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# MODULATION OF ELECTRICAL ACTIVITY BY 5-HYDROXYTRYPTAMINE IN CRAYFISH NEUROSECRETORY CELLS

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## Summary

**The effect of 5-hydroxyptryptamine (5-HT) was tested in a population of X organ neurosecretory cells in the eyestalk of the crayfish *Procambarus clarkii*. Tests were conducted both *in situ* and on isolated neurones kept in culture.**

**The application of 5-HT induced action potentials in silent cells. In spontaneously active neurones, 5-HT increased the firing rate and either induced firing or enhanced bursting activity. The effect of 5-HT was dose-dependent within the range 1–100 $\mu$mol l$^{-1}$ in cells of the intact organ. The effect persisted for 20–30 min after 5-HT had been removed from the bathing solution. Successive applications of 5-HT onto the same neurone reduced responsiveness, suggesting that desensitization had occurred. The effects of 5-HT were blocked by prior incubation with the 5-HT antagonist methysergide.**

**In X organ cells whose axons and branches in the neuropile had been severed, 5-HT induced a depolarisation associated with a slow inward current. In X organ neurones isolated from the eyestalk and kept in culture, 5-HT was capable of evoking bursts of action potentials and elicited a slow inward current. This effect was also blocked by methysergide ($10^{-4}$ mol l$^{-1}$).**

**These results suggest a direct modulatory effect of 5-HT on the pattern of electrical activity in the X organ cells.**

Key words: serotonin, neuromodulation, neurosecretory systems, bursting, neurosecretion, crayfish, Crustacea, *Procambarus clarkii*.

## Introduction

5-Hydroxytryptamine (5-HT) has been proposed as a transmitter or modulator mediating a variety of physiological functions in crustaceans. A facilitatory role on neuromuscular transmission in the lobster (Glusman and Kravitz, 1982) and in the crayfish (Fischer and Florey, 1983; Dixon and Atwood, 1985) has been described. A specific behavioural pattern of abdominal muscle flexion is induced by 5-HT injection in lobster and crayfish (Livingstone *et al.* 1980), apparently mediated by a combination of flexor motoneurone excitation and extensor motoneurone inhibition (Kravitz, 1988). In addition, 5-HT has been found to modulate the crayfish escape response (Glanzman and Krasne, 1983; Yeh *et al.* 1996).

Peripheral sensory mechanisms are also modulated by 5-HT. A facilitatory action has been described on primary mechanoreceptor afferents (Pasztor and Bush, 1989) and on receptor muscles of the lobster abdominal stretch receptor (Pasztor and Golas, 1993), as well as on crayfish peripheral mechanoreceptors (El Manira *et al.* 1991).

An enhancement of retinal responsiveness to light is also induced by 5-HT in the crayfish *Procambarus clarkii* mediated by a dual action: (a) increasing the gain of retinal photoreceptors, by acting on a light-induced conductance, and (b) promoting the retraction of intracellular pigment granules within the photoreceptors, thereby increasing the photon flux on the photosensitive membrane (Aréchiga *et al.* 1990).

Another likely target of 5-HT action in crustaceans is the neurosecretory system. The injection of 5-HT in the crayfish *Orconectes limosus* raises blood sugar levels, while this effect can be prevented by eyestalk ablation (Keller and Beyer, 1968), suggesting that 5-HT acts as a modulator of crustacean hyperglycaemic hormone (CHH) release in the X organ–sinus gland system of the eyestalk. However, a direct hyperglycaemic effect of 5-HT has been described in the shore crab *Carcinus maenas* (Bauchau and Mengeot, 1966; Luschen *et al.* 1993). It has also been suggested that 5-HT facilitates the release of red pigment dispersing hormone (RPDH) in the dwarf crayfish *Cambarellus shufeldtii* (Rao and Fingerman, 1975), of neurodepressing hormone (NDH) in the crayfish *Procambarus bouvieri* (Aréchiga *et al.* 1985), of moult inhibiting hormone (MIH) in the crab *Cancer antennarius* (Mattson and Spaziani, 1985) and of black pigment dispersing hormone (BPDH) in *Cancer maenas* (Bauchau and Mengeot, 1966; Fingerman and Nagabhushanam, 1992). A 5-HT-induced increase in the number of exocytotic figures in

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neurosecretory endings of the sinus gland of the crayfish *Astacus leptodactylus* has also been documented (Strolemberg and van Herp, 1977).

5-HT has been identified and quantified in the eyestalk of various crustacean species (Eloffson *et al.* 1982; Laxmyr, 1984; Kulkarni and Fingerman, 1992). Neurone somata and fibres reacting with anti-5-HT antisera have been described in all eyestalk ganglia, as well as in other central ganglia (Eloffson, 1983; Beltz and Kravitz, 1983; Bellon-Humbert and van Herp, 1988; Sandeman *et al.* 1988). 5-HT-like immunoreactivity has been located in dense-cored vesicles in nerve endings in the medulla terminalis (Andrew and Saleuddin, 1978) and in the retina (Aréchiga *et al.* 1990). 5-HT-like immunopositivity has also been detected in the crayfish, in a bundle of efferent axons running from the supraoesophageal ganglion to the medulla terminalis, and 5-HT release by electrical stimulation of the optic nerve has been documented (Rodríguez-Sosa *et al.*1997).

This evidence suggests a role for 5-HT in the control of hormonal secretion from the eyestalk. However, no direct evidence exists for an effect on the neurosecretory cells themselves. Although 5-HT has been reported to elicit changes in the electrical activity of X organ cells in the crabs *Cardisoma carnifex* and *Podophthalmus vigil* (Nagano and Cooke, 1981), no analysis has been made of its possible effects. It is the purpose of this paper to present evidence indicating a direct action of 5-HT on the pattern of electrical activity of X organ neurones, both *in situ* and in isolated neurones in culture.

## Materials and methods

The experiments were carried out in adult crayfishes *Procambarus clarkii* (Girard) or in isolated and cultured neurosecretory cells removed from the X organ. Animals were of either sex and in intermoult at the time of the experiment. The specimens were collected from Rio Conchos, Chihuahua, México, and adapted to laboratory conditions for 2 weeks, either under a natural light:dark cycle or with a 12 h:12 h light:dark programme. All experiments were conducted at room temperature (20–22 °C) and during day time.

Eyestalks were excised and placed in normal saline solution containing (in mmol $l^{-1}$): 205 NaCl, 5.4 KCl, 2.6 $MgCl_2$, 13.5 $CaCl_2$ and 10 Hepes (pH 7.4). The exoskeleton, muscles and connective tissue were carefully removed under a microscope to expose the neurone somata. The somata selected for our study are in the most external layer of the X organ. Their electrophysiological features have been reported previously (Onetti *et al.* 1990).

Cell location was visualized under a microscope and confirmed after the experiment by intracellular injection of the fluorescent dye Lucifer Yellow. As illustrated in Fig. 1, the X organ neurones send their axons in a well-defined dorso-lateral direction and are easily recognisable. Intracellular dye injections were made as previously described (Alvarado-Álvarez *et al.* 1993).

