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MULTIPLE SEROTONIN-ACTIVATED CURRENTS IN ISOLATED, NEURONAL SOMATA FROM LOCUST THORACIC GANGLIA

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Summary

1. Three different responses were evoked by pressure micro-application of serotonin onto freshly dissociated, current- and voltage-clamped neuronal somata from the thoracic ganglia of the locust *Locusta migratoria*.

2. In some neurones, an inward current, I(5HT)K, resulting from a decrease in potassium conductance, with slow kinetics and maximum activation at membrane potentials of −60 to −70 mV, was evoked by serotonin and by the 5-HT_3 agonist 2-methyl serotonin. This current was completely abolished by either 10 mmol l^{-1} caesium or 5 mmol l^{-1} rubidium and partially blocked by 50 mmol l^{-1} tetraethylammonium or 5 mmol l^{-1} 4-aminopyridine. The response was antagonised by the 5-HT_2-specific compounds, ketanserin and ritanserin.

3. In other somata, serotonin, 2-methyl serotonin and the 5-HT_3 antagonist ICS 205 930 evoked a second current, I(5HT)Na, which was due to an increase in sodium permeability and had slow kinetics similar to that of I(5HT)K. This current was inward over the membrane potential range −30 to −80 mV and increased with hyperpolarisation. The response was blocked by sodium-free saline and the 5-HT_3 receptor antagonist MDL 72222.

4. In other neurones, at membrane potentials more positive than −50 mV, serotonin pulses could activate a third current, I(5HT)X, which increased with depolarisation of the membrane potential and had comparatively fast kinetics. Activation of the current was accompanied by a decrease in membrane conductance. This response was completely blocked by 4-aminopyridine and weakly inhibited by both caesium and tetraethylammonium and is, therefore, probably a potassium current.

5. The three currents described here differ in their pharmacology, their ionic mechanisms and their dependence on membrane potential from the serotonin-activated currents reported for vertebrates and they provide evidence for the mechanism of action of serotonin as a neurotransmitter in insects.

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Key words: serotonin, invertebrate neurones, *Locusta migratoria*.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is widely distributed in the central nervous system (CNS) and neurohaemal organs of insects. It has been identified and localised in the CNS of numerous insect species (see review by Nässel, 1987) as well as in some sensory nerves (Lutz and Tyrer, 1988) and neurones innervating the corpus cardiacum of the locust *Schistocerca gregaria* (Konings *et al.* 1988), and in DUM neurones (Orchard *et al.* 1989) and neurohaemal organs (Flanagan, 1984) of *Rhodnius prolixus*. It also occurs in putatively neurosecretory cells in the suboesophageal ganglion of *Periplaneta americana* (Davis, 1987), the cricket *Gryllus domestica* (Baines and Downer, 1991) and *Locusta migratoria* where serotonin modulates mouthpart muscle contraction (Baines *et al.* 1990). Serotonin also seems to be characteristically located in the optic (e.g. Homberg and Hildebrand, 1989) and antennal lobes of *Manduca sexta* (e.g. Kent *et al.* 1987).

There is evidence for serotonin-immunoreactive terminals in the neuropile of the thoracic ganglia of the locust *Schistocerca gregaria* (Peters and Tyrer, 1987), and calcium-dependent release of sequestered, radiolabelled serotonin from nerve terminals in a neurohaemal organ of *Rhodnius prolixus* has been observed (Flanagan and Berlind, 1984). Radioligand binding studies have revealed the presence of high-affinity, specific binding sites for serotonin in the brains of *Drosophila* (Dudai and Zvi, 1984), locusts (Osborne *et al.* 1984) and the honeybee (Scheidler *et al.* 1986), and a *Drosophila melanogaster* serotonin receptor has been cloned and expressed in mouse 3T3 cells (Witz *et al.* 1990). Serotonin can be inactivated *via* the Malpighian tubules in *Calliphora erythrocephala* (Trimmer, 1985) and *Periplaneta americana* (Sloley and Downer, 1990), and its re-uptake in *Periplaneta americana* by whole nerve cords (Scott *et al.* 1985) and in cultured neurones, by means of a high-affinity, sodium-dependent transport system (Bermudez and Beadle, 1989), has been established.

These observations strongly support the hypothesis that serotonin is an important neurotransmitter and neurohormone in insects. However, the physiological effects of serotonin are less well known. The most thoroughly studied responses are those in the periphery. The salivary glands of insects are responsive to serotonin (reviewed by House, 1980). They receive serotonergic innervation (Peters *et al.* 1987), and serotonin, *via* cyclic AMP, probably plays an important part in the control of salivation (Trimmer, 1985). Serotonin also acts on the gut of *Locusta migratoria* (Osborne *et al.* 1990), the Malpighian tubules of *Aedes aegypti* (Veenstra, 1988), the heart of, for example, *Manduca sexta* (Platt and Reynolds, 1986) and the oviducts of the horsefly *Tabanus proximus* (Cook, 1981).

Reports on the effects of serotonin in the insect nervous system are few. Serotonin suppresses the dopamine-induced production of flight motor output in *Manduca sexta* (Claassen and Kammer, 1986) and it is reported to alter aggressiveness and CNS activity in the ant *Formica rufa* (Kostowski and Tarchalska, 1972) and the responsiveness to olfactory stimuli in honeybees (Mercer and Menzel, 1982). Usherwood *et al.* (1980) reported that serotonin evokes changes in membrane potential of neurones isolated from the locust

Schistocerca gregaria, and similar observations were made on cockroach neurones *in vitro* (Neumann *et al.* 1987).

In this paper, we describe the responses to serotonin of mechanically isolated neuronal somata from the thoracic ganglia of *Locusta migratoria*. These cells remain viable *in vitro* for many hours and, under voltage-clamp conditions, exhibit characteristic responses to γ-aminobutyric acid (GABA) (Lees *et al.* 1987), nicotine and muscarine (Benson and Neumann, 1987; Benson, 1988), octopamine (Kaufmann and Benson, 1991) and peptides isolated from the corpora cardiaca (Bermudez *et al.* 1991*b*). In these neurones, serotonin can evoke any of three distinct membrane currents. These responses are not activated by other aminergic neurotransmitters such as octopamine (Kaufmann and Benson, 1991). We have characterised the pharmacology and the ionic- and voltage-dependence of the receptors and ion channels mediating the serotonin-evoked responses. Preliminary reports of this work have appeared elsewhere (Bermudez *et al.* 1990, 1991*a*).

Materials and methods

The experiments were performed on freshly dissociated neuronal somata prepared from the thoracic ganglia of adult *Locusta migratoria* (Usherwood *et al.* 1980). The isolated cell bodies were maintained for periods of 2–8 h in physiological saline of the following composition (in $mmol\,l^{-1}$): NaCl, 180; KCl, 10; $CaCl_2$, 10; $MgCl_2$, 15; Hepes, 10; pH 6.8. The neuronal somata ranged between 30 and 300 μm in diameter. Only the larger neurones (100–300 μm) were selected for impalement.

