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THE MONOSYNAPTIC CONNECTIONS BETWEEN THE SEROTONIN-CONTAINING LP3 AND RPas NEURONES IN *HELIX* ARE SEROTONERGIC

BY ÁGNES VEHOVSZKY*,†, GYÖRGY KEMENES AND KATALIN S.-RÓZSA

Balaton Limnological Research Institute of the Hungarian Academy of Sciences, Tihany, Hungary, H-8237

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Summary

1. Monosynaptic connections between a giant serotonin-containing neurone and its serotonin-containing followers in the snail *Helix pomatia* were studied in isolated preparations of the central nervous system. The presynaptic cell was the LP3 cell in the left pedal ganglion and the followers were the RPa_s cells in the right parietal ganglion.

2. The light microscopical morphology of the pre- and postsynaptic cells was investigated in whole-mount preparations following intracellular injection with a nickel–lysine complex. Axons from LP3 project towards the cerebral and suboesophageal ganglia or run in peripheral nerves which innervate feeding muscles and the foot. The follower neurones (RPa_s) project into nerves which innervate the heart and other visceral organs. The axons of LP3 and the RPa_s cells run in close proximity in the visceral ganglia.

3. Ionophoretic application of serotonin onto the membrane of the postsynaptic RPa_s neurones mimicked the excitatory effect of the stimulation of the presynaptic LP3. Both the synaptic transmission between LP3 and its followers and the excitatory effect of exogenously applied serotonin on the RPa_s neurones decreased or were blocked in the presence of serotonin or the serotonin antagonists tryptamine, bufotenine, 7-methyltryptamine and MDL 72222-EFO2 in the bath. From this, we conclude that the excitatory neurotransmitter between LP3 and followers is serotonin and not some other neurotransmitter which might coexist with serotonin in LP3.

4. The serotonergic monosynaptic connections between LP3 and its right parietal followers may play a role in a variety of serotonin-mediated physiological and behavioural responses, forming a link between feeding, locomotion and visceral functions.

Introduction

In a number of molluscan species injection of the serotonin analogues 5,6 or 5,7-dihydroxytryptamine (5,6- or 5,7-DHT) into intact animals results in selective

*To whom reprint requests should be addressed.
†Present address: Department of Biology, University of York, Heslington, York YO1 5DD.

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accumulation of dark pigment granules in the somata of serotonin-containing neurones (*Helix*: Balaban *et al.* 1985; S.-Rózsa *et al.* 1986; *Aplysia*: Jahan-Parwar *et al.* 1987; *Lymnaea*: Kemenes *et al.* 1989). However, the pharmacological sensitivity and membrane properties of the pigment-labelled neurones are not affected by the presence of these pigment granules in the soma. The serotonin (5-HT) content of the axonal elements is only transiently depleted for a few days about 2 weeks after injection with 5,6- or 5,7-DHT and during these few days the ability of the serotonergic neurones to activate follower cells is abolished (Gadotti *et al.* 1986; Kemenes and S.-Rózsa, 1987; Jahan-Parwar *et al.* 1987; Vehovszky *et al.* 1988; Kemenes *et al.* 1988, 1990). Following recovery from the transient effects of these neurotoxins, the 5-HT neurones containing 5,6- or 5,7-DHT-induced pigment are easily visible and so they make good targets for electrophysiological investigations in both isolated and semi-intact preparations.

Using this novel approach, we have previously described monosynaptic connections between pigment-labelled neurones in the snail *Helix pomatia* (Vehovszky *et al.* 1989). The excitatory synaptic effects between the giant left pedal LP3 cell and its followers in the right parietal ganglion (RPa_5 cells) are chemically mediated. The presence of dark pigment in both LP3 and its followers in 5,6-DHT-treated snails suggested that these neurones contained serotonin (S.-Rózsa *et al.* 1986). This was later supported by immunocytochemistry (Hernádi *et al.* 1989). However, evidence for the serotonergic nature of neurotransmission between LP3 and the follower cells was lacking. Without this it could not be excluded that LP3 uses not 5-HT but a different neurotransmitter which might coexist with serotonin in the cell body.

In the present paper we provide pharmacological evidence to show that the monosynaptic connections between the pedal LP3 cell and its followers in the right parietal ganglion are serotonergic. In addition, we give a morphological description of the axonal branching pattern of LP3 and the follower cells, and suggest that through their widespread connections these neurones have regulatory roles in a variety of serotonergic functions in *Helix*.

Materials and methods

Adult *Helix pomatia* L. were collected locally on the Tihany peninsula (Hungary). The snails were maintained in an active state in the laboratory for several weeks prior to the experiments.

We used conventional microelectrophysiological techniques to make pairwise intracellular recordings from LP3 and its follower cells (RPa_5 neurones) in isolated preparations of the central nervous system (Vehovszky *et al.* 1989). The experimental chamber was perfused with normal *Helix* saline: NaCl, 80 mmol l^{-1}; KCl, 4 mmol l^{-1}; $CaCl_2.2H_2O$, 10 mmol l^{-1}; $MgCl_2.6H_2O$, 5 mmol l^{-1}; Tris, 4 mmol l^{-1} (pH 7.4).

For local application, serotonin was ejected ionophoretically onto the surface of the cell body of neurones. The microelectrode used for application was filled with a 0.1 mol l^{-1} solution of serotonin creatinine sulphate (Sigma) and the tip was positioned adjacent to the surface of the cell body. Ionophoretic application of 5-HT was carried out by passing 0.5–1 s, 5–20 nA positive current pulses through the electrode. To prevent the possible

desensitizing effect of the transmitter leaking from the tip of the electrode, prior to and between the applications a constant 0.5 nA negative retention current was used.