The preparations were allowed to stabilize for 1 h before recording and for another 30 min after impalements. Only those cells with stable resting potentials over –45 mV and with spikes with at least 10 mV of overshoot were selected for further testing. Data were collected from 64 neurones. A new preparation was used for each experiment. 5-HT (Sigma) and methysergide (Research Biochemicals International) were freshly prepared in normal saline solution at the time of experiments and were applied either by perfusion to the recording chamber or by pressure pulses onto the neurones in isolated X organs (using 69 kPa pressure pulses from 5-HT-containing pipettes) placed near (100–150 μm) the somata.

Intracellular impalements were made using borosilicate glass microelectrodes pulled with a Sutter P87 pipette puller; microelectrodes were filled with prefiltered (0.22 μm, Millipore) 3 mol $l^{-1}$ KCl to tip resistances of 40–50 MΩ. Recordings were made with an M-707 WPI amplifier.

For experiments on isolated neurones, the cells were removed from the X organ by dissociating them by gentle suction through fire-polished micropipettes, as previously described (García *et al.* 1990). Isolated neurones were plated

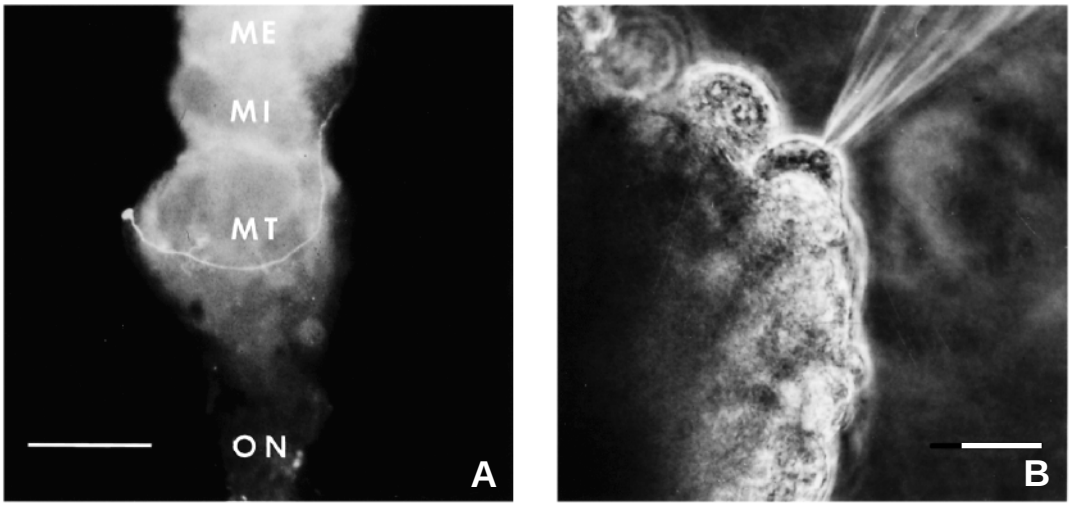



Fig. 1. Photomicrographs of the crayfish optic peduncle showing an X organ neurone filled with Lucifer Yellow after a recording has been made. (A) ON, optic nerve; MI, medulla interna; ME, medulla externa; MT, medulla terminalis. (B) Organ cell during recording. Scale bars, A, 1 mm; B, 50 μm.

in a recording chamber previously coated for 2 h with Concanavalin A (Type III, Sigma). Cells were cultured in modified Leibovitz L-15 medium, containing (in mmol $l^{-1}$): 205 NaCl; 4.5 KCl; 13.5 $CaCl_2$; 2.5 $MgCl_2$; 10 Hepes; 5.5 glucose; 2 L-glutamine; gentamycin (16 μg $ml^{-1}$, Schering Plough); streptomycin (5 μg $ml^{-1}$, Sigma); penicillin (5 i.u., Sigma). Cultured cells were kept at 20–22 °C in darkness.

Voltage-clamp experiments were performed either in the whole-cell configuration or using a perforated patch technique. Experiments were performed with an Axoclamp 2A amplifier (Axon Instruments). Voltage and current recordings were stored on a video code modulator (PCM 400, Vetter), and selected portions of the data were stored on the hard disk of an 80486 computer (Acer 433) using commercially available acquisition hardware and software (pClamp 5.1, DigiData 1200 and Axotape 2, Axon Instruments). For the perforated patch technique, a nystatin (300 μg $ml^{-1}$) patch was used.

Membrane potential and spiking activity were acquired at 10 kHz through the Axotape program. Selected portions of recordings were exported in ASCII format and analysed with a computer program developed in the laboratory to determine interspike intervals and the maximum slopes of depolarisation and repolarisation during individual spikes (see Fig. 4). From these data, instantaneous frequency graphs were prepared using SigmaPlot version 5.0 (Jandel, Inc.).

## Results

### *Effects of 5-HT on the various patterns of discharge in X organ neurones*

In 64 complete experiments, all of the impaled neurones were responsive to 5-HT in a manner that was dependent on their previous activity. As described earlier (Onetti *et al.* 1990), there are three types of activity in crayfish X organ neurones. They may be (a) silent, but capable of firing action potentials by direct depolarisation through the recording electrode, (b) tonically active, with a regular firing rate, or (c) spontaneously bursting. As seen in Fig. 2A, topical application of 5-HT elicits a prolonged depolarisation in a previously silent cell, which gave rise to a burst of spikes lasting 2–5 min. Bursting usually started approximately 10 s after perfusion of 5-HT into the bathing solution. In tonically active neurones (Fig. 2B), 5-HT induced a change in the activity pattern, eliciting prolonged bursting activity lasting for 20–30 min. All the bursting neurones recorded responded to 5-HT with an enhancement of activity. In these cells, 5-HT increased the burst duration and shortened the inter-burst interval (Fig. 2C). In most experiments, the duration of the 5-HT pulse was kept short, since long exposures to 5-HT led to a prolonged desensitization (see below). When 5-HT was applied to bursting neurones shortly before the expected burst onset, the most noticeable effect was a lengthening of the next burst, as shown in Fig. 3A. When the application was made shortly after the end of a burst, the main effect was a shortening of the interval to the next burst (Fig. 3B), although there was also an increase in the length of that burst.

To analyze the effects of 5-HT on the firing rate, the interspike interval was evaluated as the time between two successive maximum depolarisation slopes (Fig. 4A), and the instantaneous firing frequency (the reciprocal of the interspike interval) was determined before, during and after 5-HT application (Fig. 4C–E). Fig. 4B shows the effect of 5-HT on a tonically active neurone. The samples of activity used for interspike interval analysis are marked in Fig. 4B, and the results are presented in the corresponding graphs (Fig. 4C–E).