Conventional single-electrode techniques were employed to obtain voltage-clamp recordings. Thin-walled glass intracellular microelectrodes, of 10–15 MΩ resistance, were back-filled with $1\,mol\,l^{-1}$ KCl solution. Serotonin ($10^{-3}\,mol\,l^{-1}$), 2-methyl serotonin (2-methyl 5-HT; $10^{-3}\,mol\,l^{-1}$) and ICS 205 930 ($10^{-4}\,mol\,l^{-1}$) were applied to the cells by pressure ejection from a micropipette (patch-electrode) positioned 5–10 μm from the impaled somata. To obtain current–voltage (*I–V*) curves, the neuronal somata were clamped at different voltages *via* a series of 10 mV steps, held at each potential until the membrane current reached a steady level and then challenged with a pressure pulse of agonist. The ionic mechanisms and the pharmacological properties of the agonist-evoked membrane currents were investigated in cells clamped at −60 or −35 mV. These cells were bath-perfused with physiological saline containing either ion-channel blockers or putative antagonists. Dose–response curves for antagonists were obtained by bath-perfusing the compounds, beginning at low concentrations and, after a constant effect had been achieved (18–20 min for all compounds tested), at increasing concentrations. Na^+-free medium was made by substituting *N*-methyl-D-glucamine for NaCl (Ichinose and McAdoo, 1988). In this saline, the pH was adjusted to 6.8 with concentrated HCl. The chloride concentration of the resulting solution was about 30 % lower than that of the normal saline. The bath

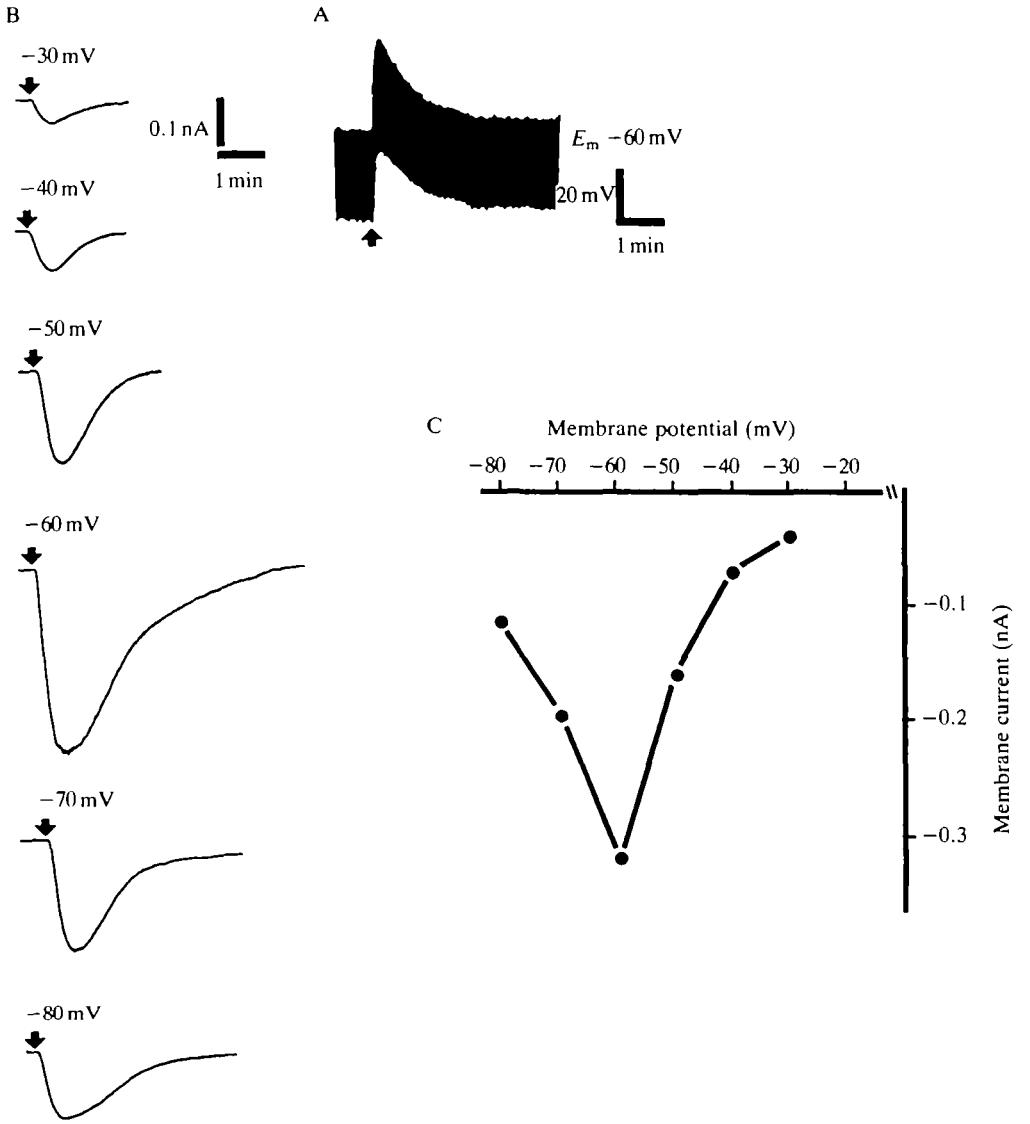

Fig. 1. Conductance effects and voltage-dependence of I(5HT)K. Current- or voltage-clamped neuronal somata were challenged with pressure pulses of serotonin (10^{-3} mol l^{-1}, 500 ms; application indicated by arrows). (A) In current-clamped neurones, activation of I(5HT)K resulted in a depolarisation and a decrease in membrane conductance. In this experiment, the membrane conductance of the impaled neurone was monitored by repeated injection of constant-amplitude current pulses. Membrane potential (E_m) was −60 mV. (B) In voltage-clamped neuronal somata, the amplitude of I(5HT)K increased as the membrane potential was hyperpolarised from −30 mV to −60 mV, but then decreased on further hyperpolarisation. (C) Current–voltage curve for the serotonin-evoked current shown in B.

was continuously perfused at approximately 0.8 ml min^{-1}. Experiments were performed at room temperature (22–24°C).

All pharmacological compounds were obtained from Semat Technical Ltd, England, except ICS 205 930, which was kindly provided by Sandoz Ltd, Basel, for use in this study.

Results

The results are based on intracellular recordings from about 100 neurones to which serotonin or related substances were pressure- or bath-applied. Pressure micro-application of serotonin (10^{-3} mol l^{-1}, 500 ms) elicited three different inward currents, designated I(5HT)K, I(5HT)Na and I(5HT)X, not all of which were recorded in every neurone. To determine the threshold concentration of these currents, impaled cells were bath-perfused with physiological saline containing increasing concentrations of serotonin. Dose–response curves were not obtained owing to desensitization of the responses. To characterise the voltage-dependence of the serotonin-evoked currents, the isolated locust somata were voltage-clamped and the amplitude of the serotonin-induced currents measured at a series of holding potentials. The holding current became unstable at potentials more positive than −35 mV or more negative than −90 mV. Therefore, in most experiments, responses to serotonin were studied only at potentials between −35 and −90 mV.

