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SEROTONIN DIFFERENTIALLY MODULATES TWO K^+ CURRENTS IN THE RETZIUS CELL OF THE LEECH

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Summary

The effects of 100 μmol l^{-1} serotonin (5-HT) were investigated on the Na^+- and Ca^{2+}-dependent action potential and distinct K^+ currents in the Retzius (R) cells of the hirudinid leeches *Macrobdella decora* and *Hirudo medicinalis* by conventional current-clamp and two-microelectrode voltage-clamp techniques.

1. In normal Na^+-containing Ringer, 5-HT decreased the duration of the action potential prolonged by 5 mmol l^{-1} tetraethylammonium (TEA^+) chloride.

2. In Na^+-free saline containing 25 μmol l^{-1} TEA^+ to block I_K, 5-HT reduced the amplitude and duration of Ca^{2+} spikes evoked by intracellular current injection.

3. Under voltage-clamp, 5-HT enhanced the peak amplitude of an early transient 4-aminopyridine (4-AP)-sensitive, voltage-dependent outward current, termed I_A. A small but significant increase in the time constant of inactivation (τ_{off}) of I_A was also measured after exposure to 5-HT.

4. 5-HT suppressed the peak and steady-state amplitudes of a delayed TEA^+-sensitive, voltage-dependent outward current, termed I_K.

These results demonstrate differential simultaneous modulation of distinct K^+ currents in the Retzius cell of the leech by the endogenous transmitter serotonin. These cells contain and release 5-HT, and are believed to be multifunction neurons implicated in feeding and swimming. This modulation may change the excitable properties of the cell, leading to a negative feedback autoregulation of its transmitter output.

Introduction

There is a large body of work on the role played by serotonin in the modulation

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of complex behavior in vertebrates (Jacobs & Gelperin, 1981) as well as invertebrates (Beltz & Kravitz, 1986; Evans & Myers, 1986; Harris-Warrick & Flamm, 1986; Mackey & Carew, 1983). At the cell membrane level, 5-HT has been most intensively studied in molluscan neurons by means of microelectrode voltage-clamp and patch-clamp techniques. These studies have shown that 5-HT exerts a multitude of modulatory effects on distinct ionic conductances (Acosta-Urquidi, 1986; Benson & Levitan, 1983; Crow & Bridge, 1985; Deterre *et al.* 1982; Ewald & Eckert, 1983; Jacklet & Acosta-Urquidi, 1985; Lemos & Levitan, 1984; Paupardin-Tritsch *et al.* 1986; Pellmar, 1984; Siegelbaum *et al.* 1982; Walsh & Byrne, 1984). However, the functional significance of the modulation has been established in only a few cases (Farley & Auerbach, 1986; Kandel & Schwartz, 1982; Klein *et al.* 1982; Pollock *et al.* 1985).

In the nervous system of the leech, 5-HT is localized in a limited number of neurons (Kerkut & Walker, 1967; Lent, 1982; Lent *et al.* 1979; Rude, 1969), but there is a growing body of evidence suggesting that serotonin can modulate transmission at central and peripheral synapses, with important functional consequences (Belardetti *et al.* 1982; Mason *et al.* 1979; McGlade-McCulloh, 1984). The bilateral pair of Retzius cells contain the largest concentration of 5-HT (Lent *et al.* 1979; McAdoo & Coggeshall, 1976; McCaman *et al.* 1973). These cells are believed to be multifunction neurons playing an important role in the control of essential behavior, such as feeding (Lent & Dickinson, 1984; Lent, 1985) and swimming (Kristan & Nusbaum, 1983; Willard, 1981) as well as affecting mucus release from the skin (Lent, 1973) and the relaxation of body wall musculature (Mason *et al.* 1979; for a review, see Leake, 1986). Most known actions of Retzius cells can be mimicked by 5-HT applied in the vicinity of presumed targets. It is therefore believed the cells' functions are largely exerted through release of this transmitter into the hemolymph. Moreover, the spontaneous activity of the Retzius cell is inhibited by short applications of 5-HT, presumably *via* extrasynaptic autoreceptors located on the neuronal soma (Kerkut & Walker, 1967). This inhibitory response has been attributed to an increase in chloride conductance (Walker & Smith, 1973) and is thought to desensitize rapidly (Kerkut & Walker, 1967). A similar rapidly desensitizing hyperpolarizing response can be evoked in another neuron, the mechanosensory cell responsive to pressure (P), by focal application of 5-HT or by R cell stimulation (Henderson, 1983; Drapeau & Sanchez-Armass, 1988). In the course of investigating the role of 5-HT in the sensitization of a defensive reflex, we observed changes in the shape of the action potential of the Retzius cell which persisted for several minutes after exposure to 5-HT. Although the properties of the 5-HT receptors in the R and P cells may not be identical, there is some evidence suggesting that they are similar, and hence that the 5-HT-induced increase in Cl^- conductance may desensitize rapidly in the R cell as well (P. Drapeau, personal communication). The persistent spike-modulating effects of 5-HT on the R cell therefore suggested to us that some other mechanism might be responsible. There have been no reports on the effects of 5-HT on the soma spike and its underlying conductances in the R cell. We

