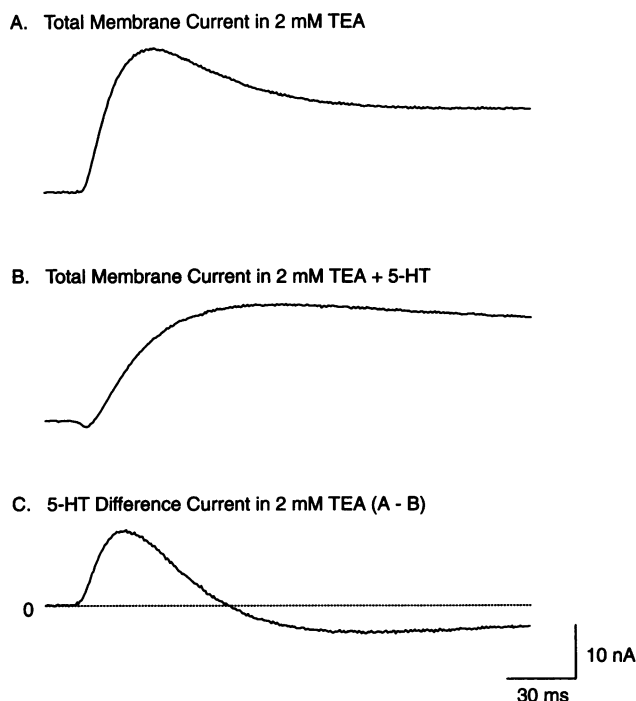


FIGURE 1 The 5-HT difference current. (*A*) Current response elicited by a voltage-clamp step from −50 mV to +20 mV in ASW containing 2 mM TEA. (*B*) Response of the same neuron 10 min after the addition of 50 μM 5-HT. (*C*) The 5-HT difference current at +20 mV, generated by subtracting trace B from trace A.

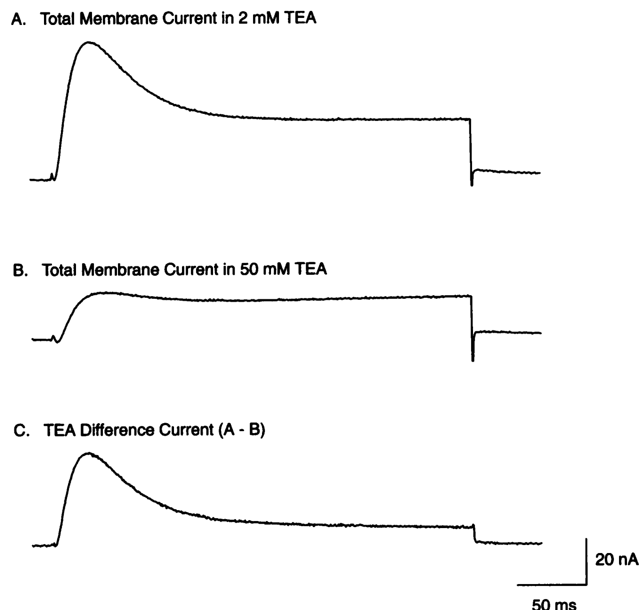


FIGURE 2 The pharmacological isolation procedure for $I_{K,V}$. (*A*) Current response elicited in a tail sensory neuron by a voltage-clamp step from −50 mV to +20 mV in ASW containing 2 mM TEA. (*B*) Response elicited in the same neuron by an identical voltage-clamp step 10 min after increasing the level of TEA to 50 mM. (*C*) The difference current (*A* − *B*), which represents $I_{K,V}$, the delayed, voltage-dependent K^+ current at +20 mV.

modulation of $I_{K,V}$ by 5-HT, we isolated $I_{K,V}$ pharmacologically and tested the effects of 5-HT on its properties.

Pharmacological isolation of $I_{K,V}$

$I_{K,V}$ was isolated using low and high concentrations of TEA. Fig. 2 *A* shows the outward current elicited by a voltage-clamp step from −50 to +20 mV in 2 mM TEA. This concentration of TEA blocks about 80% of the Ca^{2+}-activated K^+ current, whereas only about 14% of $I_{K,V}$ is blocked (Baxter and Byrne, 1989). Fig. 2 *B* shows the response elicited after increasing the concentration of TEA to 50 mM, a concentration high enough to block about 83% of the remaining $I_{K,V}$. Fig. 2 *C* shows the difference current generated by subtracting the trace in panel B from that in panel A. The kinetics and pharmacological properties of this current are, in general, consistent with those given by Rudy (1988) for delayed rectifier currents, although $I_{K,V}$ in the sensory neurons and other neurons in *Aplysia* seem to inactivate more rapidly than most other delayed K^+ currents.