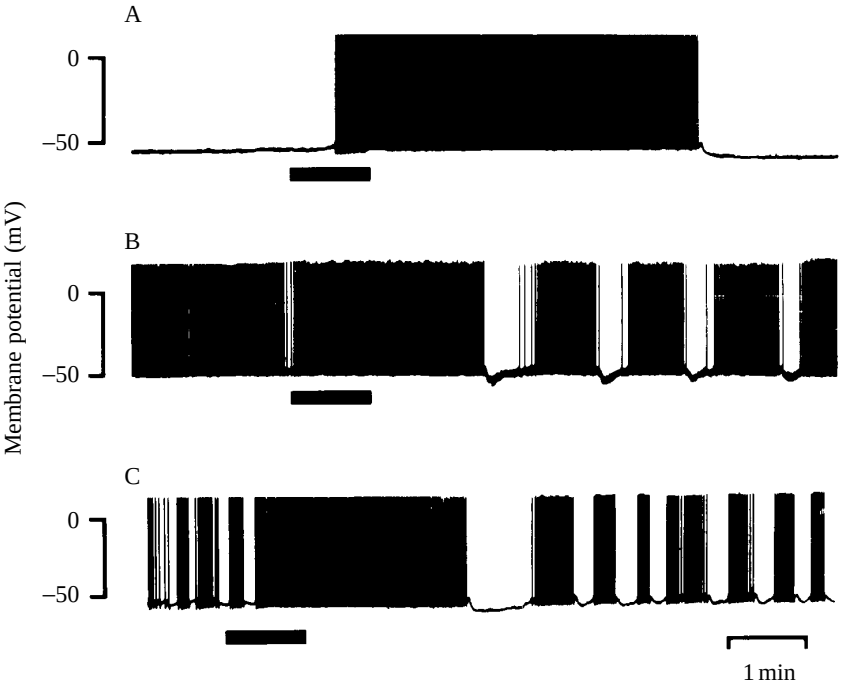



Fig. 2. Effects of 5-hydroxytryptamine(5-HT) (50 μmol $l^{-1}$) on three X organ neurones showing representative patterns of activity. (A) A previously silent neurone; (B) a neurone firing tonically; and (C) a spontaneously bursting neurone. 5-HT applications are indicated by black bars under the recordings.

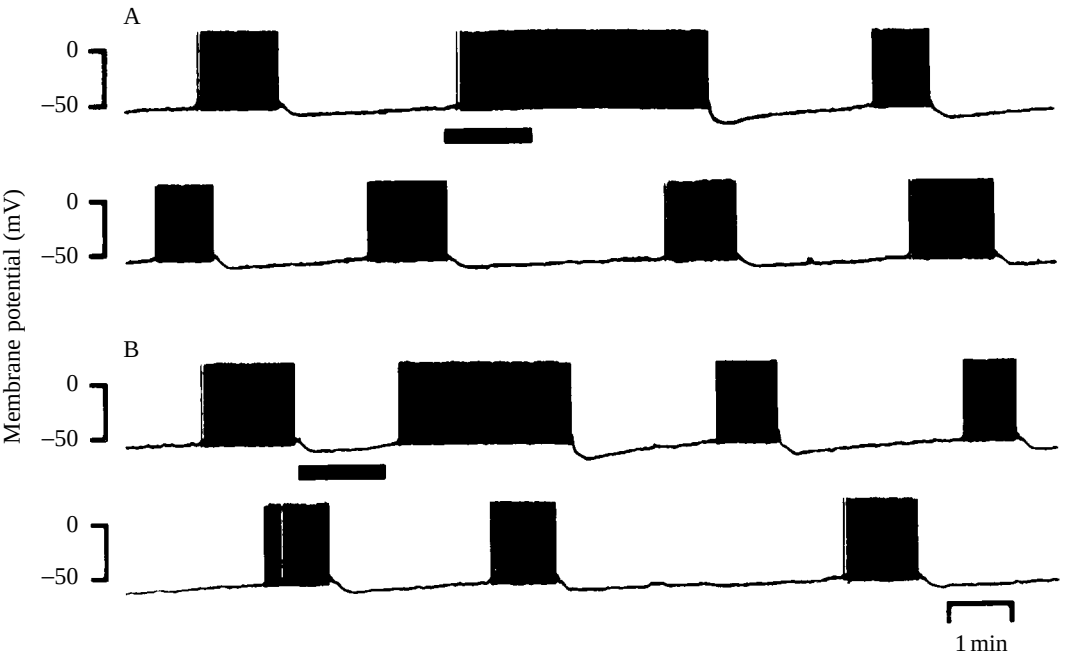



Fig. 3. Effects of 5-HT on a bursting neurone. (A) 5-HT applied at the onset of a burst prolonged the burst duration (top trace). (B) 5-HT applied after the end of a burst shortened the interburst interval and prolonged the subsequent burst (top trace). Both effects were reversed after washing (lower traces). 5-HT (50 μmol $l^{-1}$) applications are indicated by black bars.

As can be seen, the increase in the firing rate induced by 5-HT (Fig. 4D) lasted over 3 min and was followed by a prolonged hyperpolarisation. The cell was silent for a few seconds, and electrical activity was reinitiated in a bursting manner (Fig. 4E). This pattern usually lasted 20–30 min (not shown in the figure).

Although 5-HT-induced bursting was preceded by a slow depolarisation, it cannot be explained solely on that basis, since direct depolarisation of neurones through the microelectrode elicited sustained spiking activity, but not bursting. However, when bursting is prevented by hyperpolarising the cell, brief depolarising pulses may trigger action potentials, but not bursts (Fig. 5, upper traces), whereas after 5-HT application, the same depolarising pulse is capable of eliciting a long burst after the initial brief response (Fig. 5, lower traces).

Repetitive application of 5-HT resulted in a reduction of responsiveness, as shown in Fig. 6 for the effect of successive applications of 5-HT at 10 min intervals at the same