therefore undertook to analyze this effect further by means of conventional two-electrode voltage-clamp techniques.

We report for the first time in the leech, that 5-HT reduces the amplitude and duration of a Ca^{2+} spike recorded in Na^+-free saline, and that these changes are accompanied by the differential modulation of two distinct voltage-dependent K^+ currents; 5-HT enhanced the early transient outward K^+ current, I_A, and suppressed the delayed K^+ current, I_K. Although there is no direct proof that 5-HT regulates the activity of the R cell, Fuchs *et al.* (1982) have shown that the release of 5-HT from R cells in culture is reduced by hyperpolarizing current injection. Furthermore, Nusbaum & Kristan (1986) suggested that inhibition of R cells by 5-HT might be a mechanism by which a swimming episode is terminated. The modulation of the K^+ current in the R cell by 5-HT described in this study might, therefore, be one of the contributing mechanisms by which the Retzius cell titrates the amount of transmitter it releases. A preliminary account of these findings has been reported (Kleinhaus *et al.* 1988).

Materials and methods

Specimens of the hirudinid leeches *Macrobdella decora* and *Hirudo medicinalis* were obtained from commercial suppliers (St Croix Biological, Minnesota and Biopharm Ltd, Charleston, SC) and kept in distilled water with $0{\cdot}5\,g\,l^{-1}$ of dissolved Instant Ocean at 5°C. 48 leeches were used in this study. Individual segmental ganglia were dissected in leech saline containing (in $mmol\,l^{-1}$): NaCl, 120; KCl, 4; $CaCl_2$, 2; Tris–HCl, 10 or Hepes, 10; adjusted to pH 7·4 with either NaOH or HCl, as previously described (Nicholls & Baylor, 1968; Kleinhaus, 1976, 1980). After separation from the body, the ganglia were pinned to a Sylgard (Dow Corning) coated dish and the connective capsule overlying the neurons was removed by microdissection. Desheathing exposed the neurons, removed diffusion barriers and increased the yield of successful impalements. In this study we used the Retzius cells, which in *Macrobdella* are the largest neurons (50–80 μm in diameter) situated on the ventral side of the ganglia. They could be unambiguously identified on the basis of their size, position and firing properties (Keyzer & Lent, 1977; Muller *et al.* 1981; Nicholls & Baylor, 1968).

For voltage-clamp experiments, no attempt was made to isolate the soma by axonal ligation, as somatic K^+ currents in intact cells were indistinguishable from those recorded from ligated Retzius cells (Johansen & Kleinhaus, 1986). Microelectrodes were pulled from thin-walled borosilicate glass tubing (1 mm omega-dot, F. Haer) on a Brown-Flaming P-80 puller (Sutter Instr. Co.) and filled with either $3\,mol\,l^{-1}$ KCl (10–20 MΩ) or $4\,mol\,l^{-1}$ potassium acetate (20–30 MΩ). Conventional two-electrode voltage-clamp techniques were employed by using either a Dagan 8500 amplifier (Dagan Corp.) or an Axoclamp 2-A amplifier system (Axon Instruments Inc., Burlingame, CA). Most experiments used the Axoclamp 2-A (time constant 0·2 ms, gain approx. $800\,V\,V^{-1}$, 0·3 kHz output bandwidth, settling time approx. 2 ms). Membrane currents in the Dagan amplifier were

measured by a virtual ground circuit connected to a AgCl pellet immersed in the bath. In the Axoclamp the current injected across the feedback resistor in the headstage was sampled for membrane current measurement. Current–voltage (I–V) relationships for the specific K^+ current studied were generated by standardized pulse protocols and were obtained before and at various times after the addition of 5-HT. The signals were photographed directly from a storage oscilloscope, recorded on a penwriter (Gould Brush 220 or Hewlett Packard 7402A) and stored for analysis on an IBM-PC computer system interfaced with a Tecmar Labmaster A-D converter which digitized traces at 32 kHz using the appropriate software (pClamp, Axon Instruments Inc., Burlingame, CA). After off-line subtraction of leakage and capacitative currents, the data were reproduced on a digital plotter (Hewlett-Packard 7470A). Tests of significance were made using Student's *t*-test for paired correlated samples. Action potential duration (in ms) was compared in the control condition with the 5-HT condition for each cell. Likewise, currents (in nA) in the presence and absence of 5-HT were compared for each cell.