Effects of 5-HT on $I_{K,V}$

To estimate the contribution of $I_{K,V}$ to 5-HT difference currents like that in Fig. 1 *C*, measurements of $I_{K,V}$ were made both in the absence and presence of 5-HT. Thus, the isolation procedure shown in Fig. 2 was applied both in ASW and in ASW containing 50 μM 5-HT. Fig. 3 *A* shows normalized TEA difference currents (i.e., $I_{K,V}$) for voltage-clamp pulses to +20 and +10 mV under control conditions. Fig. 3 *B* shows normalized TEA difference currents collected from a different cell in the presence of 5-HT. In the presence of 5-HT, $I_{K,V}$ is smaller in magnitude. The kinetics of the traces can be compared in Fig. 3 *C*, in which records of $I_{K,V}$ in control and 5-HT-modulated conditions are superimposed and scaled to the same magnitude. In the presence of 5-HT, $I_{K,V}$ is slower to activate and inactivate.

To allow a quantitative examination of the kinetics of $I_{K,V}$ in the absence and presence of 5-HT, we used a nonlinear least-squares program (SAAM; Resource Facility for Kinetic Analysis, Seattle, WA) to fit Eq. 2 to individual TEA difference currents collected in the absence and presence of 5-HT. For a given value of p, this procedure gave us estimates of four parameters determining the Hodgkin-Huxley type description of the current (Eq. 2): the lumped parameter $I_{ss} = g_{max}A_{\infty}^{p}(V - E_K)$, the steady-state value the current would approach if there were no inactivation; B_{∞}, the steady-state inactivation function; τ_A, the time constant of activation; and τ_B, the time constant of inactivation.

An example of the curve-fitting procedure is shown in Fig. 4. The symbols show collected data points. The three smooth curves represent: the activation component of the response, $I_{ss}(1 - e^{-t/\tau_A})^2$; the inactivation component of the response, $I_{ss}[e^{-t/\tau_B} + B_{\infty}(1-e^{-t/\tau_B})]$; and the modeled current from Eq. 2. As shown in Fig. 4, I_{ss} can be much larger than the maximal value reached by the measured current. Curve fits were made to each analyzed data trace with $p = 1, 2$, and 3. A value of $p = 2$ gave the best fits to the data in 31 of 33 cases.

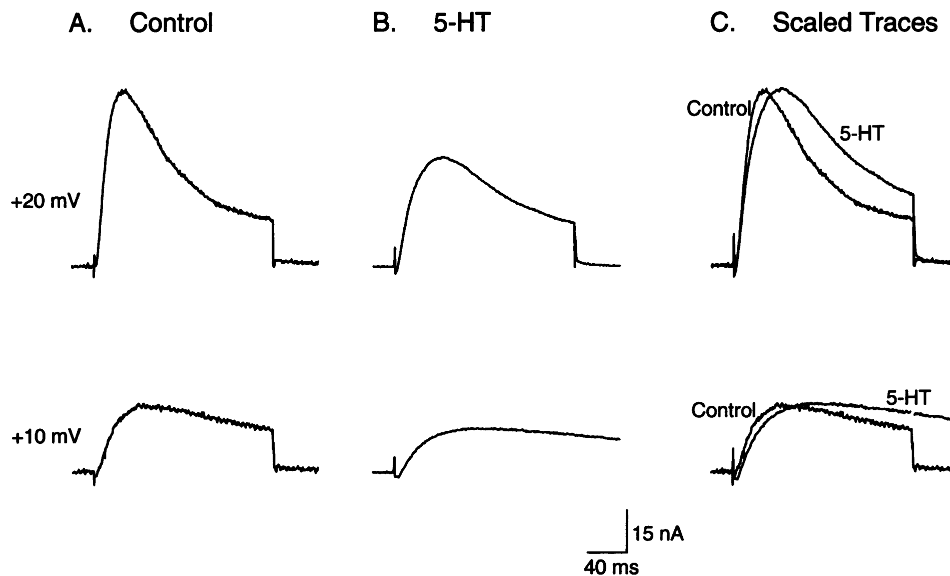


FIGURE 3 $I_{K,V}$ is modulated by 5-HT. (*A*) Control $I_{K,V}$ responses to steps from −50 mV to +20 and +10 mV. The responses are from the same neuron. $I_{K,V}$ was isolated using the procedure described in Fig. 2. (*B*) 5-HT-modulated $I_{K,V}$ responses from another neuron. The same isolation procedure was used in the presence of 50 μM 5-HT. A voltage-clamp step of longer duration was used in the lower trace. (*C*) The same responses, scaled to equal magnitudes and superimposed.

