5-HT Modulation of Hyperpolarization-Activated Inward Current and Calcium-Dependent Outward Current in a Crustacean Motor Neuron

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SUMMARY AND CONCLUSIONS

1. Serotonergic modulation of a hyperpolarization-activated inward current, I_h, and a calcium-dependent outward current, I_{Ca}, was examined in the dorsal gastric (DG) motor neuron, with the use of intracellular recording techniques in an isolated preparation of the crab stomatogastric ganglion (STG).

2. Hyperpolarization of the membrane from rest with maintained current pulses resulted in a slow time-dependent relaxation back toward rest and a depolarizing overshoot after termination of the current pulse. In voltage clamp, hyperpolarizing commands negative to approximately -70 mV caused a slowly developing inward current, I_h, which showed no inactivation. Repolarization back to the holding potential of 50 mV revealed a slow inward tail current.

3. The reversal potential for I_h was approximately 35 mV. Raising extracellular K+ concentration ([K+]_o) from 11 to 22 mM enhanced, whereas decreasing extracellular Na+ concentration ([Na+]_o) reduced the amplitude of I_h. These results indicate that I_h in DG is carried by both K+ and Na+ ions.

4. Bath application of serotonin (5-HT; 10 μM) caused a marked increase in the amplitude of I_h through its active voltage ranges.

5. The time course of activation of I_h was well fitted by a single exponential function and strongly voltage dependent. 5-HT increased the rate of activation of I_h. 5-HT also slowed the rate of deactivation of the I_h tail on repolarization to 50 mV.

6. The activation curve for the conductance (G_h) underlying I_h was obtained by analyzing tail currents. 5-HT shifted the half activation for G_h from approximately -105 mV in control to -95 mV, resulting in an increase in the amplitude of G_h active at rest.

7. Two to 4 mM Cs+ abolished I_h, whereas barium (200 μM to 2 mM) had only weak suppressing effects on I_h. Concomitantly, Cs+ also blocked the 5-HT-induced inward current and conductance increase seen at voltages negative to rest. In current clamp, Cs+ caused DG to hyperpolarize 3-4 mV from rest, suggesting that I_h is partially active at rest and contributes to the resting membrane potential.

8. Depolarizing voltage commands from a holding potential of 50 mV resulted in a total outward current (I_o) with an initial transient component and a sustained steady-state component. Application of 5-HT reduced both the transient and sustained components of I_o.

9. I_o was reduced by 10-20 mM tetraethylammonium (TEA), suggesting that it is primarily a K+ current. TEA abolished the response to 5-HT in the depolarizing range, suggesting that 5-HT acts by reducing an outward potassium current.

10. Low extracellular calcium reduced the same components of I_o as did 5-HT. Low extracellular calcium also reduced or eliminated the 5-HT response in the depolarizing range, suggesting that 5-HT specifically reduces I_{Ca}.

11. These results demonstrate that 5-HT has dual effects on the DG motor neuron, in the crab stomatogastric ganglion. We suggest that changes in the two conductances are responsible for the mixed conductance increase and decrease described in an accompanying paper in response to stimulation of peripheral serotonergic stretch receptor cells and local 5-HT application. These conductance changes are at least partially responsible for the serotonergic induction of plateau properties in DG.

INTRODUCTION

Since the discovery of serotonin (5-HT) in the nervous system, its actions on neurons have been studied in a number of different invertebrates and vertebrates. Excitatory, inhibitory, and long-lasting modulatory actions have been detected, and a variety of different currents have been shown to be modulated (see Andrade and Chaput 1990; Anwyl 1990; Kaczmarek and Levitan 1987; Kiehn 1991a; Nicoll et al. 1990; VanderMaelen 1985). It is now clear that multiple 5-HT actions also are present at the single-cell level and that these are mediated through distinct conductance changes (Andrade and Nicoll 1987; Berger and Takahashi 1990; Colino and Halliwell 1987; Gerschenfeld and Paupardin-Tritsch 1974; Takahashi and Berger 1990; Taussig et al. 1989).

In an accompanying paper we showed that 5-HT1 and stimulation of a set of peripheral serotonergic stretch receptor cells induce plateau properties in an identified motor neuron, the dorsal gastric (DG) motor neuron in the stomatogastric ganglion (STG) of the crab (Kiehn and Harris-Warrick 1992). Activity of these cells, called gastropyloric receptor (GPR) cells, and exogenous application of 5-HT excite DG by a mixed conductance increase and decrease mechanism. In this paper we provide evidence that the 5-HT-evoked conductance increase and decrease is mediated by a combined enhancement of a hyperpolarization-activated inward current, I_h, and a decrease of a calcium-dependent outward current, I_{Ca}. This combined action provides a unique mechanism for plateau induction. Part of these data have appeared in preliminary form (Kiehn and Harris-Warrick 1991).

METHODS

Methods for dissecting the STG, identifying cells, and intracellular recordings were described in the accompanying paper (Kiehn and Harris-Warrick 1992). 5-HT was either applied by pressure ejection ("puffing") from a nearby pipette (10 μM to 1 mM) or bath applied (1-10 μM). All voltage-clamp experiments were performed with two electrodes (see Kiehn and Harris-
FIG. 1. Time- and voltage-dependent rectification evoked in the crab dorsal gastric (DG) motor neuron by hyperpolarizing current pulses or voltage commands. A: under current-clamp conditions, hyperpolarizing current pulses (5 s, bottom trace; i) elicited membrane hyperpolarizations that showed a voltage- and time-dependent depolarizing sag toward the resting potential (top trace, V). Termination of the pulses was followed by depolarizing overshoots. Membrane potential, -55 mV. B: under voltage-clamp conditions, hyperpolarizing voltage step commands from -50 mV (3.5 s, bottom traces, V) activated a slow inward current (Ih) and a prolonged inward tail current when the membrane potential was stepped back to the holding potential (top traces, i). The amplitude of Ih and the rate of activation increased with increasing hyperpolarization. Different cells in A and B. Tetrodotoxin (1 μM) was added to the superfusion in A and B. The cell in B was also bathed in tetraethylammonium (10 mM) and 4-aminopyridine (2 mM). Warrick 1992). The data were digitized and stored on magnetic tape with a videocassette recorder for later analysis or collected and stored on-line with the use of the P-Clamp software program (Axon Instruments) running on an Everex STEP-386 computer. Changing the ionic composition of the bathing medium was achieved by the following equimolar substitutions: (1) extracellular sodium concentration was decreased by replacing NaCl with either choline chloride or Trisma-HCl (followed by pH adjustment); (2) extracellular potassium concentration was increased by substitution of NaCl with KCl or decreased by replacing KCl with NaCl; (3) extracellular calcium concentration was reduced by replacing CaCl₂ with MgCl₂. Tetraethylammonium chloride (TEA: 5–20 mM), 4-aminopyridine (4-AP: 2–4 mM), and cesium chloride (CsCl; 1–5 mM) were dissolved in distilled water as concentrated stocks and added to the crab saline.