In each test we first injected depolarizing current into LP3 to evoke reliable postsynaptic responses in the followers. 10 s after the test with presynaptic activation we applied 5-HT ionophoretically onto the same postsynaptic RPa_s neurone. Three replicate presynaptic stimuli and 5-HT applications were used to establish control responses in normal saline. The same procedure was then repeated in the presence of 10^{-7} and 10^{-6} mol l^{-1} 5-HT or various serotonin antagonists in the bath. Within the same experiment both the duration and frequency of the presynaptic bursts and the ionophoretic current used to apply 5-HT were the same before and after perfusion with antagonists.

The following drugs were used: MDL 72222-EF02 (Merrell), cinanserin HCl (Squibb), 7-methyltryptamine (Koch Light), tryptamine hydrochloride (Sigma), bufotenine-hydrogenoxalate (Fluka AG). Bufotenine, tryptamine and 7-methyltryptamine are known serotonin antagonists in the gastropod central nervous system (reviewed by Walker, 1986). MDL 72222-EFO2 and cinanserin (known serotonin antagonists in vertebrates: Richardson and Engel, 1986; Fozard, 1987) were also found to be selective 5-HT receptor antagonists on *Helix* neurones (Vehovszky and Walker, 1991).

The drugs were tested in the concentration range from 10^{-7} to 10^{-4} mol l^{-1} (made up in normal saline). Prior to testing, 5 min rest periods allowed the drugs to equilibrate in the perfusion chamber. The reversibility of the antagonist effect was tested by washing out with normal saline. The effect of each drug on the synaptic and 5-HT responses was tested on 5–8 different preparations. To exclude changes in the postsynaptic response due to changes in the membrane potential, we set the membrane potential of the parietal neurones to −100 mV prior to testing either the synaptic or the serotonin response.

After electrophysiological experiments the LP3 and RPa_s neurones were filled intracellularly with Ni^{2+}-lysine solution (Fredman, 1987). To develop the chemical reaction for staining, rubeanic acid solution was used according to the method employed by Quicke and Brace (1979). After dehydration in graded alcohols and clearing in methyl salicylate, the suboesophageal ganglia were mounted on slides in Canada balsam. Whole-mount preparations were photographed or stained cells were traced by using a drawing apparatus attached to a stereomicroscope.

Results

The axonal morphology of LP3 and its follower cells

The LP3 neurone is located on the dorsal surface of the medio-rostral lobe of the left pedal ganglion (S.-Rózsa and Logunov, 1981; Vehovszky *et al.* 1989 and Fig. 1). The cell body has a diameter of 100–120 μm and often contains a yellowish pigment granule.

The LP3 cell has a pseudo-bipolar shape with a thick axon trunk dividing into two main branches very close to the soma (Fig. 1). One of the main branches runs in the pleuro-pedal connective, sending collaterals to the cerebro-pedal connective and the pedal neuropile. This latter branch leaves the pedal ganglion through pedal nerves V and VI. The fine branch in the pleuro-pedal connective further divides into two collaterals, one

running in the very thin pharyngeal retractor muscle nerve (n.m.ph.: nervus musculi retractoris pharyngealis, Schmalz, 1914), the other projecting to the visceral and right parietal ganglia (Fig. 1).