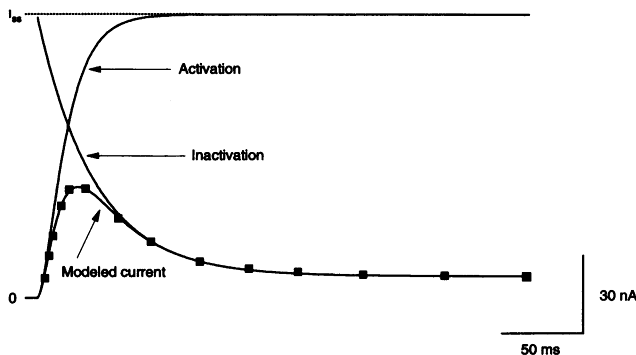


FIGURE 4 Model of $I_{K,V}$. The filled squares represent the physiological data, generated by the isolation procedure described in Fig. 2. Also depicted are the best-fit activation process, $I_{ss}(1 - e^{-t/\tau_A})^2$, the inactivation process, $I_{ss}[e^{-t/\tau_B} + B_\infty(1 - e^{-t/\tau_B})]$, and the modeled current from Eq. 2. The dashed line represents I_{ss}, the maximal value of the scaled activation process.

Table 1 shows summary results from application of the curve-fitting procedure, with $p = 2$, to data over a range of 0 to +20 mV. Listed in the table are means ± SE of I_{ss}, τ_A, B_∞, and τ_B. The number of experiments (n) is listed for each condition as well. Application of 5-HT had several effects on these parameters. First, 5-HT decreased the mean value of I_{ss}. For example, the mean value of I_{ss} was decreased by 49% at +20 mV. Second, 5-HT slowed the kinetics of activation of $I_{K,V}$ at all potentials examined. At +20 mV, the mean value of τ_A was increased by 29%. Third, 5-HT slowed the kinetics of inactivation of $I_{K,V}$ at all potentials examined. At +20 mV, τ_B was increased by 517%. 5-HT did not appear to have a consistent effect on B_∞.

The statistical significance of these changes was assessed using a two-way ANOVA, using drug treatment (Control vs. 5-HT-modulated) and voltage-clamp pulse potential (0, +10, and +20 mV) as the two factors for the analysis. The values of τ_A ($F_{2,27} = 24.17$, $p < 0.001$) and τ_B ($F_{2,22} = 11.53$, $p < 0.005$) in control conditions were significantly different from those in 5-HT. The change in normalized I_{ss} was significant as well ($F_{2,27} = 20.92$, $p < 0.001$). No significant difference in control versus 5-HT-modulated B_∞ was found ($F_{2,22} = 0.001$).

Modulation of $I_{K,V}$ accounts for the early part of the 5-HT difference current

To evaluate the contribution of modulation of $I_{K,V}$ to 5-HT difference currents (e.g., Fig. 1), we compared 5-HT difference currents for steps to +20 mV in 2 mM TEA with a physiologically-based mathematical model of 5-HT-induced changes in $I_{K,V}$. (A similar comparison could have been made with the empirical data as well.) Trace A in Fig. 5 is a model description of $I_{K,V}$ in 2 mM TEA for a voltage-clamp pulse to +20 mV, calculated using Eq. 2 and the mean parameters describing $I_{K,V}$ in control conditions from Table 1. The trace has been scaled by a factor of 1.2 to account for the fact that 50 mM TEA does not block $I_{K,V}$ completely (Baxter and Byrne, 1989). Trace B shows our model of $I_{K,V}$ in 5-HT. This modeled current has been scaled by a factor of 1.2 as well. Trace C, the difference between traces A and B, represents the predicted contribution of 5-HT-induced modulation of $I_{K,V}$ to the 5-HT difference current at +20 mV. The upward deflection for the first 45 ms indicates a reduction in net outward current due to an increase in τ_A and a reduction in I_{ss}. The downward deflection at later times is an increase in outward current caused by the 5-HT-induced increase in τ_B.