RESULTS

The voltage-clamp experiments described in this paper were undertaken to determine the conductance changes responsible for the mixed conductance increase and decreased response evoked by 5-HT in the DG motor neuron (Kiehn and Harris-Warrick 1992). To provide a background for understanding the effects of 5-HT on specific conductances, a basic description of some of the ionic currents in DG was necessary. However, especially the outward currents have already been described in detail in other STG neurons (Golowasch and Marder 1992; Graubard and Hartline 1991; Harris-Warrick 1989; Tierney and Harris-Warrick 1992), so we will not attempt to give a full account of the total set of currents in the DG motor neuron.

Hyperpolarization-activated inward current, Ih, in DG

When the DG motor neuron was hyperpolarized from rest under current-clamp conditions, a characteristic time-dependent voltage "sag" developed. This voltage response is illustrated in Fig. 1A (top) for a series of hyperpolarizing current steps. Hyperpolarizing electronic potentials larger than 10–15 mV from rest (approximately -55 mV) caused a rapid hyperpolarization followed by a slowly developing depolarizing sag response that persisted despite a main-
tained hyperpolarizing current injection. This response was voltage dependent: its absolute amplitude increased, and the delay to onset of the sag decreased with increasing hyperpolarization. Depolarizing overshoots occurred at the end of the current pulses. Figure 1B shows the sag response under voltage clamp (the cell was bathed in 1 μM tetrodotoxin (TTX), 10 mM TEA, and 2 mM 4-AP). Hyperpolarizing commands from a holding potential of -50 mV evoked an instantaneous inward current followed by a slow inward relaxation and an inward tail current on repolarization to -50 mV. The inward relaxation during maintained hyperpolarization underlies the sag voltage response, and, as with the sag, the size of inward current increased, and the delay to onset of the inward current decreased with larger negative command potentials.

In contrast to the inward relaxation associated with closure of a K\(^+\) conductance open at the holding potential (see Halliwell and Adams 1982), no reversal of the inward relaxation occurred on hyperpolarization past -80 (between -80 and -90 mV). The time-dependent current in DG therefore shares similarities with hyperpolarization-activated inward currents that have been described in other systems. We will refer to this current as I\(_h\) (also known as I\(_{\text{IR}}\) and I\(_{\text{IR}}\)) (Brown and DiFrancesco 1980; Halliwell and Adams 1982; Mayer and Westbrook 1983; Yanagihara and Irisawa 1980). With the use of hyperpolarizing steps, the threshold for activation of I\(_h\) appeared to be approximately -70 to 75 (see however, Fig. 5 and DISCUSSION), and at -100 mV the amplitude ranged from 0.8 to 2.1 nA. This inward current persisted unchanged during long hyperpolarizing voltage steps (30–60 s), indicating little or no inactivation.

\(I_h\) was not blocked by TTX (0.1–1 μM; \(n = 13\)), TEA (10–20 mM; \(n = 4\)), 4-AP (2–4 mM; \(n = 4\)), low calcium (25% substituted with magnesium; \(n = 4\)), or cobalt (10 mM; \(n = 1\)). Barium (200 μM to 2 mM; \(n = 2\)), which blocks the inward rectifying K\(^+\) current (Hagiwara et al. 1978; Mayer and Westbrook 1983), had little effect on \(I_h\). In contrast, \(I_h\) was very sensitive to low concentrations of extracellular Cs\(^+\), which is characteristic of \(I_h\) in other systems and will be described in detail below. The pharmacological profile of \(I_h\) in DG is thus very similar to that of the hyperpolarization-activated cation current described in many different cells (Angstadt and Calabrese 1989; Brown and DiFrancesco 1980; Halliwell and Adams 1982; Mayer and Westbrook 1983; McCormick and Pape 1990a; Spain et al. 1987; Takahashi 1990; Yanagihara and Irisawa 1980).

**Reversal potential and ionic dependency of \(I_h\)**

In most systems \(I_h\) is a cation current carried by K\(^+\) and Na\(^+\) ions, with a reversal potential depolarized from rest (-40 to -20 mV) (Angstadt and Calabrese 1989; Bader and Bertrand 1984; DiFrancesco 1985; Halliwell and Adams 1982; Mayer and Westbrook 1983; Yanagihara and Irisawa 1980). To determine the reversal

![FIG. 3. Serotonin (5-HT) increases \(I_h\) in the dorsal gastric (DG) motor neuron. A and B: family of currents (top traces) elicited in DG by hyperpolarizing voltage steps from -50 mV in increments of 10 to -140 mV (bottom traces) in control (A) and after superfusion of 10 μM 5-HT. The dashed line indicates a 5-HT-evoked inward shift in holding current compared with A. The cell was maintained in saline with tetrodotoxin (1 μM), tetraethylammonium (70 mM), and 4-aminopyridine (2 mM). C: current-voltage relationship for instantaneous (\(i_i\); ○ and □) and steady-state (\(i_s\); □ and ●) currents obtained in control (○ and □) and after 5-HT (● and ●). 5-HT increased both the instantaneous and steady-state currents. D: \(I_h\) was obtained as the difference curve between \(i_i\) and \(i_s\) and was enhanced in absolute amplitude after 5-HT (●) as compared with control (○) over the entire voltage range. The size of the tail current increased after 5-HT, and the rate of deactivation of \(I_h\) appeared to decrease in the presence of 5-HT (see text for further details).](image-url)
FIG. 4. Increase in \( I_h \) activation rate by serotonin (5-HT). A: graph showing the time constant, \( \tau \) (ms), of \( I_h \) activation plotted against membrane potential in control (●) and during 5-HT (10 \( \mu \)M; □). Time constants were fitted by single-exponential fits and showed strong voltage dependence. B: actual current traces for plot in A. Holding current was −50 mV, and the membrane potential was stepped to −110 mV, under control conditions (top trace) and after 5-HT (bottom trace). Currents are plotted as dots, and the fits are plotted as superimposed lines. 5-HT decreased the time constant in the entire voltage range where it was well fitted [at relative depolarized levels (less than −80 mV) the fit broke apparently because \( I_h \) was very slow and the 1st part of its activation curve was almost linear].