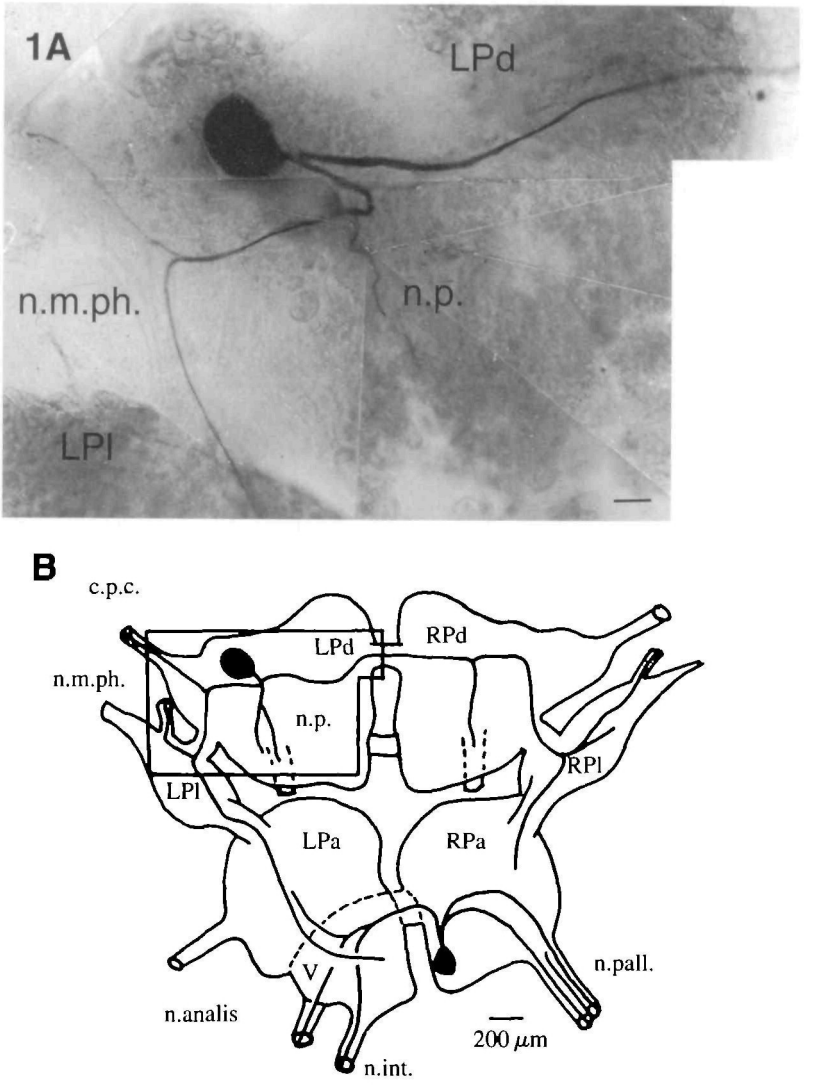

Fig. 1. Axonal branching of LP3 and the follower RPa_5 cells in the central nervous system of *Helix pomatia* (dorsal view). (A) The cell body and the main axon branches of an LP3 cell (shown in the rectangle in B) filled intracellularly with Ni^{2+} lysine (cleared whole-mount preparation). Scale bar, 100 μm. (B) A reconstruction of the axonal branching pattern of LP3 (in the rectangle) and the RPa_5 cells (only one of them is shown in the right parietal ganglion) based on drawings from whole-mount preparations (N=19 for LP3, N=11 for RPa_5 cells). LPd, RPd, left and right pedal ganglion; LPl, RPl, left and right pleural ganglion; LPa, RPa, left and right parietal ganglion; V, visceral ganglion; c.p.c., cerebro-pedal connective; n.p., pedal nerve; n.analis, anal nerve; n.int., intestinal nerve; n.pall., right pallial nerve; n.m.ph., (nervus musculi retractoris pharyngealis), pharyngeal muscle retractor nerve.

The other branch of the main axon trunk enters the contralateral pedal ganglion where its axonal branching pattern is virtually symmetrical with that on the ipsilateral side. The two main branches of the LP3 axon run through the neuropile of each of the main ganglia and seem to connect all the ganglia in the suboesophageal complex in a ring-like manner (Fig. 1B).

The follower cells of LP3 in the right parietal ganglion are located in a single cluster of 4–5 cells on the dorsal side of the ganglion and close to its medial border. Besides the similarities in their electrophysiological properties, synaptic inputs and chemical sensitivity, they are also similar in their morphology. Their somata have diameters of 100–130 μm and a similar unipolar shape. They send axonal collaterals through peripheral nerves (right pallial, anal and intestinal nerves) as well as to the visceral and left parietal ganglia (Fig. 1B). Because of the morphological and physiological similarities of these neurones they were treated as members of a homogeneous cluster and named RPa_S (serotonin-containing right parietal cells). Axon branches from LP3 and the RPa_S cells run in close proximity in the visceral ganglion (Fig. 1B).

Testing the serotonergic nature of the synaptic connection between LP3 and the RPa_S cells

Effect of extracellularly applied serotonin on LP3 and the RPa_S neurones

Serotonin, when applied to the membrane of the RPa_S cell body from a pipette, produced an excitatory response very similar to that evoked by stimulating LP3 (Fig. 2A,B). After hyperpolarizing the membrane of the RPa_S cells, the 5-HT-evoked depolarizing effect was similar to the summated excitatory postsynaptic potentials (EPSPs) following a series of action potentials (APs) in LP3 (Fig. 2C). Serotonin also depolarized LP3 when applied locally to its soma membrane. The depolarization was sufficient to generate a burst of spikes in LP3, which then excited the RPa_S followers (Fig. 2B). The similarity of the synaptic and serotonin-evoked excitation recorded in the RPa_S neurones allowed us to compare the effects of drugs on both types of responses.

To test the serotonergic nature of the synaptic connection between LP3 and the RPa_S neurones, we first compared the desensitizing effect of bath application of serotonin on the responses of the RPa_S neurones to LP3 stimulation and somatic ionophoresis of serotonin. Low concentrations (10^{-7} and 10^{-6} mol l^{-1}) of serotonin in the bath reduced or inhibited the responses of the RPa_S neurones to ionophoretic serotonin (Fig. 3). The same concentration of bath-applied serotonin also reduced the size of the compound EPSP in the RPa_S cells, but did not prevent the presynaptic spike discharge caused by intracellular electrical stimulation of the LP3 neurone (Fig. 3B,C).

Effect of serotonin antagonists on the responses of RPa_S neurones

In this second series of tests we examined the synaptic and serotonin-evoked responses of the RPa_S cells in the presence of known serotonin antagonist drugs.

All the drugs except tryptamine had some initial general excitatory effect on both the pre- and postsynaptic neurones. The general excitability of the cells increased in the presence of the drugs, resulting in more frequent firing and an increased number of