The first type of serotonin-mediated response, I(5HT)K, had a threshold of about 1 μmol l^{-1} (N=3) and was evoked in all cells tested that were between 200 and 300 μm in diameter. In current-clamped neurones, I(5HT)K was accompanied by a depolarisation and a decrease in membrane conductance (Fig. 1A). Under voltage-clamp conditions, I(5HT)K was characterised by a V-shaped I–V curve with a peak inward current at −60 mV (N>20) (Fig. 1B,C). Typically, I(5HT)K had a slow time course, the response to a 500 ms pulse of serotonin reaching its peak in more than 30 s and the membrane potential returning to the original level in more than 60 s (Fig. 1B).

A depolarisation accompanied by a decrease in conductance could result from suppression of an outwardly directed potassium current, as is the case for some serotonin-evoked excitatory responses in the CNS of vertebrates (North and Uchimura, 1989) and molluscs (Gerschenfeld and Paupardin-Tritsch, 1974). To test this possibility, we examined the effect on I(5HT)K of bath-perfusion with a range of potassium-channel blockers, including caesium (Cs^+), tetraethylammonium (TEA^+), rubidium (Rb^+) and 4-aminopyridine (4-AP) (Fig. 2). I(5HT)K was completely abolished in the presence of either 10 mmol l^{-1} Cs^+ or 5 mmol l^{-1} Rb^+ (Fig. 2A,B). 5 mmol l^{-1} 4-AP or 50 mmol l^{-1} TEA^+ also inhibited the current, though only partially (Fig. 2C,D). Higher concentrations of these blockers were not tested. To determine the involvement of other ions in I(5HT)K, we examined the effect of extracellular sodium-free saline and the calcium-channel

blocker manganese (Mn^{2+}; 2 mmol l^{-1}). As shown in Fig. 2E,F, neither of these treatments had any effect on I(5HT)K.

The second current, I(5HT)Na, had a threshold of about 0.1 μmol l^{-1} (N=4)

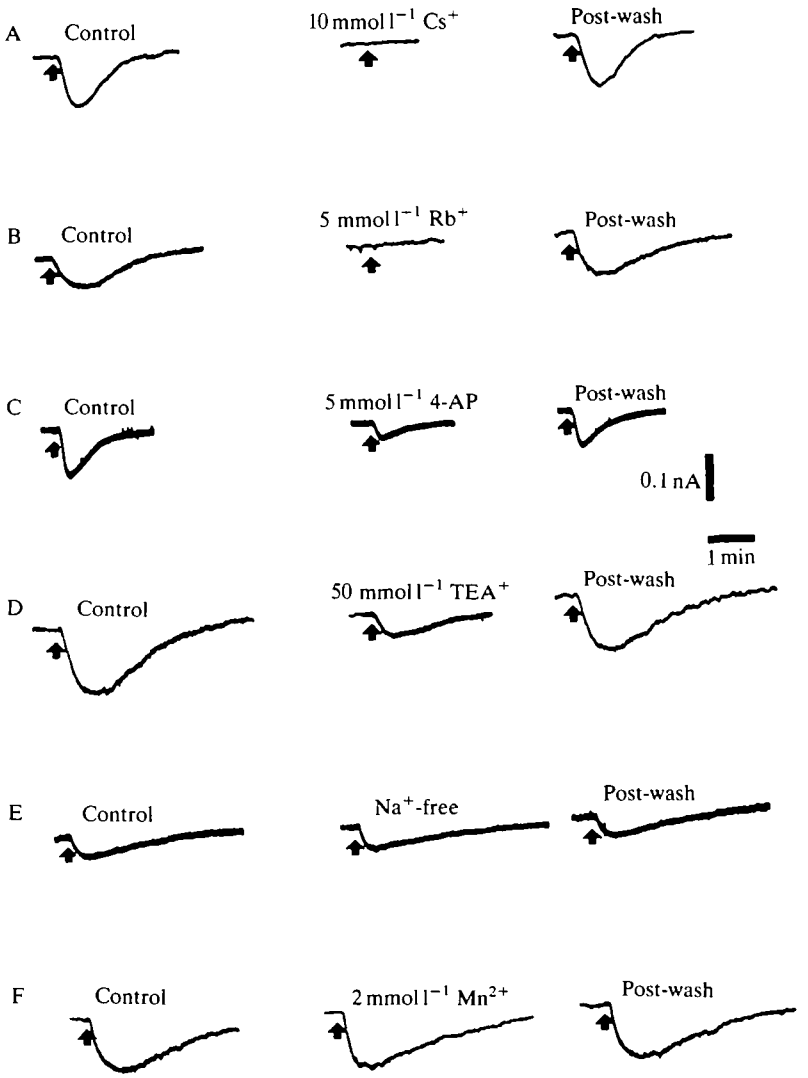

Fig. 2. Ionic properties of I(5HT)K. Neurones were voltage-clamped at −60 mV and challenged with pressure pulses of serotonin (10^{-3} mol l^{-1}, 500 ms; application indicated by arrows). (A) 10 mmol l^{-1} Cs^+ completely abolished the current evoked by serotonin in this neurone. This effect was reversible. (B) Rb^+ (5 mmol l^{-1}) reversibly blocked I(5HT)K. (C) In this neurone 5 mmol l^{-1} 4-AP reversibly decreased the serotonin-evoked current by more than 50%. (D) 50 mmol l^{-1} TEA^+ inhibited I(5HT)K by approximately 50%, and this effect was reversed after an 18–20 min wash. (E) I(5HT)K persisted when the impaled cell was bathed in a sodium-free saline. (F) Mn^{2+}, at 2 mmol l^{-1}, had no effect on I(5HT)K.

and was elicited in 70 % of the neuronal somata tested that were about 200 μm in diameter (N>30). In current-clamp conditions, I(5HT)Na was accompanied by a depolarisation and an increased conductance (Fig. 3A). The time course of