FIG. 5. Effect of serotonin (5-HT) on the voltage activation curve of the conductance, \( G_h \), underlying \( I_h \). The activation curve for \( G_h \) before (●) and during (▲) bath application of 5-HT (10 \( \mu \)M) was constructed by measuring the tail currents elicited by repolarizing the membrane potential to holding potential (−50 mV) following activating voltage commands between −60 and −150 mV, and normalizing the current to the maximal current. The cell was bathed in tetraethylammonium (20 mM), 4-amino-pyridine (2 mM), and tetrodotoxin (1 \( \mu \)M). 5-HT caused a positive shift in voltage activation of \( G_h \) (see text for further details).

FIG. 6. Extracellular cesium blocks \( I_h \) in dorsal gastric motor neuron (DG). A and B. family of currents (top traces in A and B, ▲) elicited in DG by hyperpolarizing voltage steps from −50 mV in increments of 10 to −180 mV (bottom trace in B; V) in normal saline (control; A) and in saline with 2 mM CsCl. C: current-voltage relationship for instantaneous (i; open symbols) and steady-state (i; closed symbols) current before and during Cs+. Circles represent currents under control conditions, and triangles currents in the presence of Cs⁺ as indicated in A and B. Cs⁺ reduced the steady-state current reflecting a block of \( I_h \).

potential of \( I_h \) in the DG motor neuron, the membrane potential was stepped to −140 mV and back to series of test potentials varying from −75 to −15 mV; the magnitude and polarity of the emerging tail currents during deactivation of \( I_h \) was measured and plotted against the test voltage. To minimize contamination of the \( I_h \) tail current by other currents activated at depolarized voltages, we superfused cells with TEA (10–20 mM), 4-AP (2–4 mM), TTX (0.1–1 \( \mu \)M), and cobalt (10 mM) or low calcium. Time was also allowed (Fig. 2A, 1st arrow) for fast transient outward currents, like \( I_A \) (see later), to decay before measurement. Because of the slow time course of decay of \( I_h \), only a small percentage of the actual amplitude of the \( I_h \) tail current is lost by this procedure. In this manner we estimated the reversal potential for the \( I_h \) tail current to be −35 ± 6 (SE) mV (Fig. 2A; \( n = 5 \)).

This reversal potential suggests that in DG \( I_h \) is carried by more than one ion. We have investigated the ionic dependence of \( I_h \) on K⁺ and Na⁺ by changing their extracellular concentrations. Increasing the extracellular K⁺ concentration from 11 to 22 mM evoked an inward shift in holding
current, an increase in the instantaneous current on depolarization, and an increase of \( I_h \) as measured by the differences between the steady state and instantaneous current-voltage (I-V) relationships \( (n = 2) \). The reversal potential for the \( I_h \) tail current was shifted to a more positive value: for the cell illustrated in Fig. 2, \( A \) and \( A1 \), the reversal shifted from \(-33\) to \(-23 \) mV when the extracellular \( K^+ \) concentration was doubled from \( 11 \) to \( 22 \) mM (Fig. 2A). These data suggest that a significant portion of \( I_h \) is carried by \( K^+ \) ions.

Decreasing the extracellular \( Na^+ \) concentration from \( 440 \) to \( 220 \) mM resulted in a marked reduction of the hyperpolarization-activated steady-state current and a leftward shift of the I-V relation \( (n = 2) \). If \( I_h \) is carried in part by \( Na^+ \) ions, reduction of extracellular \( Na^+ \) would shift the reversal potential to more hyperpolarized levels, reducing the driving force for the net current at hyperpolarized test potentials. Perhaps as a consequence of this shift in reversal potential, the deactivation currents, emerging as tail currents obtained after stepping back to the holding potential \((-50 \) mV\), were very small, which made it difficult to measure the reversal for the tail current with accuracy.

Although more experiments are needed to determine the actual ionic dependence of \( I_h \) in the DG motor neuron, we conclude as in other systems that \( Na^+ \) and \( K^+ \) are likely to carry at least the main part of the hyperpolarization-activated current, \( I_h \), in DG.

5-HT enhancement of \( I_h \)

In an accompanying paper \( (Kiehn and Harris-Warrick 1992) \), we showed that 5-HT had a complex excitatory effect on the DG motor neuron, mediated by a mixed conductance increase and decrease mechanism. Part of this effect is due to a 5-HT-evoked enhancement of \( I_h \) at hyperpolarized voltages. Figure 3A depicts the control response \( (1 \mu M \text{ TTX}, 20 \text{ mM TEA}, \text{ and } 2 \text{ mM } 4-\text{AP}) \) in voltage clamp for a family of hyperpolarizing voltage commands from a holding potential of \(-50 \) mV, and Fig. 3C shows the I-V relationship for the instantaneous, \( i_i \), and the steady-state, \( i_s \), currents. The difference curve for \( i_i \) and \( i_s \) shows \( I_h \) \( (\text{Fig. 3D}) \).

Bath application of 5-HT \( (10 \mu M) \) resulted in an inward shift in the holding current (compare dashed line in Fig. 3B with holding current in Fig. 3A). In addition, 5-HT induced an increase in both the instantaneous and steady-state currents evoked by hyperpolarization \( (\text{Fig. 3C}; n = 9) \), which resulted in a marked enhancement of \( I_h \) over the hyperpolarizing voltage range, as seen by the difference curve \( (\text{Fig. 3D}) \).