Na^+- and Ca^{2+}-free solutions were prepared by substituting sucrose for Na^+ and Mn^{2+} for Ca^{2+}. The outward currents I_A and I_K were separated pharmacologically with tetraethylammonium (TEA^+) chloride (Aldrich) and 4-aminopyridine (4-AP) (Aldrich), as previously described (Johansen & Kleinhaus, 1986). 5-HT (creatinine sulfate complex, Sigma) was made up as a 10 mmol l^{-1} stock solution and stored in the freezer. On experimental days samples were diluted in the appropriate saline to the final desired concentration. The volume of the experimental chamber was approximately 1·0 ml and at least 5–10 ml of a drug-containing solution was perfused onto the preparation before testing its effect. All experiments were carried out at room temperature (20–24 °C).

Results

Effect of 5-HT on the action potential

In our initial experiments, using normal Ringer, we confirmed that 5-HT caused a reversible hyperpolarization accompanied by a slowing of the spontaneous firing rate of the Retzius cell, as described by others (for a review, see Leake, 1986). Interestingly, the firing frequency recovered only partially several (12–15) minutes following drug washout. Along with the change in firing rate, we also observed that 5-HT caused a small reduction in the duration of the action potential. Alterations in the shape of the action potential produced by 5-HT have been reported in *Aplysia californica* (Klein *et al.* 1982) and in the chick dorsal root ganglion (DRG) (Dunlap & Fischbach, 1981). However, in these preparations, the small effect produced by 5-HT observed in normal saline could be dramatically enhanced when the action potential was prolonged by TEA^+, which blocks the repolarizing potassium current.

Since TEA^+ prolongs the action potential of the Retzius cell (Kleinhaus & Prichard, 1975), we examined the effect of 5-HT on the action potential of Retzius

cells in the presence of TEA^+. In normal saline, and in the presence of 5 mmol l^{-1} TEA^+, bath application of 100 μmol l^{-1} 5-HT produced a 76 ± 8% decrease in duration of the action potential: control, 25·5 ± 6 ms $N = 7$; 5-HT, 5·1 ± 1·2 ms $N = 7$; $P < 0{\cdot}01$. In contrast, the upstroke of the action potential was not significantly altered (maximum rate of depolarization: control, 20 mV, 1·9 ± 0·46 ms; 5-HT, 20 mV, 1·8 ± 0·37 ms; $t(4) = 0{\cdot}874$, $P = 0{\cdot}57$), indicating that 5-HT did not affect the Na^+ current. The shortening of the action potential observed under these conditions might therefore result from a decrease in the Ca^{2+} current underlying the plateau or from changes in the repolarizing K^+ conductance(s).

In the R cells of the leech the upstroke of the action potential is predominantly carried by I_{Na} (Kleinhaus & Prichard, 1976, 1983) but regenerative Ca^{2+} spikes in Na^+-free saline can be elicited when competing repolarizing outward currents are removed by K^+ channel blockers (Kleinhaus & Prichard, 1975; Kleinhaus, 1976, 1980). We therefore re-examined the effect of 5-HT on a Ca^{2+}-dependent action potential in the absence of Na^+.

Fig. 1A illustrates the effect of 5-HT on the Ca^{2+} spike recorded from a Retzius cell in Na^+-free saline containing 25 mmol l^{-1} TEA^+. Under these conditions the delayed rectifier, I_K, was almost completely blocked and the Ca^{2+} spike duration