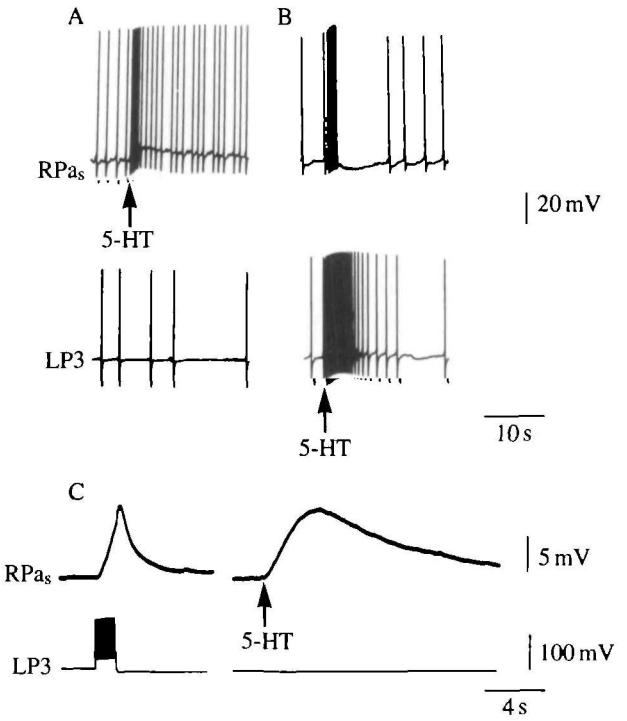

Fig. 2. Responses evoked by exogenously applied serotonin (5-HT) on LP3 and the RPa_s cells. The membrane of RPa_s was hyperpolarized to −100 mV during the tests. (A) Locally applied 5-HT excited the RPa_s cell (arrow indicates the start of the 1 s ionophoretic current pulse) but had no effect on LP3 (lower trace). (B) 5-HT, applied locally to the LP3 soma membrane (lower trace) excited this neurone, which caused synaptic excitation of the postsynaptic RPa_s cell. (C) A burst of action potentials in LP3 evoked a summated postsynaptic response in an RPa_s cell (left). This effect was mimicked by ionophoretic application of 5-HT onto the RPa_s neurone (right).

spontaneous synaptic potentials (see Figs 6, 7). However, all the drugs tested antagonized the effect of ionophoretically applied serotonin on the RPa_s cells, and all but one antagonized the synaptic response as well. This was found in a minimum of four replicate preparations for each drug.

Tryptamine (10^{-5} mol l^{-1}) abolished the serotonin response (Fig. 4B), while 10^{-4} mol l^{-1} tryptamine blocked both the synaptically evoked and serotonin responses (Fig. 4C). The blocking effect of tryptamine was reversible (Fig. 4D).

The serotonin analogue 7-methyltryptamine had a weaker effect than tryptamine but still blocked both the synaptic and the 5-HT-evoked responses (Fig. 5). After washing, the summated EPSPs reappeared but the serotonin response was only partially restored (Fig. 5D).

Bufotenine had the strongest general excitatory effect on both the LP3 and RPa_s neurones. In the presence of 10^{-6} mol l^{-1} bufotenine the spontaneous activity of LP3 and the number and amplitude of spontaneous EPSPs from other neurones seen in RPa_s cells both increased, as did the amplitude of excitatory potentials in the RPa_s neurones caused

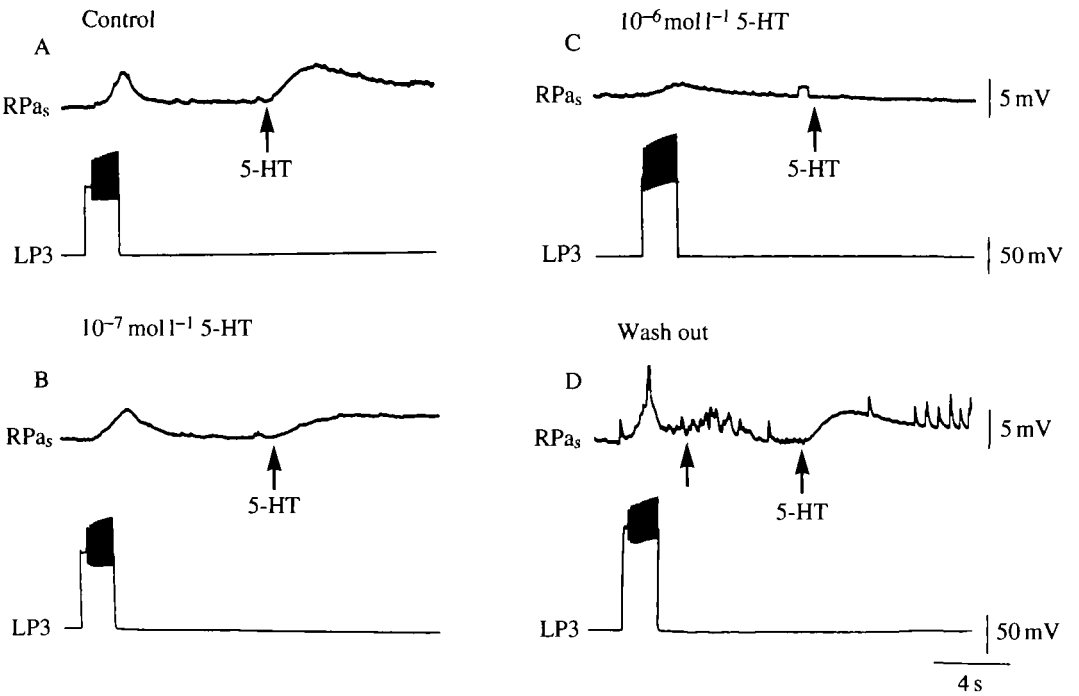

Fig. 3. Responses of RPa_5 neurones in the presence of serotonin (5-HT) in the bath. The membrane of RPa_5 was hyperpolarized to -100 mV during the tests. (A) In normal saline, an electrically evoked burst of spikes in the presynaptic LP3 neurone evoked an excitatory response (summated EPSP) in the RPa_5 follower cell, similar to the effect of serotonin applied locally onto RPa_5 (arrow). (B) In the presence of 10^{-7} mol l^{-1} serotonin in the bath, the amplitude of the summated EPSPs as well as the depolarization evoked by local 5-HT application decreased. (C) In the presence of 10^{-6} mol l^{-1} serotonin in the bath, the amplitude of the summated EPSPs in RPa_5 was much smaller than in the control and the 5-HT response was completely abolished. (D) After washing out the serotonin from the bath, the responses of RPa_5 to activity in LP3 and to locally applied serotonin increased again. Moreover, the ongoing PSPs in RPa_5 (evident in all the traces of Fig. 3) were clearly enhanced in amplitude.

by LP3 activity (Fig. 6). In contrast, the depolarization evoked by locally applied serotonin decreased at the same time (Fig. 6B). After a longer time [or in a higher concentration of bufotenine (10^{-5} mol l^{-1})] both the synaptically and pharmacologically evoked responses were blocked (Fig. 6B). After washing, the summated EPSPs reappeared again, but the depolarization evoked by serotonin was only partially restored (Fig. 6C).