A close examination of the current traces before and in the presence of 5-HT revealed that the time to activation of \( I_h \) appeared to decrease. \( I_h \) was well fitted \( (r > 0.95) \) by a single exponential function \( (I_t = A_0 + A_1 e^{-t/\tau}, \text{ where } I_t \text{ is the amplitude of the current at time } t, A_0 \text{ and } A_1 \text{ are constants, and } \tau \text{ is the activation time constant}), \text{ and the time constant } (\tau_h) \text{ for activation of } I_h \text{ exhibited strong voltage dependence (Fig. 4, A and B). 5-HT induced a substantial decrease of } \tau_h (n = 5; \text{ Fig. 4, A and B}) \text{ in the voltage range where } I_h \text{ could be well fitted and the overall effect was an apparent depolarizing shift in the voltage dependence of } \tau_h \text{.}

The tail currents after termination of the hyperpolarizing voltage step were substantially increased and markedly prolonged in time course during 5-HT (Fig. 3). This was not merely due to the increased current evoked during 5-HT: if

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**Fig. 3.** Effects of extracellular cesium on the serotonin (5-HT)-evoked inward current and 5-HT enhancement of \( I_h \). A-C: inward shift in holding current \( (\text{top traces in A-C}) \) after a short puff of 5-HT \( (1 \text{ mM}) \) in control \( (A) \), after 4 mM CsCl \( (B) \), and after washout of Cs\(^+\) \( (C) \). The dashed line between \( A \) and \( B \) indicates an outward shift in holding current during Cs\(^+\). \( I_h \) was elicited by a 2-s step from \(-50 \) to \(-100 \) mV every 10 s. A puff of 5-HT enhanced \( I_h \) and increased the size of the instantaneous current, as well as causing a slow inward shift in holding current \( (A \text{ and } C) \). The 5-HT-evoked inward shift in holding current and enhancement of \( I_h \) was markedly reduced by extracellular Cs\(^+\) \( (B) \). 5-HT still evoked a small inward current, but now accompanied by a decrease in apparent input conductance. The 5-HT-evoked effects recovered after removal of Cs\(^+\) \( (C) \).
the tail current was compared for two voltage commands evoking an equivalent $I_h$ amplitude before and during 5-HT (obtained with different voltage commands), the decay of the tail was still prolonged in the presence of 5-HT. This indicates that the rate of deactivation for $I_h$ was slowed down by 5-HT.

**5-HT effect on $G_h$ voltage activation curve**

5-HT could enhance $I_h$ by causing a positive shift in the voltage activation curve for the underlying conductance ($G_h$). Such a positive shift in the activation range of a hyperpolarization-activated current has been observed with β-adrenergic agonists and/or 5-HT stimulation in the heart (DiFrancesco 1985), in sympathetic ganglion cells (Toki-masa and Akasu 1990), and in thalamic cells (McCormick and Pape 1990b).

The activation curve for $G_h$ before and during bath application of 5-HT was constructed by measuring the tail currents elicited by repolarizing the membrane potential to holding potential (−50 mV) after activating voltage commands to between −60 and −150 mV. To minimize contamination of the $I_h$ tail currents by other currents, we sometimes superfused the preparation with TTX (0.1–1 μM), and 4-AP (2–4 mM), and TTX (0.1–1 μM). The magnitude of the tail current after different voltage commands was normalized to the maximal tail current amplitude and plotted against the voltage for the command step. The data were fit by the Boltzmann equation

$$I/I_{\text{max}} = \frac{1 + \exp[(V_m - V_{0.5})/s]}{1 + \exp[(V_m - V_{0.5})/s]}^{-1}$$

where $I$ is the amplitude of the tail current after a given voltage command, $I_{\text{max}}$ is the maximal tail current evoked, $V_m$ is the command potential, $V_{0.5}$ is the membrane potential for half activation of $G_h$, and $s$ is a slope factor that determines the steepness of the fitted curve.

As illustrated in Fig. 5, 5-HT caused a positive shift in the $G_h$ activation curve. The shift was in the range of 9–11 mV, with a half activation occurring at −106 ± 9.8 mV in control and at −96 ± 8.9 mV after 5HT ($n = 3$). The half activation for $G_h$ in DG was low but comparable with that observed in other cells (see Takahashi 1990). If $I_h$ is distributed to electrically distant dendrites (which is most likely), we would underestimate both its actual amplitude and voltage activation range when voltage clamping in the soma (see DISCUSSION). Here, however, it is worth noticing that, because of the strong voltage dependence of $I_h$, a positive shift of the activation curve along the voltage axis would contribute to the 5-HT-evoked enhancement of the $I_h$ amplitude (Fig. 3). Note also that the activation threshold for $I_h$ measured with this method appears to be slightly more positive (in the range of −60 to −55 mV) than observed with long hyperpolarizing downsteps.

**Sensitivity of $I_h$ and 5-HT–induced current to cesium**

The 5-HT–evoked depolarization of DG, seen in current-clamp experiments, was sensitive to low concentrations of extracellular CsCl (Kiehn and Harris-Warrick 1992). We have therefore tested the effect of CsCl on $I_h$ and the 5-HT–evoked inward current in voltage-clamp experiments.

As illustrated in Fig. 6, $I_h$ was greatly reduced after adding 2 mM CsCl ($n = 2$) to the extracellular medium (Fig. 6, A and B). The I–V curves for $i_h$ and $i_w$ were nearly superimposed (Fig. 6C). Higher concentrations of Cs$^+$ (3–5 mM; $n = 2$) caused a complete block of $I_h$ (not shown). In current-clamp recordings, bath application of Cs$^+$ resulted in a complete or near-complete block of the depolarizing voltage sag activated by hyperpolarizing steps and of the depolarizing overshoot at the end of the current pulse, confirming that these features are due to activation of $I_h$.