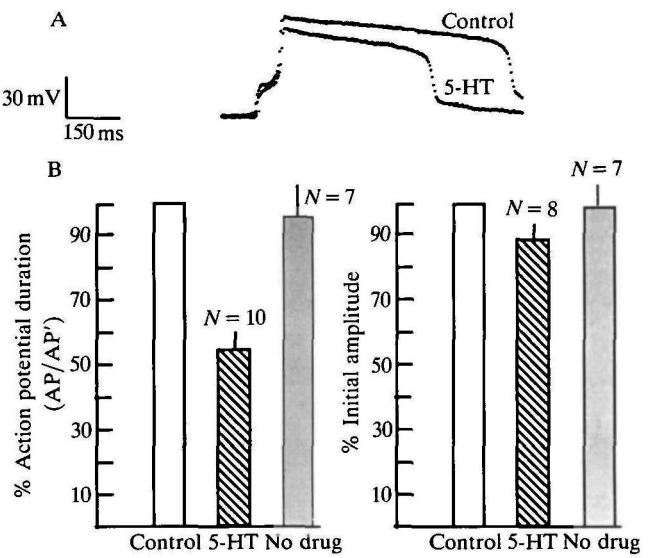

Fig. 1. Serotonin decreased the amplitude and duration of the Ca^{2+}-dependent action potential in Retzius cell. (A) A Ca^{2+}-dependent action potential evoked by depolarizing current injection (0·5–2 nA) in Na^+-free, 5 mmol l^{-1} Ca^{2+}, 25 mmol l^{-1} TEA^+ saline before (control) and after addition of 100 μmol l^{-1} 5-HT. Spike amplitude and duration were decreased by 13% and 40%, respectively, by 5-HT. (B) Graph from pooled data shows significant reduction in Ca^{2+} spike duration (left panel) and amplitude (right panel) in the presence of 5-HT (cross-hatched bar) compared with untreated cells stimulated for the same period of time (stippled bar/no drug).

varied between 0·5 and 1·0 s (Kleinhaus & Prichard, 1975). The cells were stimulated at 30 s intervals until the action potential duration stabilized (usually 3–5 min after impalement). Since membrane potential (V_M) is important in controlling spike duration (a 5 mV reduction of V_M can increase spike duration by about 20%; A. L. Kleinhaus, unpublished observation), we held the membrane potential constant near −50 mV by injection of direct current through the recording microelectrode. Measurements of action potential amplitude and duration were then made for 10 min and these values were used as controls. 5-HT (20–100 μmol l^{-1}) was subsequently added to the bath while the cell was continuously stimulated at the same rate. Between 5 and 10 min after the addition of 5-HT to the bath, Ca^{2+} spike amplitude and duration were both reduced (Fig. 1A). A comparison of the average duration of Ca^{2+} spikes recorded in R cells perfused with Na^+-free saline for at least 10 min with that of spikes recorded from R cells exposed to 100 μmol l^{-1} 5-HT for the same time, revealed that 5-HT reduced spike duration from an average of 1152 ± 230 ms (S.E.M.) to 633 ± 167 ms, a reduction of 45 ± 6% [$t(9) = 6{\cdot}7$; $P < 0{\cdot}001$], and spike amplitude from 62 ± 6 mV to 55 ± 5·7 mV, an average reduction of 11·3% [$t(7) = 6{\cdot}3$; $P < 0{\cdot}001$]. These statistically significant changes are compared in Fig. 1B with measurements on a separate group of seven cells which were perfused for the same period with saline free of 5-HT, and where the spike changed in duration from 1003 ± 190 ms to 990 ± 135 ms [$t(6) = 1{\cdot}01$; $P = 0{\cdot}4$], and in amplitude to 98 ± 3% of the initial value, showing that the changes recorded in the presence of 5-HT were not simply due to passage of time.

In molluscan giant neurons, alterations of spike duration by 5-HT have been related to modulation of Ca^{2+} and K^+ conductances (Acosta-Urquidi, 1986; Deterre *et al.* 1982; Klein *et al.* 1982; Paupardin-Tritsch *et al.* 1986). Under the experimental conditions of Fig. 1, the action potential resulted from the flow of Ca^{2+} and K^+ exclusively, since there was no Na^+ present in the saline. Therefore, a reduction of spike amplitude and duration could result either from a decrease in inward Ca^{2+} current or from an increase in outward K^+ current(s). Voltage-clamp experiments were undertaken in an attempt to establish which ionic conductances were affected by 5-HT in the TEA^+-prolonged action potential.