The average of six normalized 5-HT difference currents is shown in trace *D* of Fig. 5. Each 5-HT difference current (see

TABLE 1 Parameters describing $I_{K,V}$

		Control				5-HT				
V	n	I_{SS}	τ_A	B_∞	τ_B	n	I_{SS}*	τ_A*	B_∞	τ_B*
mV		*nA*	*ms*		*ms*		*nA*	*ms*		*ms*
0	4	3.8 ± 0.8	29.3 ± 6.3	1.0‡	‡	3	3.5 ± 0.2	56.9 ± 10.1	1.0‡	‡
+10	7	27.7 ± 3.4	17.4 ± 0.7	0.08 ± 0.04	296.2 ± 122.7	7	16.7 ± 1.6	34.1 ± 5.0	0.13 ± 0.13	1354.7 ± 317.9
+20	6	120.7 ± 11.4	15.5 ± 0.8	0.10 ± 0.02	40.2 ± 3.1	6	61.6 ± 5.3	20.0 ± 0.9	0.04 ± 0.03	248.0 ± 46.7

Values are means ± SE.
* Significantly different from control (two-way ANOVA; $p < 0.005$). See text.
‡ No inactivation was detected for voltage-clamp steps to 0 mV. In the curve fits, B_∞ was fixed to 1.0, and no estimate of τ_B was obtained in these cases.

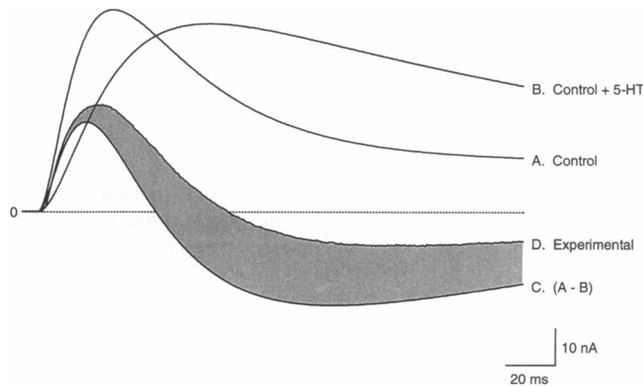


FIGURE 5 Contribution of $I_{K,V}$ to the 5-HT difference current at +20 mV. (*Trace A*) The model description of $I_{K,V}$ in ASW, based on the mean Hodgkin-Huxley type parameters (see Table 1 and Eq. 2). (*Trace B*) The model description of $I_{K,V}$ in ASW containing 5-HT. (*Trace C*) The difference current ($A - B$), representing the predicted contribution of serotonergic modulation of $I_{K,V}$ to the 5-HT difference current. (*Trace D*) the mean 5-HT difference current, based on averaged data from six neurons. The hatched region is the portion of the 5-HT difference current not accounted for by the serotonergic modulation of $I_{K,V}$.

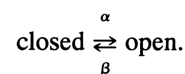
Fig. 1) was normalized via the same procedure used for $I_{K,V}$ traces (i.e., each value was divided by I_p for that particular neuron and multiplied by $\bar{I}_p$). The hatched region between traces C and D represents the portion of the 5-HT difference current for which our description of the modulation does not account.

Examination of Fig. 5 reveals two important facts about serotonergic modulation of membrane currents in sensory neurons. First, Fig. 5 implies that at least one membrane current other than $I_{K,V}$ contributes to 5-HT difference currents in 2 mM TEA. This result is not surprising; both $I_{K,S}$ and $I_{K,Ca}$ are known to be modulated by 5-HT (see Introduction). Because $I_{K,Ca}$ is mostly blocked in 2 mM TEA (Baxter and Byrne, 1989; Walsh and Byrne, 1989), we would expect that the hatched region represents mostly $I_{K,S}$. Indeed, the current represented by the hatched region has characteristics compatible with previous descriptions of $I_{K,S}$. It has a magnitude of about 16 nA and an activation time constant of about 35 ms, and it exhibits little inactivation (cf., Klein et al., 1982; Baxter and Byrne, 1990a, b; Canavier et al., 1991). In addition, this remaining component can be fairly well described by a Hodgkin-Huxley model of a slowly activating, noninactivating K^+ conductance with a g_{max} of 0.5 μS (Baxter and Byrne, 1990; Byrne et al., 1990; Canavier et al., 1991). A component of the Ca^{2+} current is also modulated by 5-HT (Braha et al., 1993). The magnitude of this modulation is small (1–2 nA), however, and thus such modulation probably constitutes a relatively small component of the current not accounted for by $I_{K,V}$.