Figures 7 and 8 illustrate that extracellular CsCl concomitantly blocked $I_h$ and the 5-HT–evoked inward current in the hyperpolarized voltage range. The DG motor neuron was held at −50 mV, and 2-s-long voltage commands to −100 mV were used to evoke $I_h$. A short puff of 5-HT evoked a slow inward current of prolonged duration with an enhancement of $I_h$ (Fig. 7A). Both responses reversed with the same time course. Superfusion of the ganglion with

![Fig. 8. Effects of extracellular cesium on the serotonin (5-HT)-evoked inward current on instantaneous conductance. A: current-voltage relationship for the 5-HT–evoked inward current at different holding potentials in the presence of 2 mM CsCl. The cell was stepped to holding potentials between −85 and −5 mV; after the current had stabilized, a brief puff of 5-HT (1 s) was applied, and the 5-HT–induced current measured at the peak. B: relative conductance changes (in %) at different holding potentials after the 5-HT–evoked inward current in the presence of Cs$^+$.](image-url)
4 mM CsCl caused a small outward shift in the holding current and eliminated the time-dependent inward relaxation during the hyperpolarizing step, reflecting a block of \( I_h \) (Fig. 7B). Under these conditions, 5-HT induced a much smaller inward current and failed to evoke an inward relaxation on hyperpolarization (Fig. 7B), suggesting that 5-HT indeed acts by specifically enhancing \( I_h \).

The fact that CsCl in current clamp caused a 4- to 5-mV hyperpolarization from rest (Kiehn and Harris-Warrick 1992) and induced a small outward current when the DG was voltage clamped (Fig. 7B; \( n = 2 \)) indicates that \( I_h \) is open at rest, where it contributes to the resting membrane potential. This is further supported by the fact that 4 mM CsCl reduced the instantaneous input conductance at \(-50\) mV, as seen by the reduced current at the beginning of the hyperpolarizing voltage step (Fig. 7, A and B). As described earlier (Fig. 3) and also seen in Fig. 7, A and B, the 5-HT-evoked increase in \( I_h \) was accompanied by an increase in the instantaneous input conductance (Fig. 7, A and C). This increase was blocked by 4 mM CsCl (Fig. 7B) and was indeed replaced by an apparent conductance decrease (Fig. 8B; see below). The Cs\(^+\) effects on the 5-HT-evoked changes were reversible (Fig. 7C). This suggests that 5-HT enhances \( I_h \) at rest, and that 5-HT enhancement of the instantaneous input conductance reflects an enhancement of \( I_h \). The 5-HT-evoked positive shift of the voltage activation curve for \( G_h \) (Fig. 5) could also increase the size of this conductance at resting membrane potentials and account for the 5-HT-evoked increase in instantaneous input conductance.

To further demonstrate this, we investigated the ability of Cs\(^+\) to block the 5-HT responses throughout the hyperpolarizing voltage range. In an accompanying paper we showed that 5-HT evoked an inward current with an inverted U-shaped I-V relationship over the entire voltage range (Kiehn and Harris-Warrick 1992). In the lower voltage range the inward current was accompanied by a conductance increase (measured with brief 10-mV hyperpolarizing steps), whereas at depolarized levels it was accompanied by a conductance decrease (Kiehn and Harris-Warrick 1991). As shown in Fig. 8, A and B, the 5-HT-evoked inward current and the conductance increase in the lower voltage range were reduced by 2 mM Cs\(^+\) (\( n = 2 \)), whereas the inward current and the conductance decrease in the upper voltage range were unaffected by Cs\(^+\) ions. This provides further evidence that the 5-HT-evoked inward current in the lower voltage range is mainly mediated by an enhancement of \( I_h \). However, the inward current evoked at depolarized voltages appears to use another ionic mechanism, as we describe below.

**Fig. 9.** Serotonin (5-HT) reduces depolarization-evoked outward current. A and A1: family of currents (top traces) evoked in dorsal gastric motor neuron by a series of depolarizing voltage commands (actual voltage values are indicated for steady-state values (1 s) above the current traces) from a holding potential of \(-50\) mV in control (A), during bath application of 5-HT (10 \( \mu \)M; A1), and after recovery (A2). B and B1: current-voltage relationship for data obtained in A and A1. The initial peak current (B) and the sustained steady-state current (B1) were reduced by 5-HT (○ and ●), compared with control (○ and ●) and recovery (△ and ▲). Notice also that an inward shift in holding current accompanied 5-HT application.
Effects of 5-HT on depolarization-evoked outward currents

The somata of stomatogastric neurons appear to be essentially devoid of depolarization-evoked inward currents (Hartline et al. 1985; Ross and Graubard 1989). In agreement with this, we found that, when depolarizing voltage commands were applied from a holding potential of -50 mV, the total outward current ($I_o$) in DG displayed a large transient component that decayed to a smaller sustained component (Fig. 9A). The $I-V$ relationships for the two components were concave upward (Fig. 9A and B1; control). Bath-applied 5-HT (10 nM) caused an inward shift in holding current and reduced the size of both the transient and the sustained depolarization-evoked outward currents (Fig. 9, A1, B, B2; 5-HT). After removing 5-HT from the bath, $I_o$ recovered to its initial values (Fig. 9, A2, B, and B1; recovery).

These experiments indicated that 5-HT reduces an outward current and/or increases an inward current activated at depolarized voltage levels. As shown in Fig. 8, A and B, and in our accompanying paper (Kiehn and Harris-Warrick 1992), 5-HT evokes an inward current and a net conductance decrease at moderately depolarized holding potentials. Here we will provide further evidence that at least part of this 5-HT-evoked effect is mediated through a reduction of an outward current.

5-HT reduces a calcium-dependent outward current

Detailed voltage-clamp studies have shown that three outward currents account for most of the currents activated by depolarization of the somata of STG neurons: 1) a calcium-activated potassium current [$I_{KCa}$], 2) a transient potassium current ($I_K$), and 3) a delayed rectifier [$I_{Kv}$] (Golo-
wasch and Marder 1992; Graubard and Hartline 1991; Harris-Warrick 1989; Russell and Graubard 1987; Tierney and Harris-Warrick 1992). At a holding potential of -50 mV, \( I_A \) is almost completely inactivated (Golowasch and Marder 1992; Graubard and Hartline 1991; Harris-Warrick 1989; Tierney and Harris-Warrick 1992; O. Kiehn, unpublished observations). The delayed rectifier activates slowly and thus contributes only slightly to the initial peak \( I_o \). The most likely target conductance for the 5-HT reduction is therefore \( I_{\text{KCa}} \), which contributes the major component of the peak \( I_o \) when STG cells are stepped from -50 mV.