Effects of 5-HT on calcium current

When I_K and I_A are blocked in a Na^+-free solution, R cells can sustain action potentials carried by either Ca^{2+}, Sr^{2+} or Ba^{2+} and which can be blocked by Mn^{2+} (Kleinhaus, 1976). Under these conditions, a voltage-dependent inward divalent cation current can be recorded under voltage-clamp in the R cell (A. L. Kleinhaus, unpublished observation). We examined the effect of 5-HT on I_{Ca} in eight cells and did not observe a significant effect on this current, $t(7) = 1{\cdot}4$, $P = 0{\cdot}2$. I_{Ca} was evoked from a holding potential (V_H) of −40 mV with voltage steps to 0 mV. 5-HT increased the Ca^{2+} current on average by 1·2 ± 0·9 nA. This increase represents a 7·4 ± 7·8% difference. However, of the eight cells none showed a decrease in I_{Ca} following treatment with 5-HT. The variability of the response may have been due

in part to the incomplete block of all K^+ currents by TEA^+ and 4-AP. Among the possible candidates are a TEA^+-resistant $I_{K(Ca)}$ and/or a 'residual background' current with ranges of activation which overlap with I_{Ca}. Such currents may be present in varying proportions in different preparations and therefore complicate the analysis of the effect of 5-HT on I_{Ca}. However, although the experiments were inconclusive, they did not support the hypothesis that the reduction of Ca^{2+} spike amplitude and duration seen in current-clamp recordings resulted from a decrease in I_{Ca}. Therefore, it appeared that 5-HT might shorten the amplitude and duration of the Ca^{2+} spike by enhancing outward repolarizing K^+ current(s).

Effects of 5-HT on outward currents

The voltage-sensitive outward currents in the Retzius cells of *Macrobdella* consist of at least two distinct components with different voltage dependence and activation–inactivation kinetics. Furthermore, the two K^+ currents can be distinguished by their selective sensitivity to K^+ channel blockers (one is blocked by 4-AP and the other by TEA^+). By analogy with similar currents in other preparations, these have been termed I_A and I_K, respectively (Johansen & Kleinhaus, 1986).

I_A

I_A can be distinguished from I_K by its faster activation–inactivation kinetics, its specific sensitivity to 4-AP block, and by the fact that it undergoes complete steady-state inactivation at a V_H of about -30 mV. For these experiments V_H was set at -80 mV and command voltage steps to elicit I_A were kept at or below 0 mV to minimize the possible contribution of a small TEA^+-resistant fraction of I_K. There is potentially a substantial overlap of these two currents at V_M values more positive than 0 mV. In some experiments, an example of which is shown in Fig. 2, I_A was studied after blocking I_K with TEA^+. In this study, the range of magnitude for I_A evoked by a step to 0 mV from a V_H of -80 mV was 7–24 nA, and for the inactivation time constant (τ_{off}) the range was 88–160 ms. Nonetheless, 5-HT consistently enhanced the peak amplitude of I_A in all Retzius cells studied by an average of 9 ± 1 nA [$t(8) = 6{\cdot}5$, $P < 0{\cdot}001$]. This represents a 108 ± 37 % change. However, it appeared that if I_A was initially large, 5-HT produced only a small additional measurable enhancement. In contrast, if I_A was small initially, the 5-HT-induced enhancement was robust. The mean current for the control condition was $12 \pm 2{\cdot}3$ nA, whereas following 5-HT the mean current was $21{\cdot}6 \pm 2{\cdot}7$ nA. Representative results for each case are illustrated in Figs 2 and 3, respectively. In the example of Fig. 3, a washout produced partial reversibility of the 5-HT effect, revealed by the complete I–V activation plots before, during and after 5-HT application. We did not see reversibility of the enhancement of I_A in all cases. However, this result may have been biased because we were not always able to maintain the electrodes in the cell throughout the successive solution changes. When reversibility occurred, the time frame was similar to that of the return of the spontaneous rate of firing observed in normal Ringer under current-clamp. A