Second, the results shown in Fig. 5 imply that 5-HT-induced changes in the voltage-clamp response at +20 mV are dominated by modulatory changes in $I_{K,V}$ at the beginning of the voltage-clamp response. Modulation of $I_{K,V}$ by 5-HT accounts for 100% of the 5-HT-induced decrease in outward current at $t = 5$ ms, 91% of the 5-HT difference current at $t = 10$ ms, 87% of the difference current at $t = 20$ ms, and 67% of the 5-HT difference current at $t = 30$ ms. The result that the early components of 5-HT difference currents are mainly due to changes in $I_{K,V}$ suggests that serotonergic modulation of $I_{K,V}$ in sensory neurons is a key contributor to 5-HT-induced spike broadening (see Discussion) (Baxter and Byrne, 1990a, b; Belkin et al., 1992; Byrne et al., 1990; Canavier et al., 1991).

Modulation of $I_{K,V}$ by 5-HT can be accounted for by changes in g_{max} and reaction rate constants

Although our range of data is limited and we do not know that the complex effects of 5-HT on $I_{K,V}$ represent the modulation of a single type of channel molecule, we thought it would be useful to examine further whether relatively simple modifications in a kinetic model could explain complex changes in the magnitude and kinetics of $I_{K,V}$. To this end, we derived descriptions of $I_{K,V}$ based on rate theory (Borg-Graham, 1991; Hille, 1992; Hodgkin and Huxley, 1952; Tsien and Noble, 1969). In this scheme, the state of each channel is determined by the state of two identical gating particles that govern activation (i.e., $p = 2$ in Eqs. 1 and 2) and one gating particle that governs inactivation. Each gating particle alternates between open and closed states with first-order kinetics



$$\text{closed} \underset{\beta}{\overset{\alpha}{\rightleftarrows}} \text{open.}$$

From rate theory, the rate constants α_A and β_A governing activation are given:

$$\alpha_A = \alpha_{AO} e^{-z_A \gamma_A (V - V_A)F/RT} \qquad \beta_A = \beta_{AO} e^{z_A(1-\gamma_A)(V-V_A)F/RT}$$

where z_A is the effective valence of the gating particle, γ_A

is the normalized position in the membrane of the transition state between open and closed states ($0 \leq \gamma_A \leq 1$), V_A is the half-activation voltage, F is Faraday's constant, R is the gas constant, and T is absolute temperature. The terms α_{A0} and β_{A0} represent the values of α and β, respectively, at the half-activation voltage. Because $V = V_A$ implies that $A_\infty = 0.5$ and, hence, $\alpha_A = \beta_A$, it follows that $\alpha_{A0} = \beta_{A0}$. From these relationships, the following equations for $A_\infty = \alpha_A/(\alpha_A + \beta_A)$ and $\tau_A = 1/(\alpha_A + \beta_A)$ can be derived

$$A_\infty = \frac{1}{1 + e^{z_A(V-V_A)F/RT}}, \tag{3}$$

$$\tau_A = \frac{1}{\alpha_{A0}} \frac{e^{z_A\gamma_A(V-V_A)F/RT}}{(1 + e^{z_A(V-V_A)F/RT})}. \tag{4}$$

Mean values of $I_{ss} = g_{max} A_\infty^2(V - E_K)$ and τ_A were used to perform simultaneous fits to Eqs. 3 and 4 for $I_{K,V}$ with free variables g_{max}, V_A, z_A, γ_A, and α_{A0}. These fits were performed separately for control data and 5-HT-modulated data. For control data, the following set of parameters describe activation: $g_{max} = 3.19$ μS, $V_A = 15.8$ mV, $z_A = -3.23$, $\gamma_A = 0.97$, and $\alpha_{A0} = 32$ s^{-1}. For 5-HT-modulated data, parameters obtained are: $g_{max} = 1.63$ μS, $V_A = 15.1$ mV, $z_A = -2.72$, $\gamma_A = 1.0$, and $\alpha_{A0} = 18$ s^{-1}.