In agreement with previous observations (Golowasch and Marder 1992; Graubard and Hartline 1991), we found \( I_{\text{KCa}} \) and \( I_{\text{Kv}} \) were greatly reduced or abolished by 10–20 mM extracellular TEA. This is briefly illustrated for a series of depolarizing voltage steps in Fig. 10, A and B. When the ganglion was superfused with 20 mM TEA, 5-HT had very little effect on the remaining total \( I_o \) (Fig. 10, C and D). This suggests that 5-HT acts by reducing a TEA-sensitive outward current, probably either \( I_{\text{KCa}} \) or \( I_{\text{Kv}} \).

Previous studies have shown that \( I_{\text{KCa}} \) is reduced when extracellular \( Ca^{2+} \) is reduced, whereas \( I_{\text{Kv}} \) is essentially unaffected (Golowasch and Marder 1992; Graubard and Hartline 1991). Accordingly, we studied the effect of low extracellular \( Ca^{2+} \) on the 5-HT response. Figure 11 illustrates that both the transient and sustained components of \( I_o \) are substantially reduced in 25% \( Ca^{2+} \) (replaced with \( Mg^{2+} \); Fig. 11 AI) compared with control (Fig. 11 A: recovery in 11 A2). This profile is similar to previous work showing that \( I_{\text{KCa}} \) in STG neurons has both a transient and a sustained component (Golowasch and Marder 1992; Graubard and Hartline 1991).

Under these conditions of reduced \( I_{\text{KCa}} \), 5-HT's effect on \( I_o \) was almost eliminated. This is illustrated in Fig. 11, B–D, for a series of depolarizing voltage steps. Low extracellular

![Image of Figure 11](image-url)

**FIG. 11.** Low extracellular calcium reduces the serotonin (5-HT) effects on total outward current. A–A2: family of currents (top traces) evoked in dorsal gastric motor neuron (DG) by a series of depolarizing voltage commands from a holding potential of -50 to -20 mV in increments of 5 mV (bottom traces) in control (A), in low (25%) extracellular calcium (calcium substituted with magnesium, A1), and after recovery (A2). Low calcium reduced both the initial and the sustained part of the total outward current. B and B1, outward current (top traces) elicited in a different DG motor neuron by a series of depolarizing voltage commands (bottom traces) from -50 mV in low (25%) extracellular calcium (B1) and in low extracellular calcium plus 5-HT (10 μM; B2). C and D: current-voltage relationships for initial peak current (● and ○) and steady-state currents (● and ○) in low calcium (○ and ○) and in low calcium plus 5-HT (● and ●). 5-HT only caused a minor reduction of the initial peak and steady-state currents in low calcium, indicating a main effect on a calcium-dependent outward current. Notice that 5-HT still caused an inward shift in holding current (B and B1) reflecting activation of \( I_h \).
calcium nearly eliminated the 5-HT reduction of both the peak component and the sustained component of total outward current. These results indicate 5-HT reduces a calcium-dependent outward current, \( I_{out(Ca)} \).

Because of the small and rapidly declining tail current after the outward voltage commands, we have not been able to show that the \( I_{out(Ca)} \) reduced by 5-HT, is in fact a potassium current. However, the total outward current shifted as expected when the extracellular K\(^+\) concentration was decreased or increased, suggesting that \( I_{out(Ca)} \) is in fact a potassium current.

Given this evidence, we suggest that the 5-HT reduction of a calcium-activated potassium current in DG and that this reduction is primarily responsible for the 5-HT-evoked conductance decrease at depolarized voltages described in the previous paper (Kiehn and Harris-Warrick 1992).

**DISCUSSION**

In an accompanying paper (Kiehn and Harris-Warrick 1997) we showed that 5-HT and stimulation of a set of peripheral serotonergic/cholinergic stretch receptors (called GPR cells) induce plateau potential capability in the DG motor neuron of the crab STG. Here we show that this response is due to enhancement of a hyperpolarization-activated inward current and a depression of a calcium-dependent outward current in the DG motor neuron.

5-HT enhancement of inward rectification in the DG motor neuron

Hyperpolarization of the DG motor neuron from rest activates a slow voltage- and time-dependent inward current. This current, \( I_h \), has very slow activation kinetics, a reversal potential positive to rest (around -35 mV), is carried by both K\(^+\) and Na\(^+\) ions, and is blocked by low concentrations of extracellular Cs\(^+\), but not by a range of other K\(^-\) channel blockers including Ba\(^2+\). The characteristics of this inward rectifying current in DG thus correspond with \( I_h \) (also known as \( I_\text{Cl} \) or \( I_\text{Ca} \)) in cardiac cells (Angstadt and Calabrese 1989; Brown and DiFrancesco 1980; DiFrancesco 1985; Yanagihara and Irisawa 1980) and a wide range of peripheral and central neurons (Bader and Bertrand 1984; Edman et al. 1987; Halliwell and Adams 1982; Mayer and Westbrook 1983; McCormick and Pape 1990a; Spain et al. 1987; Takahashi 1990). The characteristics of \( I_h \) in DG also resemble an inward rectifying current, which has been briefly characterized in the lateral pyloric (LP) neuron in the crab (Golowasch and Marder 1992).

The time course for activation of \( I_h \) in DG was much slower than for the corresponding current in hippocampal and cortical neurons (Halliwell and Adams 1982; Spain et al. 1987) and also slower than \( I_h \) in heart cells (Angstadt and Calabrese 1989; DiFrancesco 1985), thalamic neurons (McCormick and Pape 1990a), and dissociated bullfrog sympathetic neurons (Tokimasa and Akasu 1990). A comparable slow \( I_h \) has been seen in the crab LP motor neuron (Golowasch and Marder 1997). It is possible that the differences in activation rates reflect a subclassification of \( I_h \), related to species and neuron types.