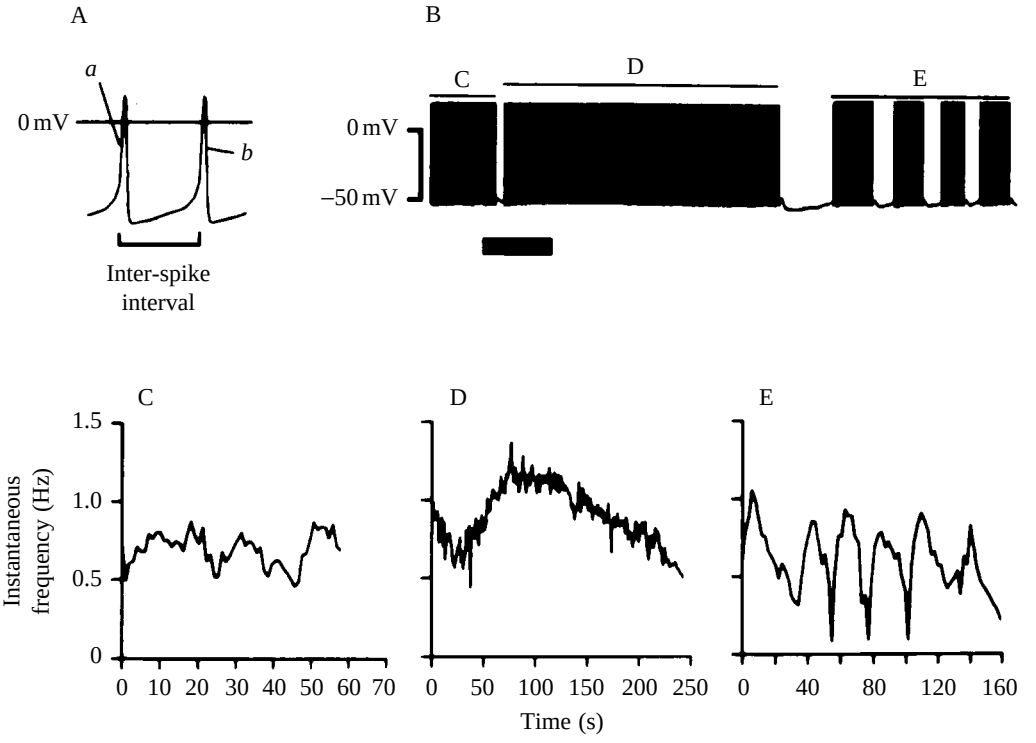



Fig. 4. Effect of 5-HT on the instantaneous spike frequency. (A) The interspike interval was defined as shown (see text). *a*, Maximum depolarization slope; *b*, maximum repolarization slope. (B) Recording of a tonically active neurone before, during and after a 1 min 5-HT application (indicated by black bar below trace) (50 μmol $l^{-1}$). (C–E) Instantaneous firing rates for the events indicated on the recording shown in B. Note the changes in the firing pattern.

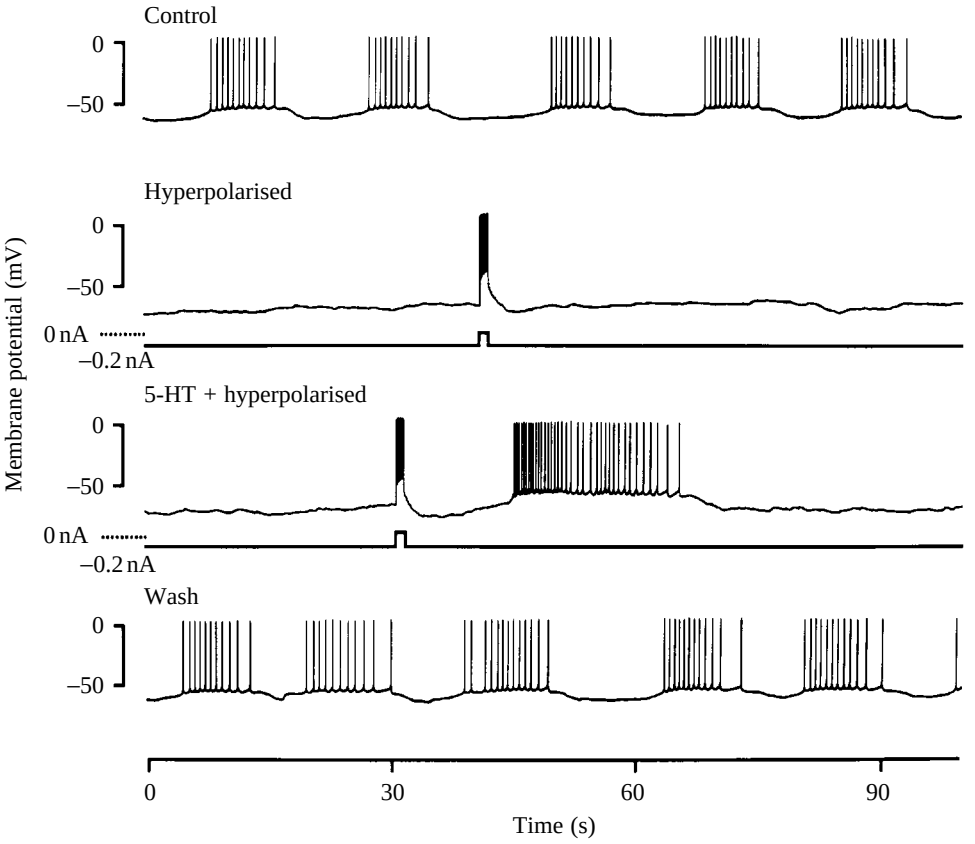



Fig. 5. Hyperpolarisation prevents bursting, but brief depolarising pulses (0.2 nA) (lower traces) elicit spiking activity (upper traces). In the presence of 5-HT (50 μmol $l^{-1}$), spiking is evoked in the hyperpolarised neurone. After washing out 5-HT, the membrane potential returns to its resting value and bursting is resumed.

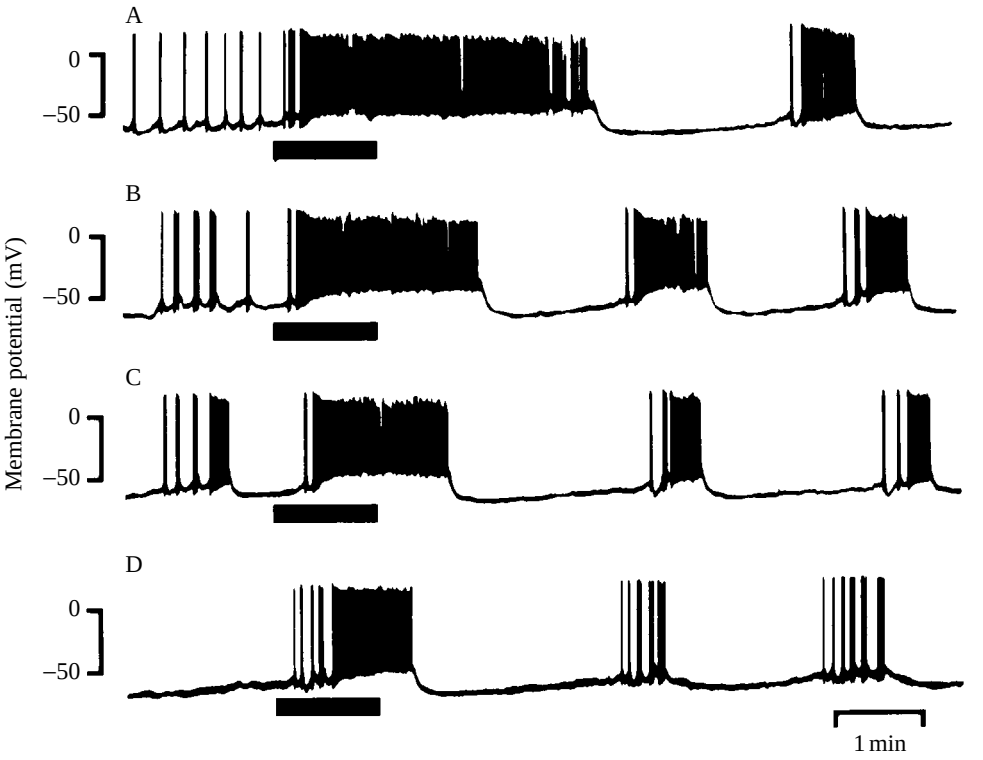



Fig. 6. Desensitization to 5-HT. Response of an X organ bursting neurone to successive 5-HT applications (50 μmol $l^{-1}$) at 10 min intervals. Notice the shortening of the 5-HT-associated burst and the splitting of successive bursts. 5-HT pulses are indicated by black bars under the recordings.

concentration (50 μmol $l^{-1}$) to a bursting neurone. Notice that the bursts evoked by the 5-HT pulses were progressively reduced in duration. After 5-HT removal, bursts were also split into shorter trains of action potentials. These observations suggest that a desensitization occurs.