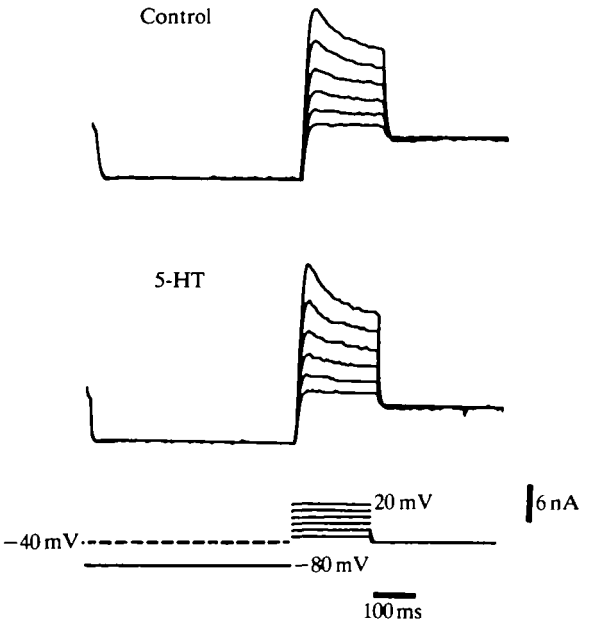

Fig. 2. Serotonin enhanced I_A in Retzius cell. A family of I_A current responses before (control) and 10 min after addition of 100 μmol l^{-1} 5-HT. Currents were evoked by 200 ms depolarizing steps in 10 mV increments, following a 500 ms conditioning prepulse to −80 mV from a holding potential (V_H) of −40 mV. Saline was Na^+- and Ca^{2+}-free with 25 mmol l^{-1} TEA^+ added to remove I_K and $I_{K(Ca)}$ contamination. This example illustrates the smallest increase in I_A produced by 5-HT (approx. 11 % change at +20 mV) observed in this study. Current traces reproduced from stored digitized data. In these and all subsequent voltage-clamp records, upper traces are currents (calibration bar in nA), and lower traces show command voltage protocol. In this experiment leakage current was not subtracted from the records.

small but statistically significant increase in τ_{off} was also measured [$t(8) = 3{\cdot}4$; $P < 0{\cdot}01$] in all the cells studied. In the control condition τ_{off} was $119{\cdot}1 \pm 8$ ms, whereas in the presence of 5-HT it was $129{\cdot}1 \pm 8$ ms. On average we observed a $9{\cdot}3 \pm 2{\cdot}7$ ms increase in τ_{off} in the presence of 5-HT. This represents a 8 ± 2 % change.

I_K

Since the steady-state inactivation of I_A is complete at −30 mV, we separated I_A from I_K by setting V_H between −30 and −10 mV and by adding 3 mmol l^{-1} 4-AP to the saline. In contrast to its effect on I_A, 5-HT consistently suppressed the peak and steady-state amplitude of I_K in Retzius cells at all voltage steps in 10 of the 10 cells studied [$t(9) = 4{\cdot}4$; $P < 0{\cdot}002$]. Pooled data from the 10 cells indicated that I_K was reduced on average by $15{\cdot}7 \pm 3{\cdot}6$ nA (control $36{\cdot}15 \pm 4$ nA; 5-HT = $20{\cdot}45 \pm 4$ nA). This represents a mean decrease of 44 ± 6 % (Figs 4 & 5). This

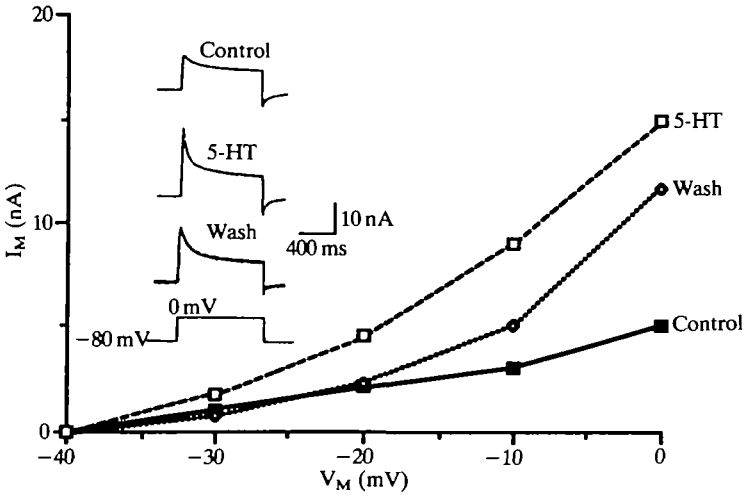

Fig. 3. Serotonin reversibly enhanced I_A in Retzius cell. Current–voltage (I–V) activation plot of peak I_A amplitude (leakage corrected) before (control), during (5-HT) and 20 min after washout. Current responses were evoked by command voltage steps in increments of 10 mV, 800 ms in duration from a V_H of −80 mV. This experiment illustrates one of the largest serotonin-induced increases (380 % change at 0 mV) in I_A observed in this study. The inset shows sample I_A traces evoked at 0 mV, recorded with a penwriter.

effect was not readily reversible within the time course of our observations (10–20 min following drug removal).

Discussion

Our results show that application of 100 μmol l^{-1} 5-HT reduced both the amplitude and the duration of Retzius cell Ca^{2+} spikes and differentially modulated two distinct voltage-dependent outward K^+ currents: I_A was enhanced and I_K was suppressed.