Millimolar concentrations of Cs\(^+\) block \( I_h \) (Fig. 6) and cause the DG motor neuron to hyperpolarize from rest (-55 to -60 mV) under current-clamp conditions (Kiehn and Harris-Warrick 1992), or evoke an outward shift in holding potential during voltage clamp at -50 mV (Fig. 7). Concomitantly, Cs\(^+\) reduces the apparent input conductance measured as the instantaneous current during hyperpolarizing voltage steps (Fig. 8B). These results all suggest that \( I_h \) is partially activated at -50 mV and contributes to the normal resting membrane potential. However, with voltage-clamp steps, we were unable to elicit a detectable \( I_h \) at voltages more depolarized than -65 to -70 mV (although measuring the tail current gave slightly more positive values (around -55 to -60 mV for the activation threshold). Although it is possible that \( I_h \) is not responsible for the effects of Cs\(^+\) at these voltages, a more likely explanation is an inadequate space clamp of the DG neuropil. Neurons in the STG have a large neuropil connected to the soma by a single process (King 1976), and complete voltage control of the distant neuropil seems unlikely. A hyperpolarizing voltage command from electrodes in the soma will thus affect distant regions less than regions near the soma. If \( I_h \) is located in the neuropil, the measured voltage activation curves will be more hyperpolarized than the real values obtained in the neuropil. It is thus possible that we underestimate both the actual amplitude and activation time constants of \( I_h \) located in the neuropil distant from the soma. However, we can still compare the response with and without 5-HT, because 5-HT did not change the input resistance apart from the two effects that are the subject of this paper.

We have found that 5-HT has at least four effects on \( I_h \) in the DG motor neuron: 1) it markedly enhances \( I_h \) over its full voltage range; 2) it causes a decrease in the time to activation of \( I_h \); 3) it slows down the rate of deactivation of \( I_h \); and 4) it causes a positive shift of 7-10 mV in the voltage activation curve for the underlying conductance. 5-HT also enhances the initial instantaneous current on large hyperpolarizing steps (Figs. 3 and 7) and causes an inward shift in holding current at -50 mV. We suggest that these changes reflect activation of \( I_h \) at -50 mV, because the 5-HT-mediated effects were blocked along with \( I_h \) by Cs\(^+\). Activation of \( I_h \) can thus explain the 5-HT- and noncholinergic GPR cell-evoked conductance increase at hyperpolarized potentials described in our accompanying paper (Kiehn and Harris-Warrick 1992). This view is also in accordance with the finding that the 5-HT-evoked apparent conductance increase recorded in current clamp in DG was suppressed and reverted to an apparent conductance decrease by reducing extracellular Na\(^+\) (Kiehn and Harris-Warrick 1992). This would shift the reversal potential for \( I_h \) to more negative potentials and reduce the driving force for the current at rest.

The results in this paper are to our knowledge the first demonstration that \( I_h \) is subject to transmitter regulation in invertebrates. It is therefore of interest to compare the 5-HT enhancement of \( I_h \) found in the DG motor neuron with the well known \( \beta \) receptor mediated enhancement of a hyperpolarization-activated cation current in heart cells (Brown and DiFrancesco 1980; DiFrancesco 1985) and the recently described serotonergic and/or noradrenergic enhancement of \( I_h \) in vertebrate central neurons (Bobker and Williams 1989; McCormick and Pape 1990b; Pape and
Mc Cormick 1989, Takahashi and Berger 1990). In heart cells (DiFrancesco 1985; McCormick and Pape 1990b) and thalamic neurons (Mc Cormick and Pape 1990b), $I_h$ enhancement is accompanied by a positive shift in the voltage. Potent modulation of $I_h$ around the resting membrane potential was also seen in thalamic neurons (Mc Cormick and Pape 1990b), neurons in the prepositus hypoglossus nucleus in the brain stem (Bobker and Williams 1989), and neonatal motoneurons (Takahashi and Berger 1990). These data, combined with our results, thus suggest a strong conservation in the mode of $I_h$ transmitter regulation throughout phylogeny.

5-HT-evoked effects on outward currents

In addition to the enhancement of $I_h$, we have shown that 5-HT reduces the total outward current ($I_o$) evoked with depolarizing steps from a holding potential of -50 mV. Detailed voltage-clamp studies of the LP motor neuron in the crab STG (Golowasch and Marder 1992) and of stomatogastric gastric cells from the lobster (Graubard and Hartline 1991; Harris-Warrick 1989; Russell and Graubard 1987; Tierney and Harris-Warrick 1992) have shown that three outward currents dominate $I_o$: 1) a delayed rectifying current, $I_{K(Ca)}$; 2) a transient A-like current, $I_A$, similar to that found in molluscan cells (Neher 1971); and 3) a calcium-dependent outward current, $I_{o(Ca)}$. On the basis of the following observations, we suggest that the main effect of 5-HT on outward current in the DG motor neuron is a reduction of $I_{o(Ca)}$. First, $I_h$ is essentially inactivated at a holding potential of -50 mV (Golowasch and Marder 1992; Graubard and Hartline 1991; Harris-Warrick 1989; Tierney and Harris-Warrick 1992; O. Kiehn, unpublished observations) and thus contributes very little to the total outward current when DG is stepped from -50 mV. Second, 5-HT reduces both the initial and sustained components of $I_o$ (Fig. 9), an effect that is mimicked by low calcium saline and cadmium ions (Fig. 11) (Golowasch and Marder 1992; Graubard and Hartline 1991). Third, low calcium saline, which has no effect on $I_h$ and $I_{K(Ca)}$ (Golowasch and Marder 1992), markedly reduced the 5-HT depressing effect on outward current (Fig. 11). Fourth, 10-20 mM TEA, which reduces or blocks $I_{o(Ca)}$ and $I_{K(Ca)}$ but has little effect on $I_h$ (Golowasch and Marder 1992; Graubard and Hartline 1991), reduces the 5-HT-evoked effect on $I_o$ (Fig. 10). Finally, the delayed rectifier activates slowly and contributes very little to the initial peak $I_o$ (Golowasch and Marder 1992; Graubard and Hartline 1991). The 5-HT effect on the early peak of $I_o$ therefore cannot be attributed to reduction of $I_{K(Ca)}$ and the low calcium experiments speak against a role for $I_{K(Ca)}$ in the 5-HT-evoked reduction of the sustained current. We therefore argue that 5-HT selectively reduces a calcium dependent outward current.