The similar values of V_A, z_A, and γ_A obtained under control conditions and in the presence of 5-HT suggest that 5-HT can exert its effects on the process of activation by modifying only g_{max} and α_{A0}. Fig. 6 illustrates this point. Fig. 6 *A* shows measured and theoretical values of $I_{ss} = g_{max} A_\infty^2 (V - E_K)$ for control and 5-HT-modulated conditions. (Corresponding theoretical values of A_∞^2, which are the same in control and modulated conditions, are included in Fig. 6 *B*.) Fig. 6 *C* shows measured and theoretical values of τ_A for control and modulated conditions. In Figs. 6 *A* and 6 *C*, both control and modulated theoretical traces were calculated using $V_A = 15.45$ mV, $z_A = -2.95$, and $\gamma_A = 1.0$. Values of $g_{max} = 3.19$ μS and $\alpha_{A0} = 31$ s^{-1} were used for the control traces, whereas values of $g_{max} = 1.63$ μS and $\alpha_{A0} = 18$ s^{-1} were used for the 5-HT-modulated traces. The good fits to the measured data indicate that the data are consistent with a mechanistic model in which 5-HT exerts its effects by modifying only g_{max} and α_{A0}.

We derived equations describing B_∞ and τ_B in terms of rate theory as well. In this derivation, it was assumed that there exists a minimum level of inactivation (B_{min}). The equations are

$$B_\infty = \frac{1 - B_{min}}{1 + e^{z_B(V-V_B)F/RT}} + B_{min}, \tag{5}$$

$$\tau_B = \frac{1}{\alpha_{B0}} \frac{e^{z_B\gamma_B(V-V_B)F/RT}}{1 + e^{z_B(V-V_B)F/RT}}. \tag{6}$$

Mean values of B_∞ and τ_B were used to perform simultaneous fits to Eqs. 5 and 6 with free variables B_{min}, V_B, z_B,