10^{-5} mol l^{-1} MDL 72222-EFO2 had a general enhancing effect on the EPSPs similar to that of bufotenine. At a higher concentration (5×10^{-5} mol l^{-1}), this drug had a blocking effect on both the synaptic and 5-HT-evoked responses, which was irreversible (Fig. 7A), unlike that of the previous drugs.

Cinanserin (5×10^{-5} mol l^{-1}) strongly and irreversibly blocked the 5-HT-evoked response of the RPa_5 cells, but had no effect on the synaptic response evoked by presynaptic discharges of LP3 (Fig. 8).

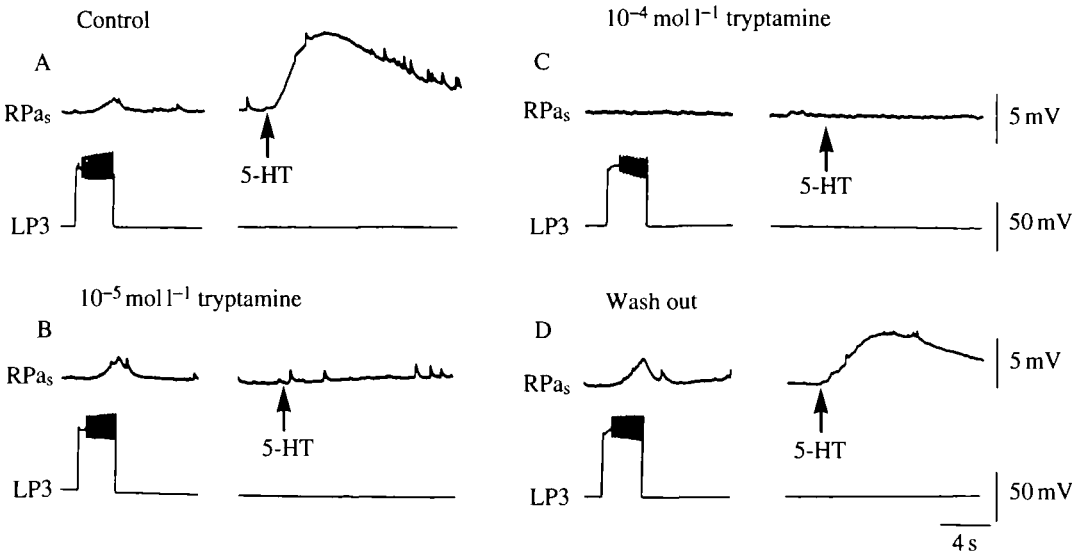

Fig. 4. The effect of tryptamine on the synaptic and serotonin-evoked responses recorded in the RPa_S neurones. The membrane of RPa_S was hyperpolarized to −100 mV during the tests. (A) In normal solution (Control), firing of the presynaptic LP3 neurone evoked summated EPSPs, and local application of serotonin onto RPa_S evoked depolarization. (B) Tryptamine (10^{-5} mol l^{-1}) blocked only the serotonin response. (C) 10^{-4} mol l^{-1} tryptamine inhibited both types of responses in the postsynaptic cell, while intracellular current injection into the LP3 neurone still evoked a presynaptic discharge. (D) Both the synaptically and the 5-HT-evoked responses were restored after washing out with normal saline.

Discussion

We have shown that the monosynaptic excitatory connection from the serotonin-containing left pedal LP3 neurone to its right parietal followers in *Helix pomatia* (described by Vehovszky *et al.* 1989) is mediated by serotonergic mechanisms. This is important because a variety of transmitters can coexist in neurones, and the observation that a cell contains serotonin does not necessarily mean that it uses it as a transmitter at a particular synapse.

Serotonin (5-HT) applied locally onto the cell body of RPa_S neurones depolarized these cells (Figs 2–8) and so mimicked the excitatory effect of the electrical stimulation of the presynaptic LP3 cell. The desensitizing effect of serotonin in the bath on both the synaptically evoked and the serotonin responses (Fig. 3) suggests that the same receptors are involved in the mediation of both types of responses. Furthermore, the excitatory effect of locally applied 5-HT and the synaptic responses evoked by presynaptic stimulation could be similarly blocked by bath application of serotonin antagonists at concentrations of 10^{-6}–10^{-4} mol l^{-1}. All but one of the drugs also had a general excitatory effect on both LP3 and the RPa_S cells. We suggest that this may be due to an initial serotonin agonist effect of the bath-applied antagonists (Vehovszky and Walker, 1991; Walker, 1985, 1986).