Action potential duration and amplitude

Action potential duration and amplitude have been shown to be critical variables in the release of transmitter, since only small changes in action potential shape can produce dramatic changes in transmitter release (Jessell & Iversen, 1977; Kandel & Schwartz, 1982). The observed decrease in amplitude and duration of the Ca^{2+} spike caused by 5-HT would be expected to decrease the output of neurotransmitter from the Retzius cell. The simplest explanation for these effects of 5-HT is to attribute them to a reduction of I_{Ca}, as has been demonstrated in the DRG (Holz *et al.* 1986; Dunlap & Fischbach, 1981). Interestingly, Dunlap & Fischbach (1981) reported that 5-HT decreased the duration of spikes recorded from embryonic DRG cells in culture in the absence of TEA^+, and they attributed the effect to suppression of I_{Ca}. However, our

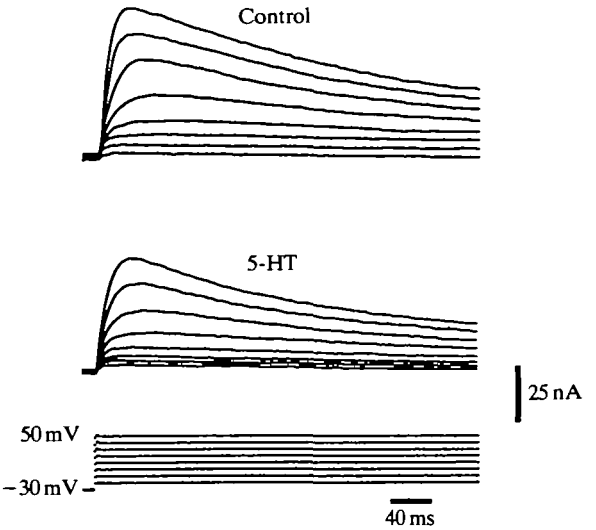

Fig. 4. Serotonin suppressed I_K in Retzius cell. A family of I_K currents evoked by 10 mV incrementing depolarizing steps from a V_H of −30 mV in Na^+- and Ca^{2+}-free saline containing 3 mmol l^{-1} 4-AP to remove contamination from I_A and $I_{K(Ca)}$, before (control) and 10 min after adding 100 μmol l^{-1} 5-HT. This experiment illustrates a moderate serotonin-induced decrease in peak current (24 % change at +50 mV). The traces are reproduced from digitized data after leakage and capacitative transients had been subtracted.

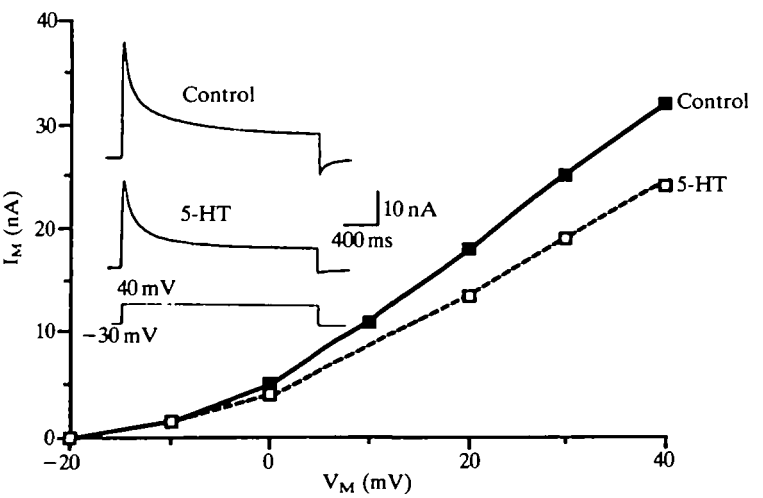

Fig. 5. Serotonin suppressed peak and steady-state components of I_K in Retzius cell. I–V plot of peak I_K current (leakage-corrected), before (control) and after 10 min of exposure to 100 μmol l^{-1} 5-HT. The inset shows sample I_K responses that have reached steady state (2 s pulse duration), recorded with a penwriter.

preliminary experiments indicated that, in the absence of I_K and I_A, 5-HT produced variable effects on I_{Ca} in the Retzius cell. Therefore, the 5-HT-induced reduction of the Ca^{2+} spike amplitude and duration recorded under current-clamp is more likely to be due to the enhancement of I_A by 5-HT. This conclusion is supported by the observation that, under these recording conditions, the spike repolarizing current is probably mostly I_A, since I_K was mostly blocked by 25 mmol l^{-1} TEA^+, and resting potential values were about −50 mV, at which voltage substantial I_A would still be available for activation.