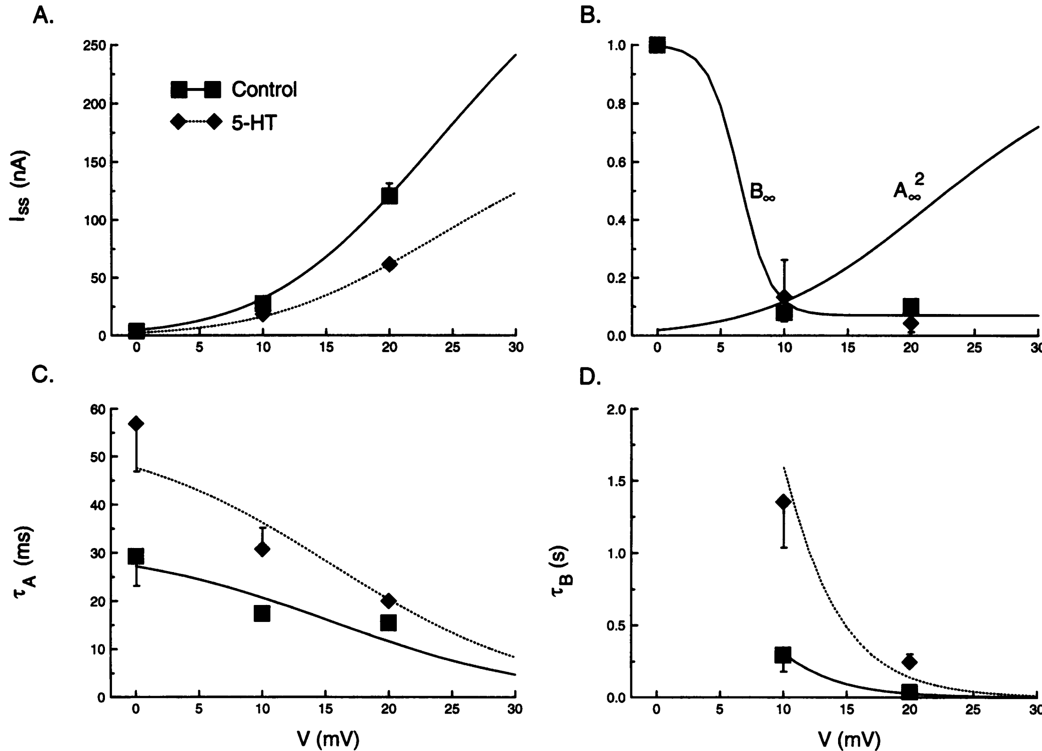


FIGURE 6 Hodgkin-Huxley parameters describing $I_{K,V}$. In each panel, the solid squares represent experimental data collected under control conditions. The solid diamonds represent data collected in the presence of 50 μM 5-HT. Error bars represent ±SE. In some cases the ±SE is smaller than the symbols (see Table 1). The solid lines represent model descriptions of the control data. The dashed lines are model representations of data in 5-HT. See text for more details. The plots show steady-state current (*A*), the steady-state activation and inactivation variables (*B*), the activation time constant (*C*), and the inactivation time constant (*D*) as functions of membrane potential. (The curve for A_∞^2 in panel *B* was derived from the equation $I_{ss} = g_{max} A_\infty^2(V - E_K)$ and the control data for I_{ss} illustrated in panel *A*.)

γ_B, and α_{B0}. Because 5-HT exerted no significant effect on B_∞, control and 5-HT-modulated values of B_∞ were lumped together in these curve fits. Control and 5-HT-modulated values of τ_B were fit separately.

Fig. 6 *B* shows measured and theoretical values of B_∞ as functions of membrane potential. Fig. 6 *D* shows measured and theoretical values of τ_B. The theoretical values of B_∞ and τ_B were computed using Eqs. 5 and 6 with $B_{min} = 0.07$, $V_B = 6.5$ mV, $z_B = 20.5$, and $\gamma_B = 0.7$. The control curve in panel *D* was calculated using $\alpha_{B0} = 1.3\ s^{-1}$, whereas the 5-HT-modulated curve was calculated with $\alpha_{B0} = 0.25\ s^{-1}$. Although the data are from a somewhat limited range of potentials, the fact that the data can be accounted for by changing only α_{B0} is further evidence in favor of a model in which 5-HT affects only the rate constants between the inactivated and noninactivated states.

DISCUSSION

Previous studies of serotonergic modulation of membrane currents in sensory neurons of *Aplysia* have focused mainly on modulation of the S-current ($I_{K,S}$; Klein et al., 1982; Pollock et al., 1985). Here we have described 5-HT-dependent modulation of $I_{K,V}$, a K^+ current that can be distinguished from $I_{K,S}$ based on its steep voltage-dependence and its sensitivity to the K^+ channel blockers TEA and 4-AP (Baxter and Byrne, 1989; Camardo et al., 1983; Critz et al., 1991; Klein and Kandel, 1980; Klein et al., 1982; Pollock et al., 1985; Pollock and Camardo, 1987; Scholz and Byrne, 1987, 1988; Shuster and Siegelbaum, 1987; Shuster et al., 1991; Siegelbaum et al., 1982; Siegelbaum, 1987; Walsh and Byrne, 1989). Although both $I_{K,S}$ and $I_{K,V}$ are modulated by 5-HT, the details of this modulation are different for the two membrane currents. For example, application of 5-HT seems to affect only the magnitude of $I_{K,S}$ (Camardo et al., 1983; Klein et al., 1982; Pollock and Camardo, 1987; Shuster et al., 1985; Siegelbaum et al., 1982; Siegelbaum, 1987). In contrast, 5-HT leads to more complex changes in $I_{K,V}$, affecting not only its magnitude but also its kinetics of activation and inactivation (see also Goldsmith and Abrams, 1992; Hochner and Kandel, 1992). In addition, the serotonergic modulation of $I_{K,S}$ is mediated via the cAMP/PKA second messenger system (e.g., Siegelbaum et al., 1982), whereas modulation of $I_{K,V}$ appears to be mediated by both cAMP/PKA and DAG/PKC (Goldsmith and Abrams 1992; Hochner and Kandel, 1992; Sugita et al., 1991, 1992a, b, 1993).