Since 5-HT has been reported to affect the duration of individual spikes in other systems, an analysis of spike duration was made in our preparations. However, no effects were found on the waveform of individual spikes, other than those related to the higher level of depolarisation during 5-HT application compared with control activity. In samples of 300 spikes in each of three experiments, neither the depolarising and repolarising phases of individual spikes nor the overshoot and total duration of the spikes were affected by 5-HT.

### *Dose-dependency of 5-HT effect*

Although the long duration of the effect of 5-HT and the resulting desensitization hampered the analysis of dose–response relationships, it was possible in some preparations to explore a wide range of doses in a single neurone. Intervals between successive 5-HT applications had to be longer than 10 min (various interval lengths were tested). As seen in Fig. 7A, for a bursting neurone subjected to pulses of 5-HT applied over an increasing range of concentrations from 1 to 100 μmol $l^{-1}$ at intervals that also increased (15, 30 and 45 min). The number of bursts evoked and the duration of the effect were proportional to the dose of 5-HT. The interspike intervals within bursts were also shortened in proportion to 5-HT concentration. As shown in Fig. 7B, the mean frequency of spikes within a burst was almost doubled over the dose range tested. The value shown in the figure is the mean for all the bursts during the time of 5-HT application. A proportional relationship can also be seen between inter-burst interval and the dose applied (Fig. 7C).

### *Pharmacological blockage*

The effect of 5-HT could be blocked by methysergide topically applied at the same doses (10–100 μmol $l^{-1}$) previously found to suppress 5-HT modulatory action on the retina (Aréchiga *et al.* 1990). In the experiment illustrated in Fig. 8 (representative of three experiments with the same dose), the preparation was incubated in 100 μmol $l^{-1}$ methysergide for 10 min prior to the pulse of 50 μmol $l^{-1}$ 5-HT. Both the depolarisation and the increase in bursting activity

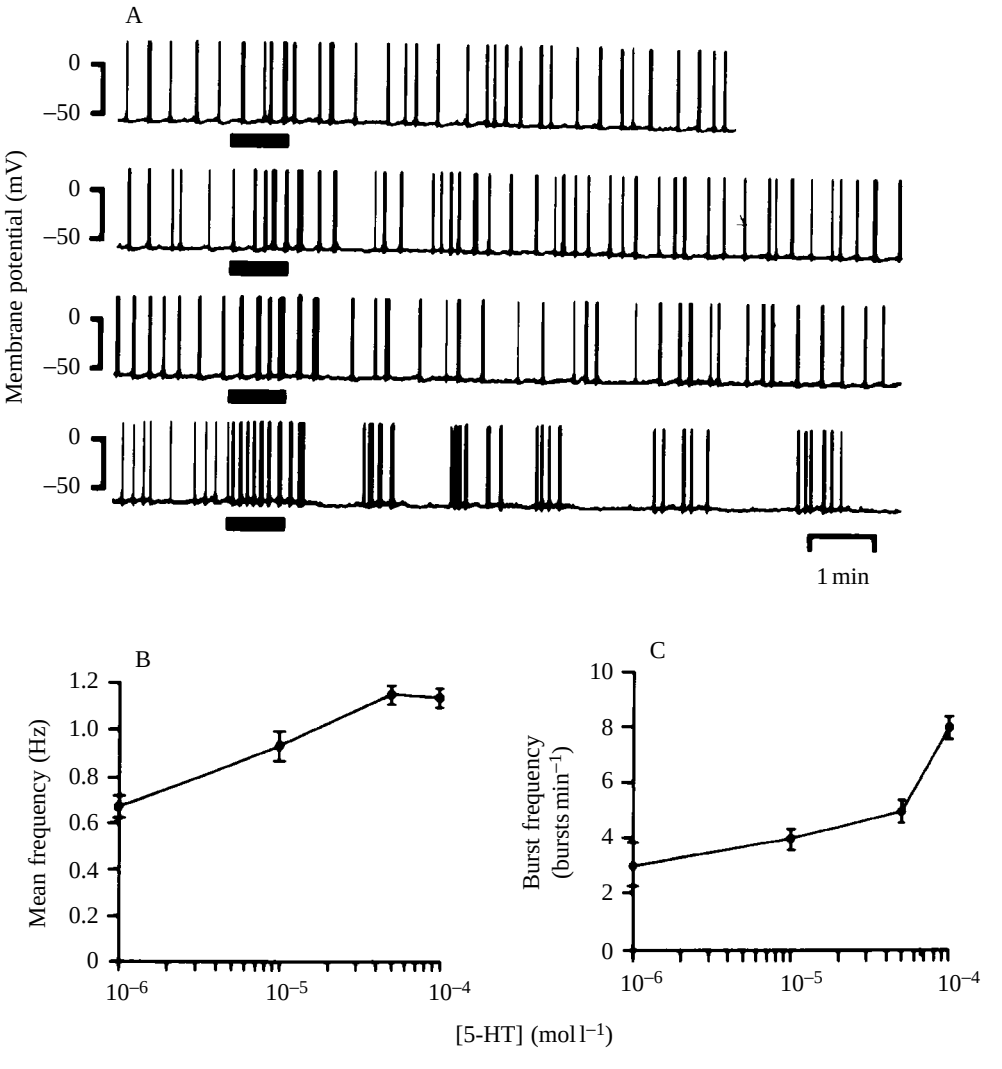



Fig. 7. Dose–response relationship for 5-HT on a bursting neurone. (A) 5-HT pulses of increasing concentrations (1, 10, 50 and 100 μmol $l^{-1}$) given at increasing intervals of 15, 30 and 45 min, respectively, from top to bottom. (B) The response was evaluated as the mean frequency of spikes within each burst evoked during 5-HT perfusion. (C) Effect of 5-HT concentration on burst frequency, in the 5-HT responses shown in A. Each point represents the mean value of four experiments. Error bars indicate standard deviation.

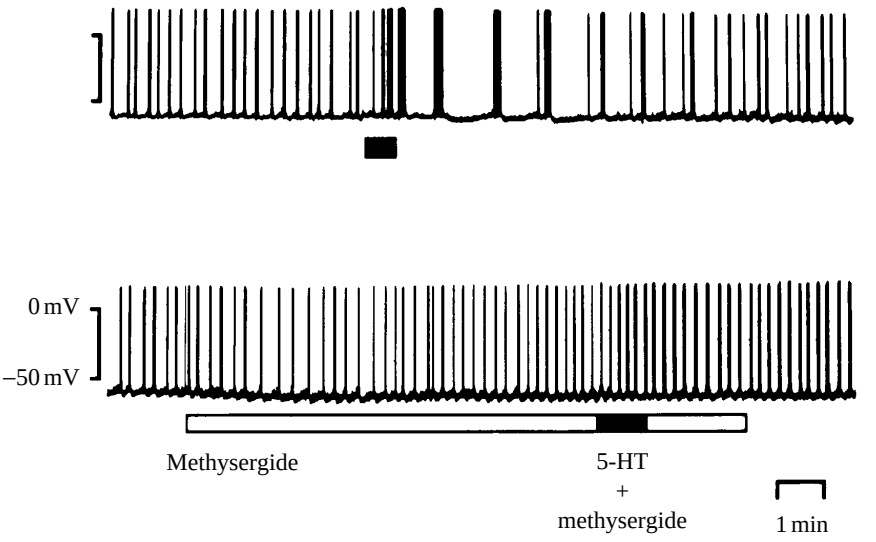



Fig. 8. Blockade of the effect of 5-HT by methysergide. As seen in the control (top trace), bursting activity was enhanced by 5-HT (black bar, 50 μmol $l^{-1}$) and this response was suppressed by continuous perfusion with methysergide (100 μmol $l^{-1}$), indicated by the open bar beneath the lower trace (recorded 30 min after the control).

were blocked. It is interesting to note that no effect of methysergide was detected on spontaneous synaptic input to X organ cells.