In *Helix aspersa*, the parietal F4, F5 and F6 cells (Kerkut *et al.* 1975) are homologous

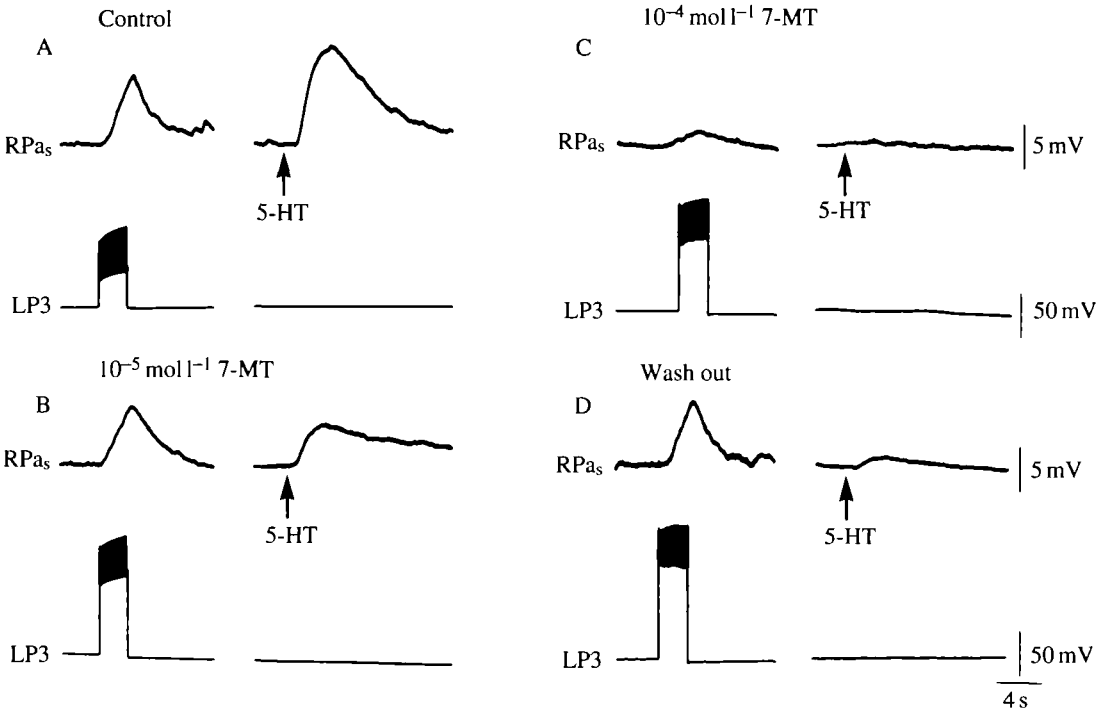

Fig. 5. The effect of 7-methyltryptamine (7-MT) on the synaptic and serotonin-evoked responses recorded in the RPa_S neurones. The membrane of RPa_S was hyperpolarized to −100 mV during the tests. (A) In normal solution (Control), firing of the LP3 neurone evoked summated EPSPs in the RPa_S neurone, similar to the depolarizing response after serotonin application directly onto RPa_S (arrow). (B) In the presence of 10^{-5} mol l^{-1} 7-MT, the serotonin response was decreased. (C) 10^{-4} mol l^{-1} 7-MT decreased the synaptic response and inhibited the depolarizing response after serotonin application. (D) After washing out with normal saline, the synaptic connection was restored, but the serotonin response remained reduced.

with the RPa_S neurones of *Helix pomatia*. Exogenously applied serotonin evoked a Na^+- and Ca^{2+}-dependent depolarization of the *H. aspersa* neurones (Wright and Walker, 1984; Bokisch and Walker, 1986) similar to that seen in the RPa_S cells following either the application of 5-HT or stimulation of LP3 (this study). The serotonin-evoked responses of the F cells were blocked by the application of the same serotonin antagonists that we used (Wright and Walker, 1984; Walker, 1985). In voltage-clamp studies of the *Helix aspersa* neurones, the two-component inward current evoked by serotonin could also be blocked with 7-methyltryptamine (Paupardin-Tritsch *et al.* 1981).

The serotonin analogue tryptamine had a highly selective antagonist effect on the serotonin-evoked excitatory responses (Gerschenfeld and Paupardin-Tritsch, 1974; Vehovszky and Walker, 1991), and in our study proved to be an effective blocker of both the serotonin and synaptically evoked responses of RPa_S neurones.