It is still uncertain whether $I_{o(Ca)}$ in DG is carried only by $K^+$ ions, but, if we consider the pharmacology and the evidence from other studies of $I_{o(Ca)}$ in other STG neurons (Golowasch and Marder 1992; Graubard and Hartline 1991), we suggest that $I_{o(Ca)}$ is indeed carried by $K^+$ ions, and that 5-HT suppresses a calcium-dependent potassium current.

A 5-HT-evoked reduction of $I_{o(Ca)}$ is in accordance with the conclusion of our accompanying paper (Kiehn and Harris-Warrick 1992) that 5-HT and GPR stimulation evoke a conductance decrease, which is pronounced at depolarized voltages from rest and reduced by lowering the extracellular calcium. This indicates that activity of the sensory GPR cells specifically reduce $I_{o(Ca)}$ in DG.

$I_{o(Ca)}$ in crab stomatogastric motor neurons resembles the voltage- and calcium-dependent large conductance $K^+$ current that has been described in many central and peripheral neurons (Blatz and Magleby 1987). This current, often called $I_C$, has recently been shown to have a fast-inactivating and a non-inactivating component in Helix neurons (Crest et al. 1990). $I_{o(Ca)}$ in DG also has an early transient and a sustained component (Fig. 11); it is possible that more than one calcium-dependent $K^+$ current is present in DG, in which case 5-HT would simultaneously reduce several calcium-dependent outward currents in DG.

The 5-HT-evoked reduction of $I_{o(Ca)}$ described here shares similarities with the effects of 5-HT on myenteric neurons (Grafe et al. 1980). Like DCs, these neurons have a high resting Ca2+-dependent outward current, which is reduced by 5-HT, resulting in a substantial depolarization with a decrease in resting input conductance (Grafe et al. 1980). This contrasts with a 5-HT-evoked reduction of a calcium-dependent $K^+$ conductance generating the slow post-spike afterhyperpolarization in hippocampal neurons (Andrade and Nicoll 1987; Colino and Halliwell 1987) and spinal motor neurons (Hounsgaard and Kiehn 1989; Hounsgaard et al. 1988; Van Dongen et al. 1986; Wallén et al. 1989). In these cells, 5-HT elicits a small or negligible change in input conductance or membrane potential (cf., however, White and Fung 1989), indicating that the contribution of calcium-dependent outward currents to the overall electrosensitivity differs substantially from cell type to cell type.

The data presented here do not preclude the possibility that 5-HT affects other DG currents in addition to enhancing $I_h$ and reducing $I_{o(Ca)}$. Of special interest are the inward voltage-dependent current(s) responsible for generating the plateau potential, which is carried at least in part by Ca2+ ions (Kiehn and Harris-Warrick 1992). However, in our voltage clamp studies of the DG cells, we never observed inward currents, even after substantial reduction of outward currents with TEA (Fig. 10 and unpublished observations). Previous studies have shown that the somata of neurons in the STG appear to be essentially devoid of inward currents (Hartline et al. 1985; Ross and Graubard 1989); apparently the inward currents are generated in the neuropil beyond the effective range of the soma voltage clamp.

Functional consequences of 5-HT regulation of DG

The 5-HT-evoked enhancement of $I_h$ and reduction of $I_{o(Ca)}$ have important consequences for the electrophysiological behavior of the DG motor neuron. Because both $I_h$ and $I_{o(Ca)}$ contribute to the resting membrane potential in the quiescent DG motor neuron, the 5-HT-evoked enhancement of $I_h$ and reduction of $I_{o(Ca)}$ cause DG to depolarize. This action may itself be adequate to alter the balance between inward and outward conductances to uncover a plateau potential in DG (see DISCUSSION in Kiehn and Harris-Warrick 1992). It is interesting that, when $I_h$ is...
blocked by 5 mM CsCl, 5-HT can still induce a regenerative plateau-like response in DG (Fig. 7 in Kiehn and Harris-Warrick 1992). This indicates that 5-HT enhancement of \( I_h \) is not necessary to induce plateau potentials in DG. This is consistent with the fact that \( I_h \) in any case will slowly deactivate at the membrane potential of the plateau. However, when \( I_h \) is active (i.e., in the absence of Cs⁺) the 5-HT-evoked positive shift in the voltage dependence of \( I_h \) activation and the increase in rate of activation for \( I_h \) cause the DG plateau to be initiated more rapidly (Fig. 7, Kiehn and Harris-Warrick 1992). 5-HT’s enhancement of \( I_h \) therefore seems to be important in the initiation of the plateau in DG. \( I_h \) will also help sustain the plateau, because 5-HT reduces its rate of deactivation. Once activated, the strength of the plateau potential is strongly influenced by \( I_{\text{o(Ca)}} \) activity (Hermann and Wadephul 1987). A 5-HT-induced reduction of \( I_{\text{o(Ca)}} \) will not only increase the amplitude but also increase the duration of the plateau response by postponing the plateau termination. Thus 5-HT modulation of the two conductances plays complementary roles in induction and maintenance of the DG plateau. This unique combination of enhancing \( I_h \) and decreasing \( I_{\text{o(Ca)}} \) might be of importance both in other STG neurons and in the increasing number of cells, where plateau properties are important in neuronal processing.

We thank our colleagues for critical comments on an earlier version of this manuscript.

The research was supported by National Institute of Neurological Disorders and Stroke Grants NS-17323 and NS-32501, United States Department of Agriculture Hatch Act Grant NYC-191410 to R. Harris-Warrick, and the Danish Medical Research Council supported O. Kiehn.

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Received 22 October 1991; accepted in final form 24 March 1992.

REFERENCES


MAVER, M. I. AND WESTBROOK, G. I. A voltage-clamp analysis of inward