*Site of action of 5-HT*

From the experiments described so far, no definite view can be derived as to the site(s) of action of 5-HT on X organ neurones. The issue is of interest since it may help to clarify whether 5-HT acts as a neurotransmitter at specific synaptic sites or as a modulator on wide areas of the neuronal surface. To explore this issue, 5-HT was tested on isolated clusters of X organ cells. Fig. 9C shows the isolated X organ cluster in which at least five neurone somata can be distinguished. Their axons were severed before the emergence of the branches in the neuropile of the medulla terminalis. As seen in Fig. 9A, the axotomized neurone was no longer capable of generating fast spikes or spontaneous bursting activity but, in response to depolarising pulses, did produce slow action potentials, that are known to be $Ca^{2+}$-dependent (Onetti *et al.* 1990). During 5-HT perfusion (1 μmol $l^{-1}$), a 5 mV depolarisation was evoked (Fig. 9B, upper trace). This effect was reversible after 5-HT removal. The time course of the depolarisation evoked by 5-HT corresponds to the slow inward current recorded under voltage-clamp conditions (Fig. 9B, middle trace). Both traces

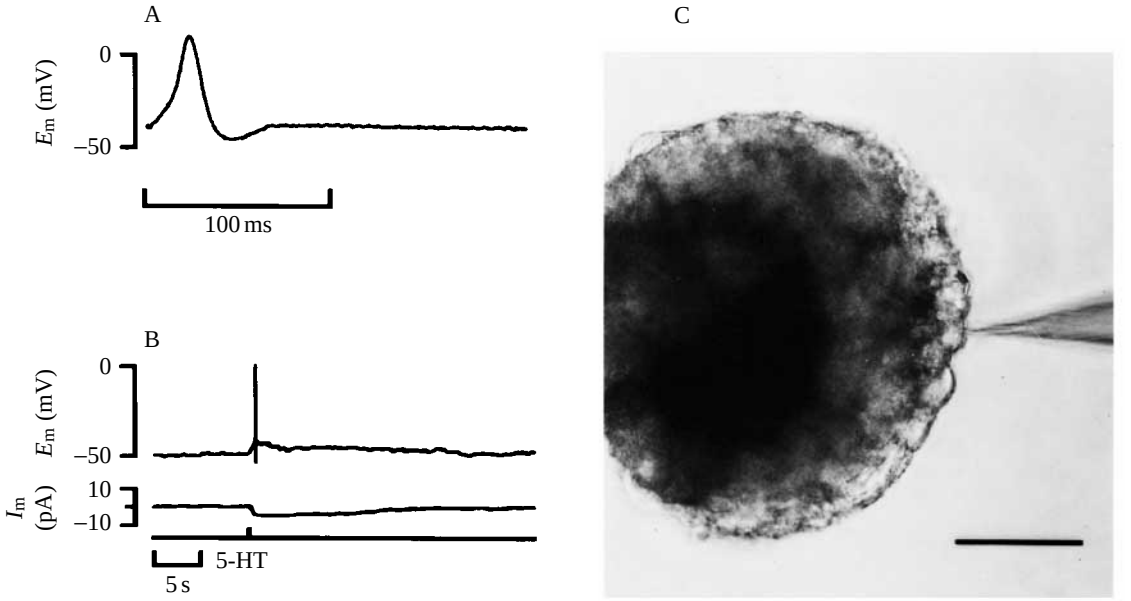


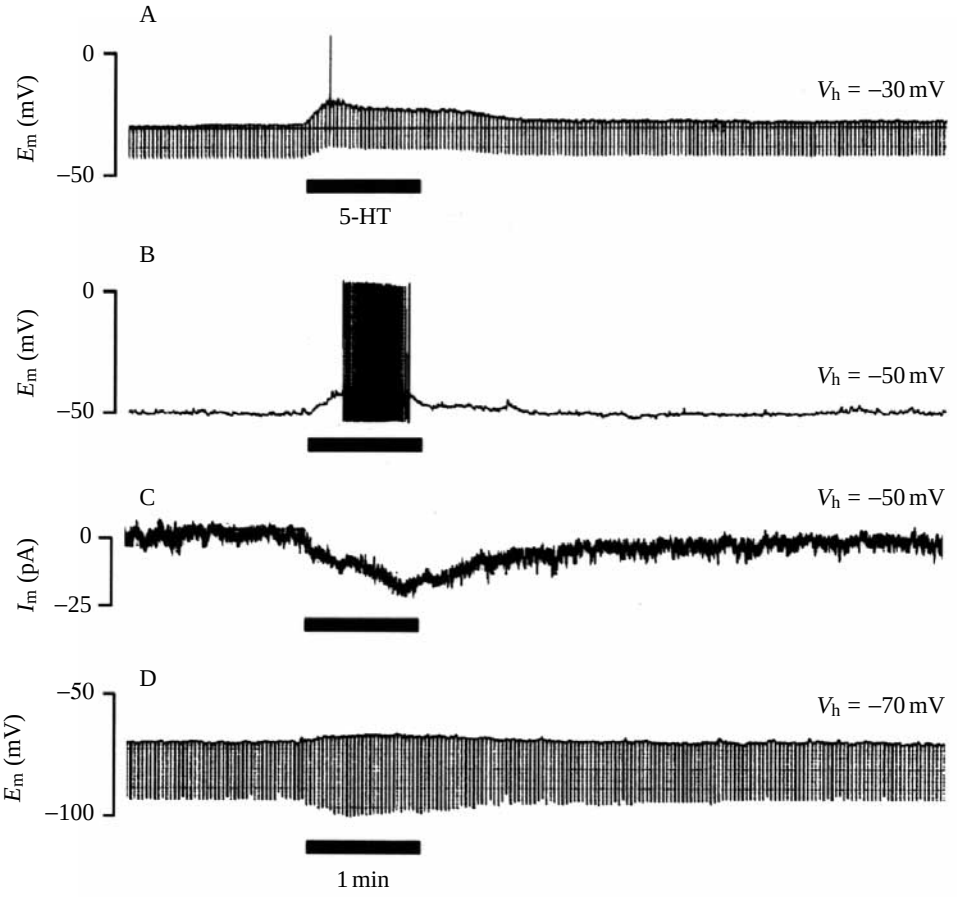

Fig. 9. Effect of 5-HT (1 μmol $l^{-1}$) on the response of a neurone in the isolated X organ. (A) Control recording; a single spike is evoked by a brief depolarising pulse. (B) A 5-HT pulse (1 μmol $l^{-1}$, 69 kPa, 100 ms, shown in the bottom trace) evokes, under current-clamp mode, a 5 mV depolarisation and a single spike (upper trace). In the whole-cell configuration, 5-HT evoked a slow inward current ($I_m$) measured at a holding potential of –50 mV. (C) A photomicrograph of an isolated X organ from the eyestalk. Scale bar, 110 μm.

were obtained after applying 5-HT by pressure pulses onto the somata of the cells.