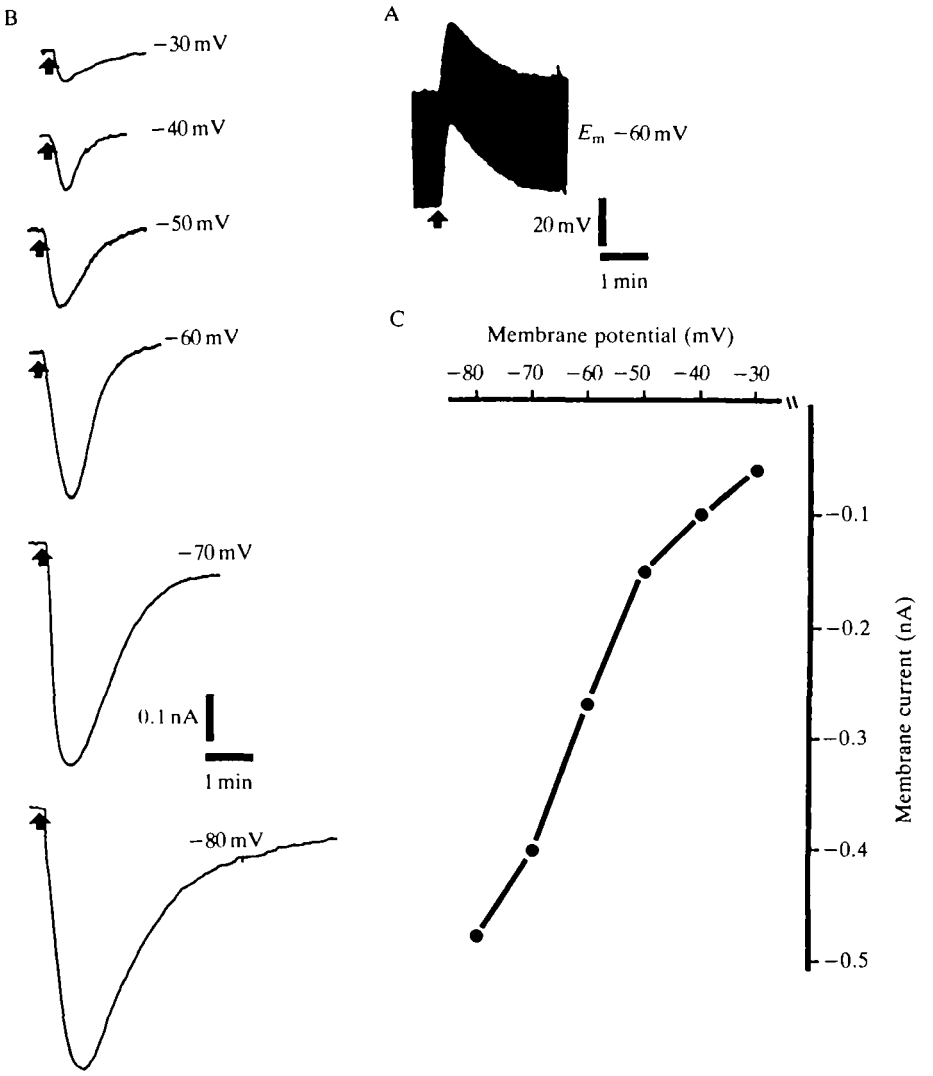

Fig. 3. Conductance effects and voltage-dependence of I(5HT)Na. Current- or voltage-clamped neuronal somata were challenged with pressure pulses (500 ms) of serotonin (10^{-3} mol l^{-1}). Arrows indicate serotonin application. (A) In this current-clamped cell, the response evoked by serotonin was a depolarisation accompanied by a marked increase in membrane conductance. Membrane potential (E_m) was −60 mV. (B) Under voltage-clamp conditions, the current underlying the serotonin-evoked response shown in A was inwardly directed and its amplitude increased with hyperpolarisation. At potentials more depolarised than −50 mV the current showed outward rectification. (C) Current–voltage curve of the serotonin-evoked current shown in B.

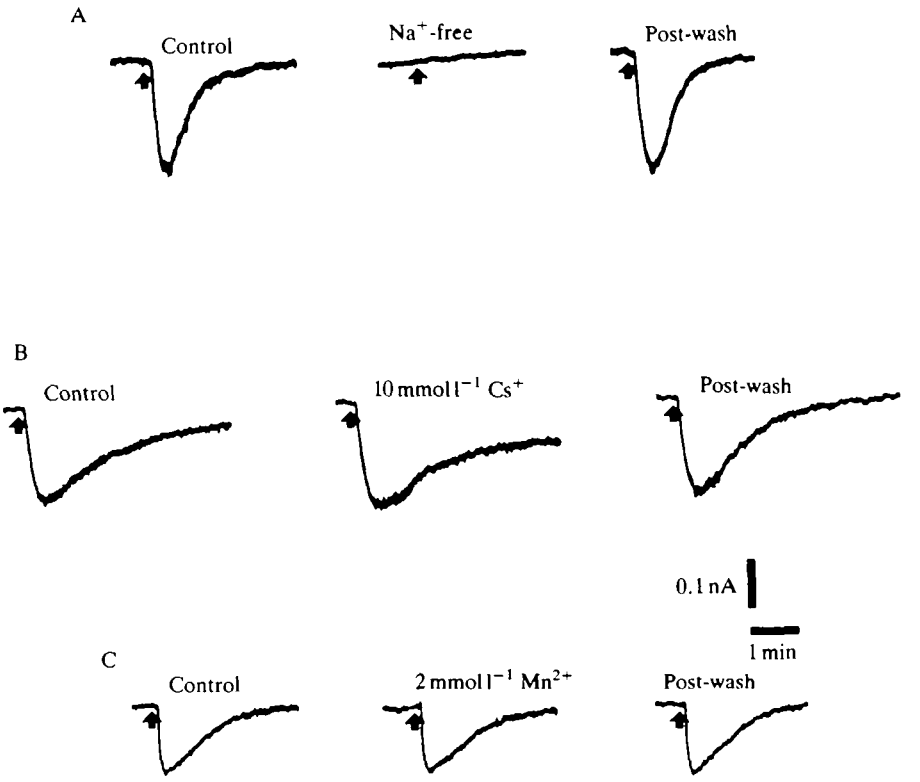

Fig. 4. Ionic properties of I(5HT)Na. Impaled cells were voltage-clamped at −60 mV and bath-perfused with salines of different ionic composition as indicated in Materials and methods. (A) Sodium-free (Na^+-free) saline abolished the current activated by serotonin in this neurone. (B) 10 mmol l^{-1} Cs^+ had no effect on the current evoked by serotonin in this cell. (C) 2 mmol l^{-1} Mn^{2+} had no effect on I(5HT)Na. Arrows indicate serotonin application (pressure application, 500 ms, 10^{-3} mol l^{-1}).

I(5HT)Na was comparable to that of I(5HT)K. In voltage-clamped cells, I(5HT)Na increased with membrane hyperpolarisation over the range −30 to −80 mV (N=20; Fig. 3B,C). To investigate the ions mediating this current, we bath-perfused the impaled cells with sodium-free medium and salines supplemented with either potassium-channel blockers or calcium-channel blockers (Fig. 4). I(5HT)Na was completely abolished in sodium-free medium (Fig. 4A) and was insensitive to both Cs^+ and Mn^{2+} (Fig. 4B,C). These observations indicate that I(5HT)Na results from an increase in sodium permeability.