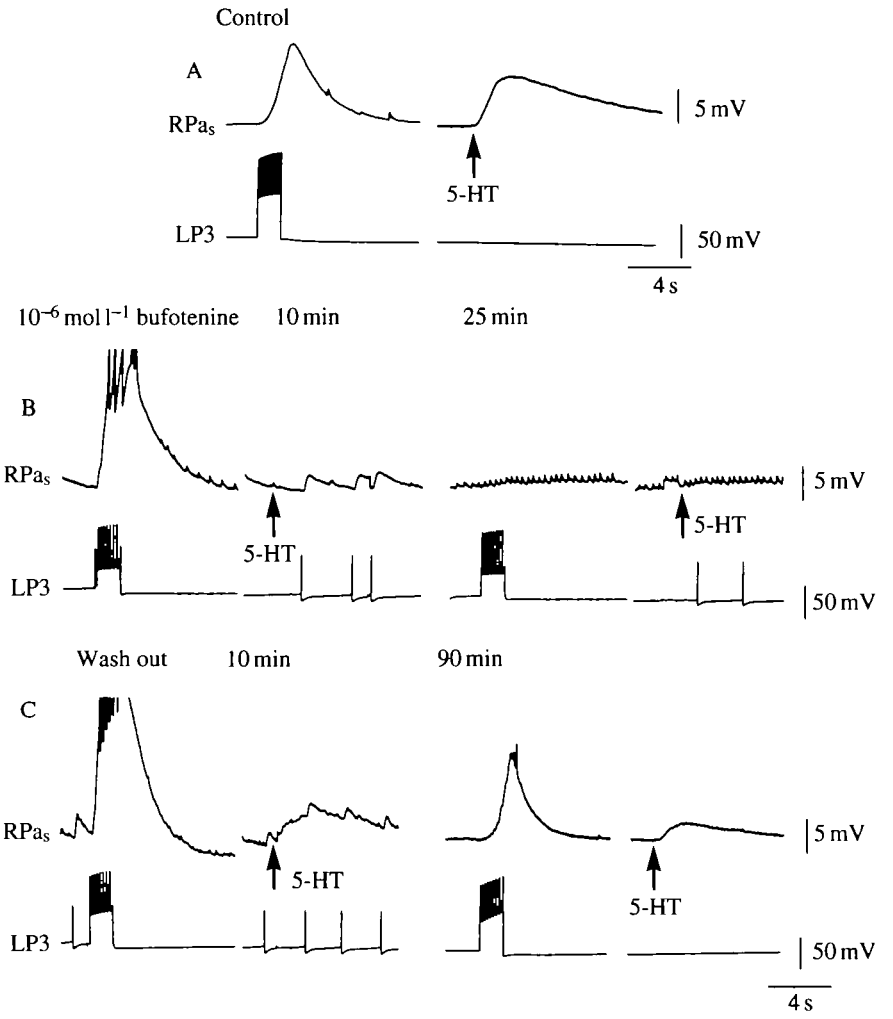

Fig. 6. The effect of bufotenine on the synaptic and serotonin-evoked responses recorded in the RPa_5 neurones. The membrane of RPa_5 was hyperpolarized to -100 mV during the tests. (A) In normal saline, firing of the presynaptic LP3 neurone evoked an excitatory response in the follower neurone similar to the action of serotonin applied locally to RPA_5. (B) 10 min after application, bufotenine (10^{-6} mol l^{-1}) enhanced the summated EPSPs evoked by LP3 discharge, increased the synaptic excitability of both neurones, but inhibited the depolarizing effect of locally applied serotonin (left). After 25 min both the synaptic response to LP3 stimulation and the serotonin response were inhibited, while spontaneously occurring enhanced postsynaptic potentials were still visible (right). (C) Ten minutes after starting to wash out bufotenine, the spontaneous activity of both neurones increased. The enhanced synaptic effect of LP3 discharge on the RPa_5 neurone was restored, but the response to 5-HT was still weaker than in the control (left). Ninety minutes after starting to wash, the spontaneous activity and the postsynaptic excitation were the same as in the control, but the serotonin response was still reduced (right).

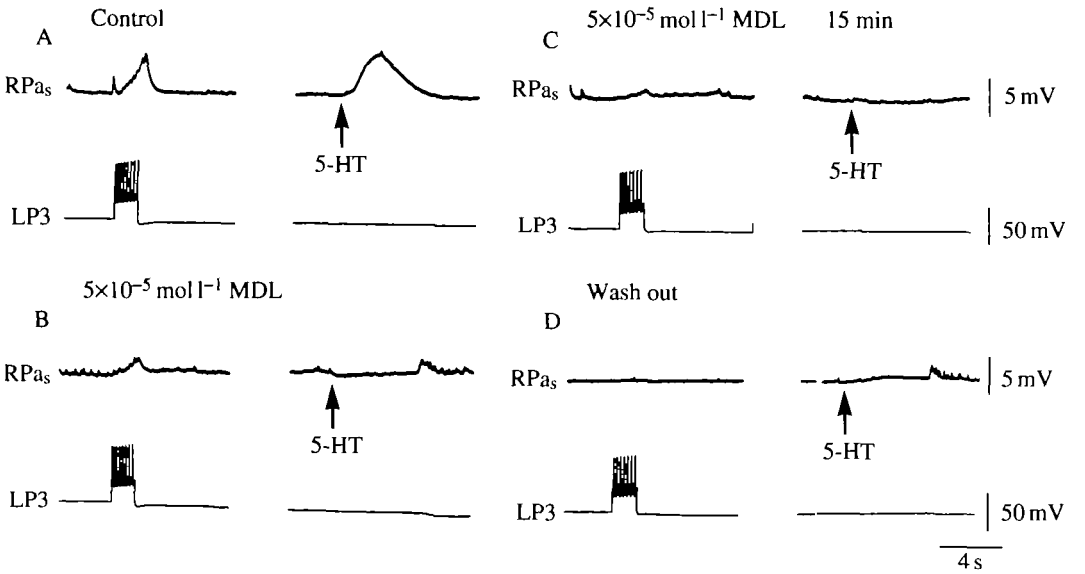

Fig. 7. The effect of MDL 72222-EFO2 (MDL) on the synaptic and serotonin-evoked responses recorded in the RPa_5 neurones. The membrane of RPa_5 was hyperpolarized to −100 mV during the tests. (A) In normal saline, stimulation of the presynaptic LP3 cell evoked summated EPSPs, while serotonin applied locally to RPa_5 evoked depolarization of the RPa_5 cell membrane. (B) MDL (5×10^{-5} mol l^{-1}) reduced the amplitude of EPSPs in the RPa_5 neurone and inhibited the excitatory response evoked by serotonin. (C) 15 min after the application of MDL, neither synaptic nor serotonin-evoked responses could be recorded in the RPa_5 neurone. (D) The effect of this antagonist was irreversible: no responses could be recorded 20 min after washing out with normal saline.

MDL 72222-EFO2 and cinanserin, often used as serotonin antagonists in vertebrates (Richardson and Engel, 1986; Fozard, 1987) are selective but nearly irreversible 5-HT receptor antagonists in *Helix* neurones (Walker and Vehovszky, 1989). MDL 72222-EFO2 had a similarly irreversible antagonist effect on the synaptically evoked responses of right parietal neurones after LP3 stimulation.