I_A

The results of this study show that 5-HT modulated (up-regulated) I_A in the Retzius cell. Modulation of I_A by 5-HT and other agents has been reported in a number of molluscan preparations. Suppression (down-regulation) of I_A by 5-HT and small cardioactive peptide B (SCP_B) has been reported for *Hermissenda* identified giant neurons (Acosta-Urquidi, 1988) and by 5-HT for type B photoreceptors (Farley & Auerbach, 1986).

Treatments that elevate cyclic AMP levels (including injection of cyclic AMP, cyclic AMP analogues, forskolin and phosphodiesterase inhibitors) also suppress (down-regulate) I_A in *Aplysia* bag cells (Strong, 1984), Dorid neurons (Coombs & Thompson, 1987) and *Hermissenda* neurons (Acosta-Urquidi, 1985). These studies also reported additional complex changes in I_A kinetics of inactivation induced by the adenylate cyclase activator, forskolin. The decrease in I_A was related to changes in neuronal excitability, including increased firing rate and spike broadening.

By contrast, this study reports the first example of modulation of I_A by 5-HT in the leech Retzius cell, resulting in an increased conductance (up-regulation). This increase in I_A amplitude is accompanied by a small increase in the activation time constant of I_A (τ_{off}).

Examples of a 5-HT-induced increase in diverse potassium conductances *via* activation of cyclic-AMP-dependent pathways in identified molluscan neurons are numerous. In *Aplysia* cell R15, 5-HT enhances the anomalous rectifier (Benson & Levitan, 1983; Lemos & Levitan, 1984) and $I_{K(Ca)}$ (Ewald & Eckert, 1983) and in some *Hermissenda* neurons 5-HT enhances $I_{K(Ca)}$ (Jacklet & Acosta-Urquidi, 1985; Acosta-Urquidi, 1986). Enhanced I_A conductance could thus be due either to an increase in the opening probability of single channels, and/or to an increase in the single-channel conductance and/or the number of active channels. Recordings at the single-channel level will be required to distinguish between these mechanisms.

I_K

The present study provides an example of a 5-HT-induced decrease of I_K in the leech Retzius cell. Suppression of I_K peak and steady-state current was not accompanied by any obvious changes in kinetics of inactivation. A number of studies on identified *Aplysia* neurons have demonstrated I_K modulation (down-

regulation and kinetic changes) by 5-HT and treatments that elevate intracellular cyclic AMP levels (Strong & Kaczmarek, 1986; Walsh & Byrne, 1984; Baxter & Byrne, 1986). In some *Hermissenda* giant neurons, 5-HT, SCP_B and treatments that elevate intracellular cyclic AMP levels also suppress I_K (Acosta-Urquidi, 1985, 1986, 1988). Serotonin has been reported to increase cyclic AMP levels in the nerve cord and muscle of the leech (Belardetti *et al.* 1982; McGlade-McCulloh, 1984). Although we have no direct evidence for the involvement of any internal messenger(s) in the mechanism by which 5-HT modulates I_A and I_K in the leech Retzius cells, the studies mentioned above suggest that such a mechanism may be operating in this animal as well. Further experiments are planned to explore this possibility.

Functional significance

The significance of the simultaneous modulation of diverse K^+ currents by 5-HT in the leech Retzius cell is unclear. On the one hand, suppression of I_K should promote spike broadening and an overall increase in cell excitability (Acosta-Urquidi, 1988; Klein *et al.* 1982, 1986). On the other hand, enhancement of I_A might result in an increased spike threshold, increased spike adaptation and decreased spike duration. In current-clamp recordings we found that 5-HT shortened the duration of the Ca^{2+} spike, suggesting that, under conditions of reduced I_K current, I_A may play a role in spike repolarization. Furthermore, 5-HT decreases the frequency of firing in R cells (Walker & Smith, 1973; Kerkut & Walker, 1967; Nusbaum & Kristan, 1986) which may result in part from the increase in I_A current observed in this investigation. Modelling studies are planned to gain further insight into the precise interaction of the diverse K^+ conductances studied in this paper and the desensitizing Cl^- conductance reported earlier (Kerkut & Walker, 1967; Walker & Smith, 1973), in the hope of determining the functional consequences of their modulation by serotonin.

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