The third inward current, I(5HT)X, had a threshold of about 3 μmol l^{-1} (N=2) and was evoked in 60 % of neurones tested that were approximately 100 μm across (N=15). I(5HT)X was accompanied by a depolarisation and a decreased conductance in current-clamped somata (Fig. 5A). I(5HT)X was evoked at membrane potentials more positive than −50 mV and increased with depolarisation (N=6; Fig. 5B,C). The time course of I(5HT)X was faster than those of I(5HT)K and

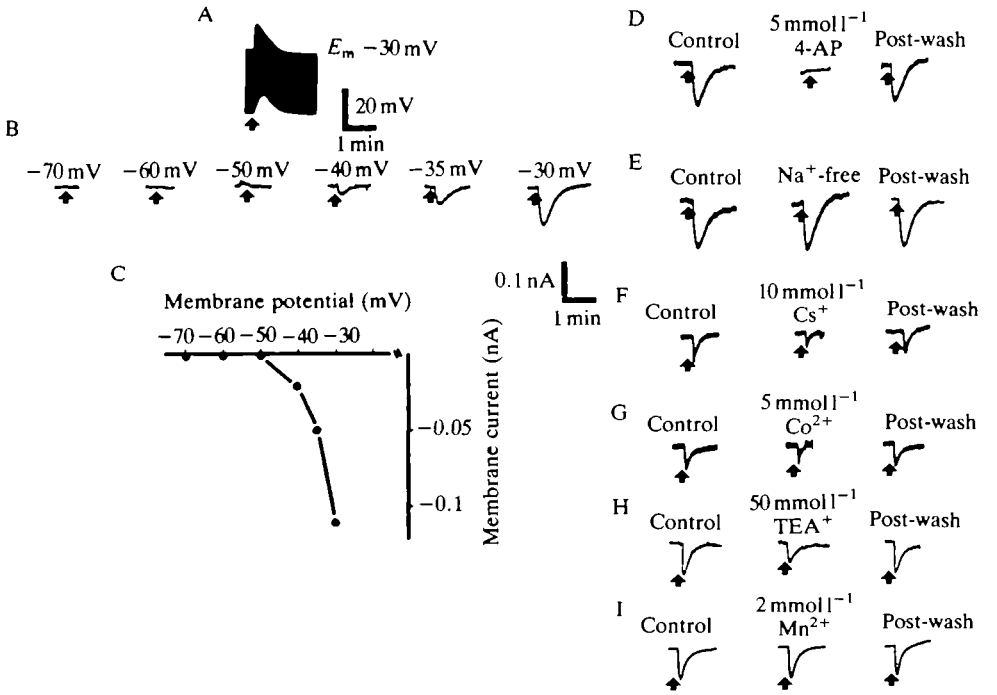

Fig. 5. Conductance effects, voltage-dependence and ionic properties of I(5HT)X. Serotonin (10^{-3} mol l^{-1}) was applied to the impaled neuronal somata by pressure application (indicated by arrows; 500 ms pulses). (A) In current-clamped neurones, I(5HT)X was accompanied by a depolarisation and reduced conductance. Membrane potential (E_m) was −30 mV. (B) In voltage-clamped neurones, I(5HT)X was activated only at voltages more positive than −50 mV and its amplitude increased with depolarisation. (C) Current–voltage curve of the serotonin-generated current shown in B. (D) I(5HT)X was reversibly blocked by 5 mmol l^{-1} 4-AP. (E) Na^+-free saline did not affect I(5HT)X. 10 mmol l^{-1} Cs^+ (F), 5 mmol l^{-1} Co^{2+} (G) and 50 mmol l^{-1} TEA^+ (H) did not produce complete blockade. (I) 2 mmol l^{-1} Mn^{2+} had no effect on I(5HT)X. The voltage-clamp recordings (D–I) were performed on cells clamped at −30 mV.

I(5HT)Na (Fig. 5B). I(5HT)X was completely blocked by 5 mmol l^{-1} 4-AP (Fig. 5D) but sodium-free saline had no effect (Fig. 5E), which suggests that potassium ions are the charge carriers. However, the current was only weakly inhibited by both Cs^+ and TEA^+ (Fig. 5F,H), which block some potassium currents, including I(5HT)K in these cells. Cobalt (Co^{2+}; 5 mmol l^{-1}), which is a calcium-channel blocker but which can also affect potassium channels (Lapied *et al.* 1989), slightly decreased the amplitude of I(5HT)X (Fig. 5G), but Mn^{2+} (2 mmol l^{-1}), which is highly specific for calcium channels, had no effect (Fig. 5I), suggesting that I(5HT)X is not dependent on calcium ions.

We attempted to characterise the pharmacology of the sites at which serotonin was acting to produce the three inward currents by using compounds that are

Table 1. *Summary of the pharmacology of serotonin-evoked currents in isolated neuronal somata from locust thoracic ganglia*

Current	Agonists	Antagonists	Ineffective ligands	Ion-channel blockers	Ineffective blockers
I(5HT)K	5-HT	Ketanserin	α-Methyl 5-HT	Cs^+	4-AP
	2-Methyl 5-HT	Ritanserin	ICS 205 930	Rb^+	TEA^+
			MDL 72222		Mn^{2+}
I(5HT)Na	5-HT	MDL 72222	α-Methyl 5-HT		Cs^+
	2-Methyl 5-HT		Ketanserin		Mn^{2+}
	ICS 205 930		Ritanserin		
I(5HT)X	5-HT		α-Methyl 5-HT	4-AP	Cs^+
			Ketanserin		TEA^+
			ICS 205 930		Co^{2+}
			MDL 72222		Mn^{2+}

selective ligands for vertebrate central serotonin receptor subtypes. The results of these pharmacological studies are summarised in Table 1: none of the compounds investigated had any effect on I(5HT)X.

I(5HT)K was insensitive to α-methyl 5-HT_2, an agonist of the vertebrate 5-HT_2 receptor (Fozard, 1987). However, the 5-HT_2 receptor antagonists ketanserin and ritanserin antagonised I(5HT)K with EC_{50} values of $6.3\times10^{-6}\pm1\times10^{-6}$ mol l^{-1} (mean±S.E.; N=3) and $6.5\times10^{-6}\pm3\times10^{-6}$ mol l^{-1} (mean±S.E.; N=3), respectively, and totally blocked I(5HT)K at 10^{-4} mol l^{-1} (Fig. 6A,B). Neither MDL 72222 nor ICS 205 930, two 5-HT_3 antagonists, had any effect on I(5HT)K at concentrations of up to 3×10^{-5} mol l^{-1} (Fig. 6C,D). In contrast, 2-methyl 5-HT, a 5-HT_3 agonist, evoked I(5HT)K (Fig. 7).