The only antagonist which at a concentration higher than 10^{-5} mol l^{-1} inhibited the pharmacological effect of locally applied serotonin but did not affect the synaptic response on the same cell was cinanserin (highest concentration tested 5×10^{-5} mol l^{-1}). However, the threshold concentrations of the other effective antagonists such as MDL 72222-EFO2, tryptamine, 7-methyltryptamine and bufotenine were also higher for blocking the synaptic response than for reducing or abolishing the serotonin-evoked depolarization. This difference could be due to differences in the concentration of the exogenously applied and synaptically released 5-HT but is more likely to be due to the different localization of the postsynaptic and soma receptors, the latter being more accessible for exogenously applied drugs.

The only other identified serotonergic monosynaptic connections in *Helix* are made by the giant serotonergic cells (GSCs) of the cerebral ganglion onto followers in the buccal ganglia. These cells and their connections have homologues in several molluscan species

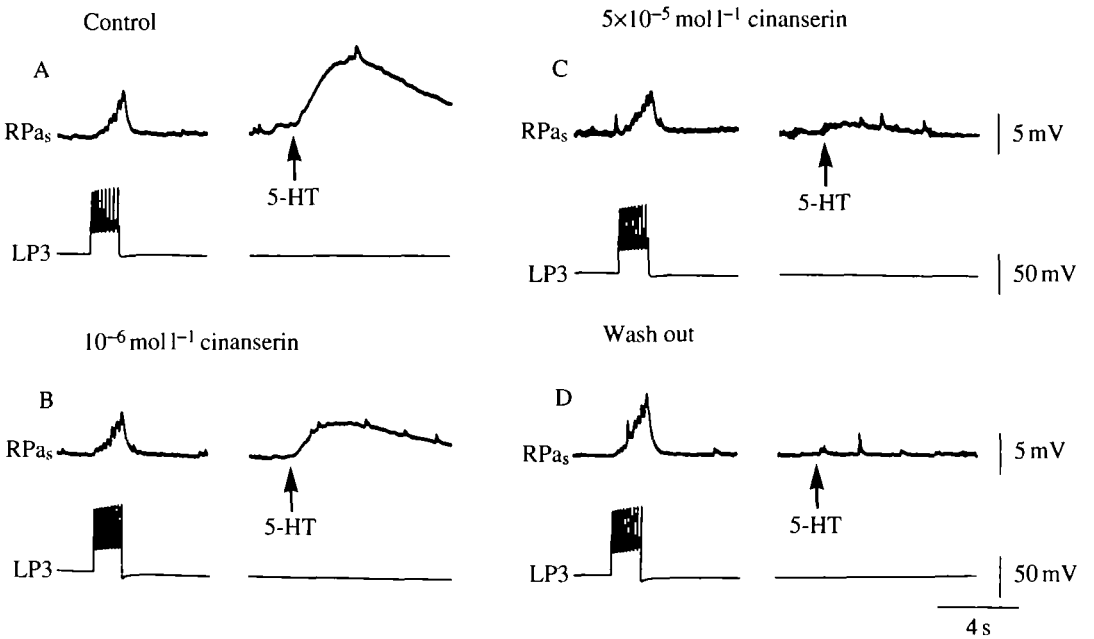

Fig. 8. The effect of cinanserin on the synaptic and serotonin-evoked responses recorded in the RPa_S neurones. The membrane of RPa_S was hyperpolarized to −100 mV during the tests. (A) Control: electrical stimulation of LP3 and application of serotonin onto RPa_S caused depolarizing responses in the RPa_S neurone. (B) 10^{-6} mol l^{-1} cinanserin did not block the synaptic response but reduced the depolarization evoked by serotonin application. (C) Cinanserin (5×10^{-5} mol l^{-1}) abolished the serotonin effect without inhibiting the synaptically evoked EPSPs. (D) After washing with normal saline, the blocking effect of cinanserin proved to be irreversible for the serotonin response.

(reviewed by Pentreath *et al.* 1982). In many respects the pedal LP3 neurone shares common features with the cerebral GSCs in *Helix*. Both cells contain serotonin, have similar electrophysiological characteristics and are excited by serotonin themselves (see Cottrell, 1982). Serotonin applied exogenously to either the RPa_S or the buccal M cells mimicked the effect of the stimulation of the presynaptic cell on both cell types. In addition, in both the RPa_S cells and the buccal follower neurones of the GSCs the fast excitatory effect of 5-HT could be blocked by bath application of bufotenine, tryptamine and 7-methyltryptamine (reviewed by Walker, 1986). However, there is one major difference between the two types of serotonergic connection: in contrast to the buccal followers of the serotonergic GSCs, the RPa_S cells themselves (like their presynaptic LP3 neurone) contain serotonin (Vehovszky *et al.* 1989; Hernádi *et al.* 1989). This means that in their connections with other neurones the RPa_S cells can also act as presynaptic serotonergic cells.

The pedal LP3 cell has a widespread axonal arborization with processes running in peripheral nerves and in the neuropile of central ganglia. The most likely site where the monosynaptic connections between LP3 and the RPa_S cells are made is in the visceral ganglion where the LP3 and RPa_S axons run in close proximity.

The identified monosynaptic connections between the serotonergic pedal LP3 neurone and its serotonin-containing right parietal follower cells could be active in a variety of serotonin-dependent functions, such as feeding, locomotion and excitation of the heart. In this way the pedal LP3 neurone or other LP3-type neurones may link several neural networks controlling different motor functions.

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