*Effect of 5-HT on isolated X organ neurones*

Although the effects so far described for 5-HT on X organ cells appear to be due to a direct action on the neurones, an indirect action cannot be ruled out while working on neurones in intact X organ. These neurones are known to receive synaptic inputs (Iwasaki and Satow, 1971) mediating the influence of light (Glantz *et al.* 1983), and GABA evokes depolarising responses and trains of action potentials in a dose-dependent manner (García *et al.* 1994). Although neither 5-HT nor methysergide was found to have any effects on the synaptic activity recorded in our preparations, the only certain way to explore a direct action of 5-HT on neurones was to test it on isolated X organ cells.

As illustrated in Fig. 10, in an isolated X organ neurone from the same population recorded *in situ*, that had been cultured for 24 h, topical application of 50 μmol l$^{-1}$ 5-HT elicited a slow depolarisation, recorded under current-clamp conditions. Concurrent with the depolarisation, there was a 33 % increase in the input resistance, as determined by the application of brief



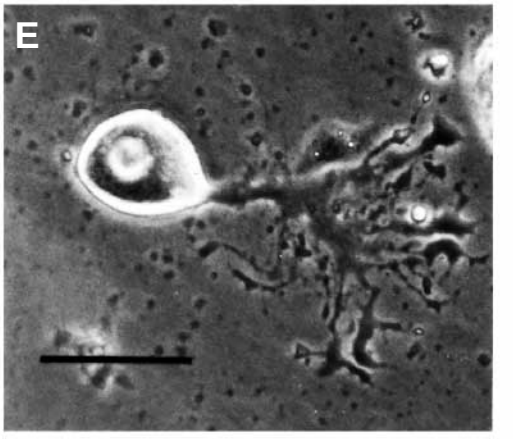




Fig. 10. Responses of a cultured X organ neurone to 5-HT (50 μmol l$^{-1}$) recorded under perforated patch-clamp (A, B and D under current-clamp mode; C under voltage-clamp mode). In A and D, input resistance is probed by brief hyperpolarising pulses applied through the recording pipette. Voltage (mV) for ordinates in A, B and D; current (pA) for ordinate in C. Black bars indicate 5-HT applications. (E) Photomicrograph of an isolated neurone from the X organ. $V_h$, holding potential; $E_m$, membrane potential; $I_m$, membrane current. Scale bar, 50 μm.

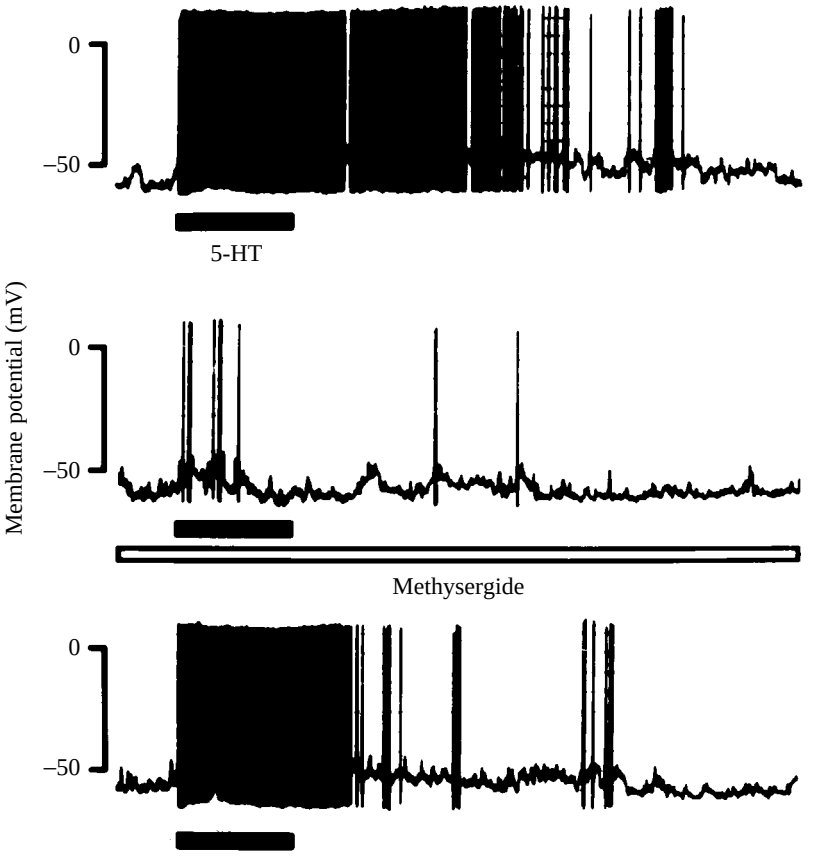



Fig. 11. Effects of 5-HT (50 μmol $l^{-1}$, 1 min) and methysergide (100 μmol $l^{-1}$) on an X organ neurone kept in culture and recorded using the perforated patch technique. The upper trace shows bursting elicited by a 1 min application of 5-HT (indicated by black bar). After a 10 min incubation in methysergide, the effect was greatly reduced (middle trace). After a 10 min wash, bursting could again be evoked by 5-HT (lower trace).

(200 ms at 0.5 Hz) pulses of 10 pA (Fig. 10A). The membrane potential was set to −30 mV by depolarising current injection. Notice the firing of one action potential during the depolarisation. When the membrane potential was shifted to −50 mV, a similar 5-HT pulse was capable of eliciting a burst of action potentials (Fig. 10B). Under voltage-clamp, at a holding potential of −50 mV, 5-HT applied under similar conditions as in the previous tests evoked a slow inward current with a similar time course to that of the conductance changes observed in the previous tests (Fig. 10C). After shifting the membrane potential to −70 mV (Fig. 10D), again under current-clamp conditions, the enhancement of input resistance was only 25 %.

Previous incubation of a cultured neurone in 100 μmol $l^{-1}$ methysergide resulted in a considerable suppression of the response to 5-HT under perforated patch conditions. As seen in Fig. 11, 5-HT (50 μmol $l^{-1}$) elicited a prolonged burst (upper trace). This response was largely blocked by prior incubation of the neurone in methysergide (middle trace). A substantial recovery of the response to 5-HT occurred after the removal of methysergide (lower trace). The small difference from the control response might be expected given the desensitization observed in the *in situ* experiments.