I(5HT)Na was insensitive to the 5-HT_2 ligands α-methyl 5-HT, ketanserin (Fig. 8A) and ritanserin (Fig. 8B) at concentrations up to 10^{-4} mol l^{-1}. In contrast, I(5HT)Na was sensitive to the 5-HT_3 ligands MDL 72222 (Fig. 8C), ICS 205 930 (Fig. 8D) and 2-methyl 5-HT. I(5HT)Na was inhibited by MDL 72222 ($EC_{50}=2\times10^{-6}\pm0.4\times10^{-6}$ mol l^{-1}; mean±S.E.; N=3) (Fig. 8C) and both 2-methyl 5-HT, a $5HT_3$ agonist, and ICS 205 930, a $5HT_3$ antagonist, were agonists at the receptor mediating the I(5HT)Na current in the locust neurones (Figs 8D, 9, 10).

Discussion

Our results show three independent actions of serotonin on neurones freshly dissociated from the thoracic ganglia of adult locusts. Each action is associated with a different ionic mechanism and pharmacology. This multiplicity in receptor type and ionic mechanism has also been found in the CNS of both vertebrates (Bobker and Williams, 1990) and molluscs (Gerschenfeld and Paupardin-Tritsch, 1974) where serotonin is known to act as both modulator and neurotransmitter.

The response-specific pharmacology and ionic charge carriers of the currents

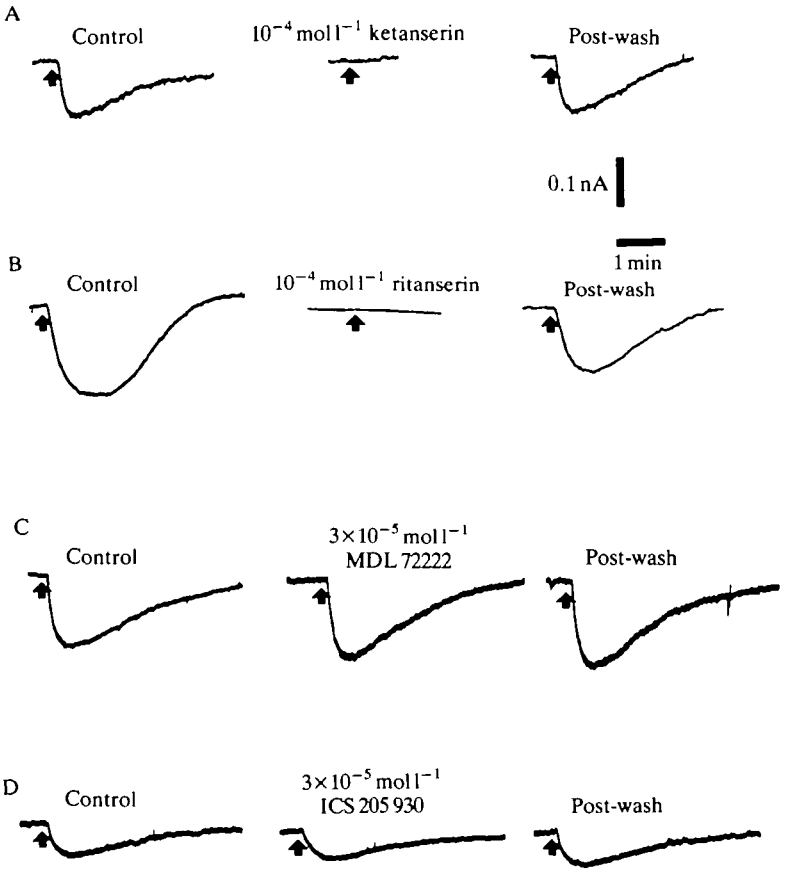

Fig. 6. Pharmacology of I(5HT)K. Serotonin-receptor-specific substances were bath-perfused to test their effects on I(5HT)K. In these experiments, cells were voltage-clamped at −60 mV. (A) 10^{-4} mol l^{-1} ketanserin completely blocked the serotonin-evoked current in this neurone. The ketanserin blockade was fully reversible. (B) Ritanserin, at 10^{-4} mol l^{-1}, completely abolished I(5HT)K. This effect was fully reversible after an 18–20 min wash-out. (C) I(5HT)K was not affected by bath-perfused MDL 72222 (3×10^{-5} mol l^{-1}). (D) ICS 205 930 (3×10^{-5} mol l^{-1}) had no effect on I(5HT)K. Arrows indicate the application of serotonin onto voltage-clamped cells (pressure application; 10^{-3} mol l^{-1}, 500 ms)

evoked by serotonin in the locust thoracic ganglionic neurones are summarised in Table 1. I(5HT)K results from the suppression of a potassium conductance. Inactivation of a potassium conductance by serotonin has been reported for a considerable number of molluscan (Gerschenfeld and Paupardin-Tritsch, 1974) and vertebrate neuronal types, including those of the rat nucleus accumbens (North and Uchimura, 1989). In these cells, the 5-HT_2 serotonin receptor subtype has been implicated. I(5HT)K is antagonised by ketanserin and ritanserin, which are 5-HT_2 antagonists. However, the 5-HT_2 agonist α-methyl 5-HT did not evoke

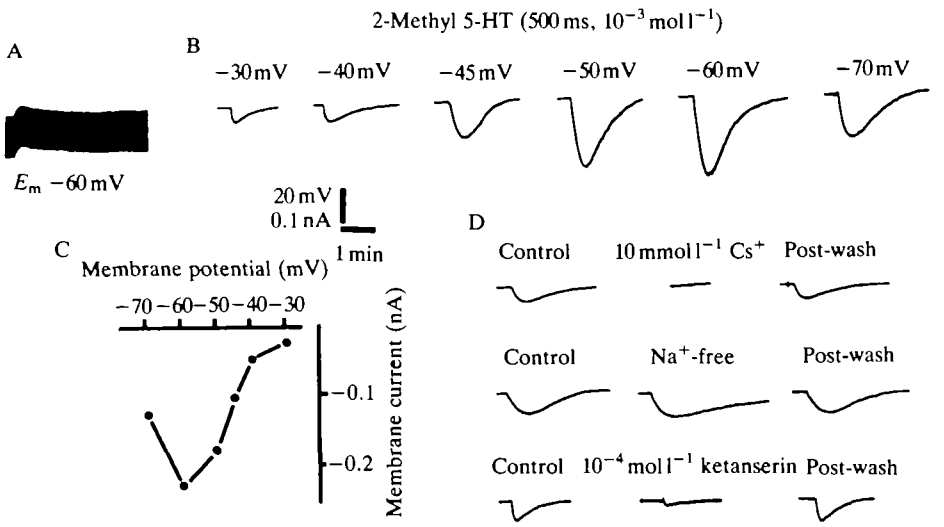

Fig. 7. A Cs^+-sensitive current evoked by 2-methyl 5-HT. (A) In this current-clamped neurone, 2-methyl 5-HT produced a depolarisation with no measurable change in membrane conductance. (B) Under voltage-clamp conditions, the voltage-dependence of the current underlying the 2-methyl-5-HT-evoked potential was similar to that shown by I(5HT)K. (C) Current–voltage curve of the 2-methyl-5-HT-evoked currents shown in B. (D) In these voltage-clamped neurones, the current evoked by 2-methyl 5-HT was reversibly abolished by 10 mmol l^{-1} Cs^+, insensitive to sodium-free saline and blocked by 10^{-4} mol l^{-1} ketanserin, as for I(5HT)K. Arrows indicate the application of 2-methyl 5-HT (10^{-3} mol l^{-1}, 500 ms).