Complex modulation of K^+ conductances has been seen in other neurons. In bag cell neurons of *Aplysia*, elevated levels of intracellular cAMP lead to an increase in the rate of inactivation of the A current (Kaczmarek and Strumwasser, 1984; Strong, 1984). In addition, cAMP has differential and complex effects on the two components of the delayed rectifier in bag cells (Strong and Kaczmarek, 1986). In squid axons, ATP-dependent phosphorylation induces a shift to more depolarized potentials in the steady-state activation and inactivation curves as well as kinetics of activation of the delayed rectifier (Perozo et al., 1989). Complex modulation of the delayed rectifier has been demonstrated in non-neural tissue as well. In atrial myocytes from the bullfrog, the β-adrenergic agonist isoproterenol shifts the steady-state inactivation curve of the delayed rectifier to more hyperpolarized potentials and modulates the time constant of activation in a complex fashion (Giles et al., 1989). In human T lymphocytes, isoproterenol decreases the amplitude of the delayed rectifier, accelerates its inactivation, and shifts the steady-state inactivation curve to more hyperpolarized levels (Soliven and Nelson, 1990).

Our macroscopic data, collected over a restricted range of membrane potentials, limit our ability to draw conclusions regarding the molecular mechanism(s) underlying the modulation of $I_{K,V}$. We can address some specific hypotheses regarding these mechanisms, however. For example, it has been proposed that ion channel gating may be modified by electrostatic effects of charges near the channel on the voltage sensor of the channel (see Frankenhaeuser and Hodgkin, 1957; Hille, 1992). This so-called *surface potential theory* was proposed originally to explain the dependence of channel gating on levels of extracellular ions (Frankenhaeuser and Hodgkin, 1957), but more recently it has been hypothesized that transferred phosphate groups exert a similar effect on the delayed rectifier in squid giant axon (Perozo and Bezanilla, 1990). Our data seem incompatible with a surface potential mechanism underlying the effects of 5-HT on $I_{K,V}$. In a Hodgkin-Huxley type model, a change in surface potential would lead to a simple shift in all voltage-dependent parameters (Hille, 1992). This effect is not observed in our data. For example, no simple shift in voltage-dependence can explain simultaneously the changes we see in I_{ss} and τ_A in the presence of 5-HT. In addition, the large change in τ_B without an apparent change in steady-state inactivation is incompatible with a simple surface potential effect. Our results do not allow us to rule out a surface-potential mechanism of modulation altogether, however. The possibility exists that 5-HT exerts its effects on only a subpopulation of the channels underlying $I_{K,V}$, leaving open the possibility that a simple change in surface potential in that subpopulation leads to a seemingly complex effect in the total macroscopic current (Perozo et al., 1989).

Our results are consistent with a 5-HT-induced reduction in g_{max}. At the level of single channels, such a change in g_{max} could indicate a change in the number of available channels, a voltage-independent change in the probability of opening of each channel, or a change in the open-state conductance of each channel (Belardetti et al., 1987; Ewald et al., 1985; Levitan, 1988; Numann et al., 1991). Changes in the both number of active channels (Bean et al., 1984; Gunning, 1987; Hurst and Hunter, 1990; Shuster et al., 1985) and voltage-independent changes in the open-channel probability of single channels (Belardetti et al., 1987) have been documented in various cell types.

At the level of single channels, scaling the rate constants of, for example, the reactions governing activation could be accomplished simply by changing the free-energy barriers of transition between activated and nonactivated states (Hille,

1992). To our knowledge, an effect by which a drug scales the free-energy barriers between states has not been described. Nonetheless, such a model seems plausible.

Previous models of plasticity in sensory neurons of *Aplysia* have ascribed 5-HT-induced spike broadening to a decrease in the magnitude of $I_{K,S}$ (Klein et al., 1982). Our results, however, are more compatible with a model in which serotonergic modulation of $I_{K,V}$ is the principal contributor to 5-HT-induced changes in spike width. In responses to voltage-clamp pulses to +20 mV, $I_{K,V}$ accounts for 100% of the 5-HT difference current at $t = 5$ ms and 91% of the 5-HT difference current at $t = 10$ ms (Fig. 5). Action potentials in tail sensory neurons have an average duration of about 3 ms at 15°C (Sugita et al., 1992b). Thus, the repolarization of individual spikes is likely to depend primarily on relatively fast voltage-dependent processes. The dominance of the early portion of the 5-HT difference current by modulation of $I_{K,V}$ implies that modulation of $I_{K,V}$ is the major contributor to 5-HT-induced changes in spike width in sensory neurons. A similar conclusion was reached by Goldsmith and Abrams (1992) and by Hochner and Kandel (1992). Our quantitative description of the modulation of $I_{K,V}$ by 5-HT will allow us to test this prediction quantitatively using a physiologically based model of sensory neurons in *Aplysia* (Canavier et al., 1991).

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