## Discussion

The sensitivity of the X organ neurosecretory cells to 5-HT supports a role for this amine in stimulating the release of neurohormones in the eyestalk, as proposed from pharmacological studies (for a review, see Fingerman and Nagabhushanam, 1992). It also accords with the abundance of 5-HT-like immunoreactive cell bodies and fibres observed in the crayfish medulla terminalis neuropile where the X organ cells receive synaptic inputs (Elofsson *et al.* 1982; Sandeman *et al.* 1988; Rodríguez-Sosa *et al.* 1997). We presume that the neurones examined in this study are those containing CHH, since their location, size and shape coincide with those of cells in which immunopositivity to antibodies against CHH has been documented (Jaros and Keller, 1979; van Herp and van Buggenum, 1979). It is reasonable to suggest that the bursting activity elicited by the applications of 5-HT on X organ neurones that we describe in this paper is the mechanism mediating the release of CHH induced by 5-HT in the crayfish *Orconectes leniusculus* (Keller and Beyer, 1968). However, since no immunocytochemical characterization was made, the effects of 5-HT could be exerted on X organ cells containing other hormones.

5-HT could mediate the effects of stress, which is known to raise blood sugar levels in the lobster *Homarus americanus* (Telford, 1986), and the effects of darkness, since both the blood sugar concentration (Gorles-Kallen and Vooter, 1986) and the 5-HT content in the eyestalk (Fingerman and Fingerman, 1977) are higher at night. Since not all eyestalk neurohormones appear to be released under the influence of 5-

HT (Fingerman and Nagabhushanam, 1992), it would be interesting to test this amine on other groups of neurosecretory cells in the eyestalk.

The induction of a bursting pattern of activity in X organ cells by 5-HT would be an effective way of triggering hormone release. In fact, bursting activity has been proposed as the most efficient temporal pattern of action potential distribution in releasing neurosecretory products from vertebrate hypothalamic neurosecretory cells (Wakerly and Lincoln, 1973; Dutton and Dyball, 1979; Poulain and Wakerly, 1982; Cazalis *et al.* 1985), although this relationship has been questioned for crustacean neurosecretory cells (Keller *et al.* 1994).

Most features of the effects of 5-HT on X organ neurones are similar to those described in other systems in which a facilitatory action has been documented for 5-HT. The persistence of the effect of 5-HT has also been described in preparations such as the lobster neuromuscular junction (Glusman and Kravitz, 1982) and the crayfish neuromuscular junction (Dixon and Atwood, 1985) and retina (Aréchiga *et al.* 1990), where it may last for over 30 min. The persistence of the effect long after 5-HT has been removed from the bathing fluid suggests the participation of an intracellular messenger stage, as has been described for many other systems involving 5-HT. Desensitization to the effects of 5-HT is another feature that has been described in invertebrate neurones in the past (Gerschenfeld and Paupardin-Tritsch, 1974).

The dose–response relationship for the effects of 5-HT on the X organ cells *in situ* is similar to that described for the behavioural responses of the lobster (Livingstone *et al.* 1980) and the retinal effects in the crayfish (Aréchiga *et al.* 1990). The effective doses are higher than those necessary for peripheral effects, such as those on the neuromuscular junction, and also higher than the 5-HT content in the haemolymph (Livingstone *et al.* 1980). This has been attributed to permeability barriers and to active uptake systems which limit the availability of the amine at the receptor sites (Livingstone *et al.* 1980). This view is consistent with our observation that the sensitivity to 5-HT increases when it is tested on isolated neurones.

Methysergide has been shown to block 5-HT effects in other crustacean systems, such as the crayfish retina (Frixione and Hernández, 1989; Aréchiga *et al.* 1990) and central neurones (see Zhang and Harris-Warrick, 1994), as well as in neurones of other invertebrates, such as the snail *Achatina fulica* (Furukawa and Kobayashi, 1988). However, its specificity is unknown, so the nature of the 5-HT receptors mediating the excitatory effect of 5-HT is still an open issue.

The sensitivity to 5-HT of the X organ neurone somata after axotomy is more consistent with a role for 5-HT as a modulator rather than as a transmitter. In fact, the regional sensitivity of these neurones to 5-HT is different from that to γ-aminobutyric acid (GABA), which is ineffective when tested on organ somata, since sensitivity is confined to the neuropile (García *et al.* 1994) where all synaptic connections to X organ neurones appear to be made (Andrew *et al.* 1978; Glantz *et al.* 1983). This is also consistent with the lack of effect of methysergide on spontaneous synaptic activity in these neurones. Besides a local modulatory role for 5-HT released at the neuropile of the medulla terminalis, it may also have a hormonal action, since this amine has been identified in crayfish haemolymph (Livingstone *et al.* 1980).

Various ionic mechanisms have been described to account for 5-HT-induced excitatory responses. Since no effects of 5-HT were detected on the amplitude or the duration of individual spikes of X organ cells, no action on fast voltage-dependent currents can be assumed. A host of slow inward currents have been described when 5-HT is introduced into a number of preparations, such as invertebrate neurones (Gerschenfeld and Paupardin-Trisch, 1974; Deterre *et al.* 1981; Boyle *et al.* 1984; Furukawa and Kobayashi, 1988; Levitan and Levitan, 1988; Baxter and Byrne, 1989; Harris-Warrick and Marder, 1991; Price and Goldberg, 1993; Pellmar, 1984) and vertebrate neurones (Andrade and Chaput, 1991; Colino and Halliwell, 1987; Hounsgaard and Kiehn, 1989; Pape and McCormick, 1989; Wallén *et al.* 1989; Stefani *et al.* 1990; Anwyl, 1992). 5-HT-induced depolarisations have also been reported as a result of inhibition of an electrogenic $Na^+$ pump (Catarsi *et al.* 1993). Of particular interest for the results reported in this paper is the induction of bursting activity by 5-HT, which has been reported in a variety of systems; 5-HT increases bursting activity in the AB/PD neurones of the stomatogastric ganglion of the crab *Cancer borealis* (Zhang and Harris-Warrick, 1994). In the same ganglion, 5-HT induces plateau potentials in the dorsal gastric (DG) motoneurone; this effect is achieved by the combination of an enhancement of a hyperpolarisation-activated inward current and a reduction of a $Ca^{2+}$-dependent outward current (Kiehn and Harris-Warrick, 1992).

Another effect, similar to the one reported here, is that 5-HT augments bursting pacemaker activity in the PON neurones of the suboesophageal ganglion of the snail *Achatina fulica*; both the depolarising and the post-burst hyperpolarising phases are enhanced by 5-HT. The ionic mechanism responsible for this effect is the enhancement of a $Na^+$-dependent negative slope resistance region (NSR) in the steady-state current–voltage relationship and the induction of a $Ca^{2+}$-dependent NSR (Funase *et al.* 1993). This effect may be of particular relevance to our results because, as proposed by Onetti *et al.* (1990), the bursting activity in X organ cells is related to an NSR mediated by $Na^+$ and modulated by intracellular $Ca^{2+}$. Given the voltage-dependence of the inward current elicited by 5-HT in our experiments, in conjunction with the evoked increase of input resistance, the reduction of a $K^+$ current appears to be a likely mechanism for the action of 5-HT. However, to establish this, a thorough search will be necessary, bearing in mind that more than one ionic mechanism may underlie the depolarising and the burst-generating responses in these neurones.

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