I(5HT)K or any other response in the locust neurones, and the 5-HT_3 agonist 2-methyl 5-HT did activate this current. [In other neurones, it also activated I(5HT)Na.] The slow time course of I(5HT)K suggests the involvement of a second messenger, such as cyclic AMP. Second-messenger systems of this type characteristically link serotonin receptors to the potassium channels mediating slow responses in both the vertebrate CNS (see Bobker and Williams, 1990) and molluscan neurones (Drummond *et al.* 1980). Serotonin receptors in insect muscle mediate an increase in the intracellular concentration of cyclic AMP (Baines *et al.* 1990) and the recently cloned *Drosophila melanogaster* serotonin receptor activates an adenylate cyclase in the 3T3 expression system (Witz *et al.* 1990). Like the receptors for I(5HT)K and I(5HT)Na, the latter receptor is activated by 2-methyl 5-HT. Also as for I(5HT)K, the serotonin-induced relaxation of the isolated foregut of the locust *Schistocerca gregaria* is blocked by ketanserin but unaffected by ICS 205 930 (Osborne *et al.* 1990), but nothing is known about the ion-dependence of this response.

I(5HT)Na is a sodium-dependent current. In the vertebrate nervous system, serotonin can also activate a sodium conductance but, in contrast to the very slow kinetics of I(5HT)Na, the vertebrate current is rapidly activated (<30 ms) and short-lasting (100–300 ms), being mediated by a receptor/ion channel complex

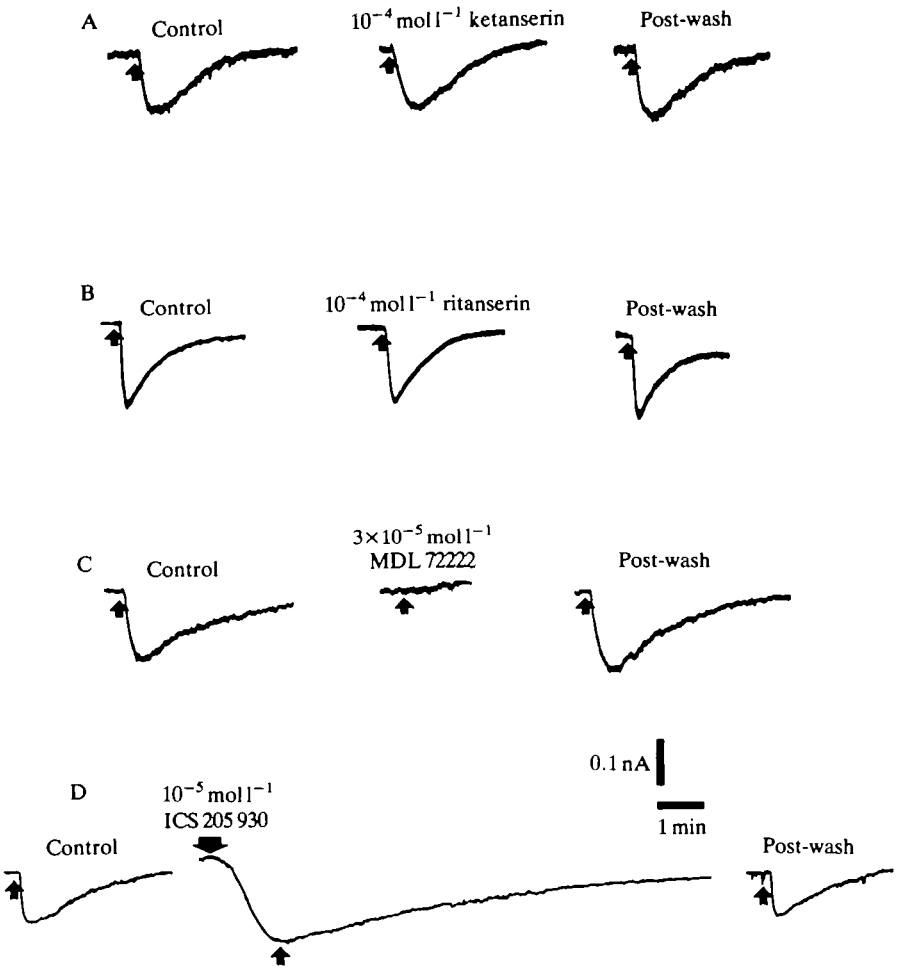

Fig. 8. Pharmacology of I(5HT)Na. Neither ketanserin (10^{-4} mol l^{-1}; A) nor ritanserin (10^{-4} mol l^{-1}; B) had any effect on I(5HT)Na. (C) I(5HT)Na was completely abolished by 3×10^{-5} mol l^{-1} MDL 72222. This effect was fully reversible after about 18–20 min of wash-out. (D) At 10^{-5} mol l^{-1}, bath-perfused ICS 205 930 induced an inward current and blocked the response to serotonin. The response evoked by ICS 205 930 was fully reversible following perfusion with control saline. Small arrows indicate the application of serotonin onto impaled neuronal somata (10^{-3} mol l^{-1}, 500 ms) while the large arrow indicates the application of ICS 205 930 by bath-perfusion.

without the intervention of a second messenger. It is antagonised by 5-HT_3 receptor antagonists (Bobker and Williams, 1990). The receptor for I(5HT)Na also has 5-HT_3 pharmacological characteristics since it is blocked by the 5-HT_3 antagonist MDL 72222 and activated by the 5-HT_3 agonist 2-methyl 5-HT. The 5-HT_3 antagonist ICS 205 930 also binds to the receptor but acts as an agonist.

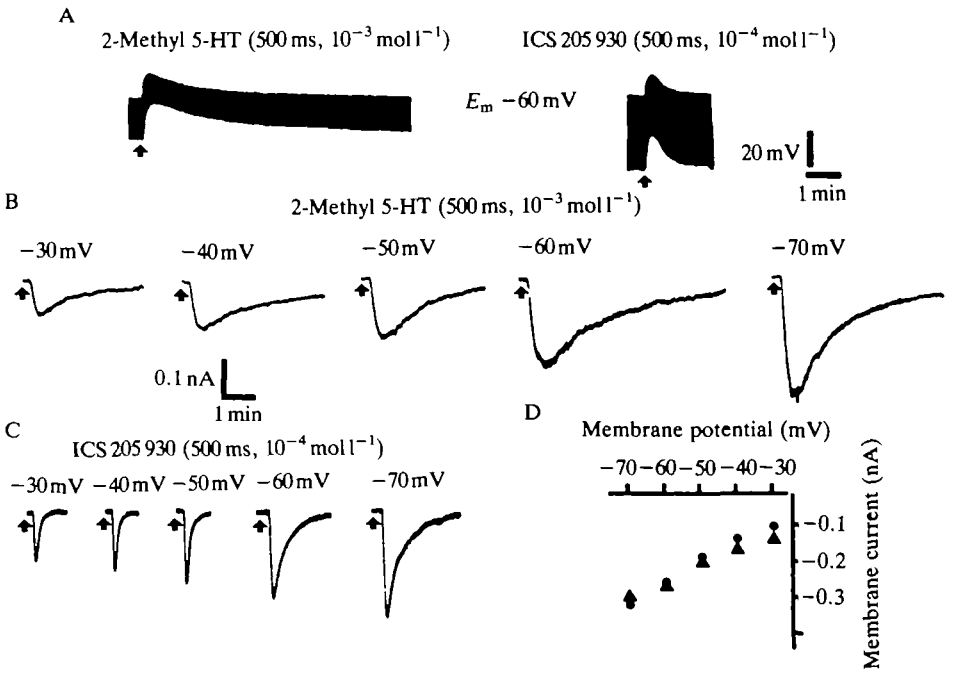

Fig. 9. Effects of 2-methyl 5-HT and ICS 205 930 on locust neurones *in vitro*. (A) In these current-clamped neurones, pressure-application (500 ms) of both 2-methyl 5-HT (10^{-3} mol l^{-1}) and ICS 205 930 (10^{-4} mol l^{-1}) evoked a depolarisation accompanied by an increase in membrane conductance, as for I(5HT)Na. Membrane potential (E_m) was −60 mV. The currents underlying the depolarising responses to both 2-methyl 5-HT (B) and ICS 205 930 (C) were inwardly directed and their amplitudes increased with hyperpolarisation, also as for I(5HT)Na. (D) Current–voltage curve of the responses elicited by 2-methyl 5-HT (●) and ICS 205 930 (▲) shown in B and C. Arrows indicate application of the agonists (500 ms).

Thus, although there is considerable resemblance in receptor pharmacological profile between the vertebrate fast, sodium-dependent 5-HT_3 response and I(5HT)Na, the kinetics and pharmacological details differ. In terms of kinetics, I(5HT)Na bears a closer resemblance to the slow sodium current A′, elicited by serotonin in molluscan neurones (Gerschenfeld and Paupardin-Trisch, 1974), than to any of the serotonin-activated currents reported so far for the vertebrate CNS (Bobker and Williams, 1990). As for I(5HT)K, the slow kinetics of I(5HT)Na suggests mediation by a second messenger.

I(5HT)X probably results from the suppression of a potassium current. The pharmacology and dependence on membrane potential of its ion channel and the pharmacology of its receptor are different from those of I(5HT)K. I(5HT)X has faster kinetics and is activated at depolarised potentials. It is completely blocked by 5 mmol l^{-1} 4-AP, which is only a partial blocker of I(5HT)K. TEA^+ is a weak blocker of both currents, but Cs^+ effectively blocks I(5HT)K and not I(5HT)X. None of the agonists, except serotonin, and none of the antagonists active at the

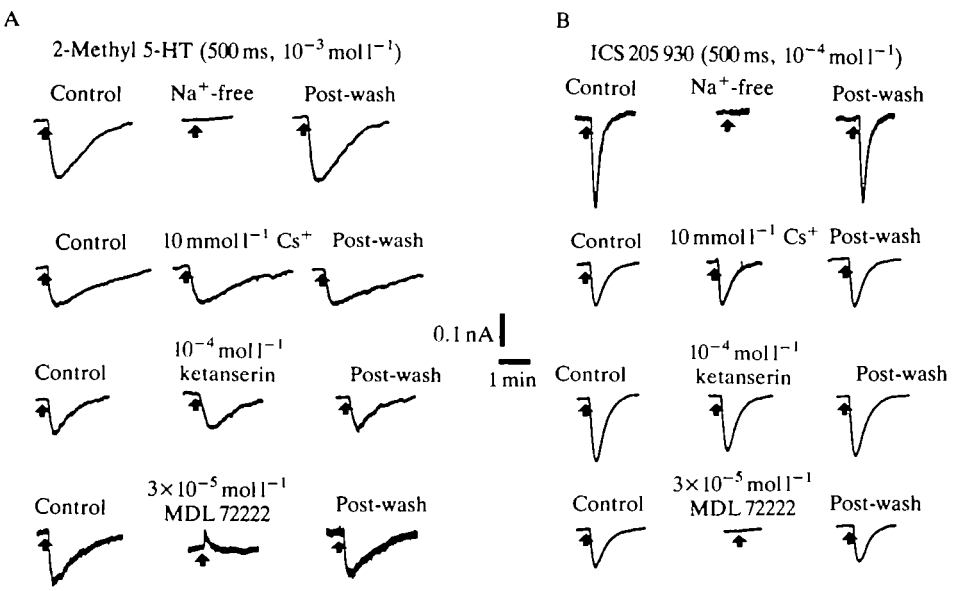

Fig. 10. Ionic properties and pharmacology of the currents generated by 2-methyl 5-HT (10^{-3} mol l^{-1}; A) and ICS 205 930 (10^{-4} mol l^{-1}; B) in locust neurones *in vitro*. The two currents were sodium-dependent and insensitive to both 10 mmol l^{-1} Cs^+ and 10^{-4} mol l^{-1} ketanserin, but were blocked by 3×10^{-5} mol l^{-1} MDL 72222, as for I(5HT)Na. The small outward current seen with pressure-application of 2-methyl 5-HT during bath-perfusion with MDL 72222 is a pressure artefact occasionally observed in these neurones. Arrows indicate application of the agonists (500 ms).

receptor for the I(5HT)K response had any effect at the I(5HT)X receptor. The voltage-dependence and potassium-channel-like pharmacology of I(5HT)X resemble those of the inward current evoked by serotonin in rat hippocampal CA1 neurones, a current that also results from the suppression of a voltage-dependent potassium conductance (I_m) (Colino and Halliwell, 1987). Although the pharmacology of these receptors has not been well-characterised, it is worth noting that they are both insensitive to ketanserin.

In insects, numerous studies have localised and mapped serotonergic neurones in the CNS. There is evidence for calcium-dependent release, serotonin-specific binding sites and receptors, and mechanisms for the re-uptake and inactivation of serotonin. Physiological responses to serotonin at peripheral sites such as the salivary glands are well-known. In this paper, we demonstrate that insect neurones also have the capability of responding to serotonin in a variety of ways mediated by ion-specific channels exhibiting distinct kinetics and voltage-dependence and activated *via* pharmacologically distinct receptors that differ in pharmacological profile from the known vertebrate serotonin receptors. These observations provide further evidence that serotonin is an important neurotransmitter and neuromodulator in insects.

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