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A comparison of fast and slow depolarizations evoked by 5-HT in guinea-pig coeliac ganglion cells *in vitro*

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1 5-Hydroxytryptamine (5-HT) was applied by pressure ejection to coeliac ganglion cells of the guinea-pig maintained *in vitro* and responses measured intracellularly.

2 Cells responded in one of three ways to 5-HT: by (a) a fast, transient depolarization (43%), (b) a fast transient followed by a slow depolarization (biphasic response, 30%) or (c) a slow sustained depolarization (25%).

3 Fast depolarizations (response (a) above)) were graded according to the duration of the ejection pulse. Maximal responses had a mean amplitude of 12 ± 0.8 mV, a duration of 6.4 ± 1.0 s, a latency of 0.4 ± 0.1 s, were associated with a fall in membrane input resistance, increased in amplitude by hyperpolarization and probably mediated by an increased conductance to Na and K. The estimated reversal potential was −22.8 ± 2.4 mV ($n = 14$). The maximal fast response seen in biphasically-responding cells (b) appeared similar to fast response (a).

4 Fast depolarizations (a) showed marked tachyphylaxis and were abolished by superfusion of the ganglion with 5-HT (100 μM). They were reduced in amplitude by tubocurarine (10–100 μM, pIC_{50} 4.4), MDL 72222 (1–5 μM, pIC_{50} 5.8), quipazine (1 μM reduced responses by 65 ± 15%, $n = 3$), ICS 205-930 (1 μM reduced responses by 64 ± 14%, $n = 7$) and metoclopramide (10 μM reduced responses by about 45%), but were unaffected by methysergide (up to 1 μM) or hexamethonium (up to 1 mM).

5 Slow depolarizations (c) varied in amplitude with the duration of the ejection pulse. Maximal responses had a mean amplitude of 6.4 ± 0.7 mV, a duration of 62 ± 6 s, a latency of 3.5 ± 0.8 s and were reduced in amplitude by methysergide (0.1–1 μM, pIC_{50} 6.5) but not by MDL 72222 (1 μM). The maximal slow component in biphasically-responding cells (b) was similar in amplitude and duration to slow response (c), was partially blocked by methysergide (1–5 μM) in 4 of 6 cells and was enhanced by tubocurarine (50 μM) which reduced the fast component.

6 Slow depolarizations (b,c) were associated with either a small reduction or no change in membrane input resistance depending on the cell studied. Hyperpolarization had variable effects on slow depolarization amplitude.

7 It was concluded that the fast, phasic depolarization is mediated by an ionic mechanism and by receptors both of which are distinct from those involved in the slow depolarization. The receptor mediating the fast depolarization is a 5-HT_3 receptor while that mediating the slow depolarization has yet to be identified.

Introduction

Certain sympathetic neurones, e.g. coeliac ganglion cells, may be innervated by 5-hydroxytryptamine (5-HT)-containing nerve fibres (Dun *et al.*, 1984; Ma *et al.*, 1985). Nerve fibres showing a positive 5-HT immunoreactivity surround many guinea-pig coeliac ganglion (g-pcg) cells and, in about 60% of cells in which a slow excitatory postsynaptic potential (e.p.s.p.) is generated by stimulation of the splanchnic nerves (20 Hz for 2 s), the mediator may be 5-HT (Dun *et al.*, 1984). The same neurones also displayed a slow depolarization with similar characteristics to the nerve evoked slow e.p.s.p., when superfused with 5-HT (Dun *et al.*, 1984). Slow e.p.s.ps thought to be mediated by 5-HT have also been described in ganglion cells of the myenteric plexus (Wood & Mayer, 1979).

In other sympathetic ganglia, e.g. rabbit superior cervical ganglia (scg) (Wallis & North, 1978), where

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there is no known 5-HT innervation, 5-HT applied by iontophoresis evokes rapid, phasic depolarizations. The phasic depolarization to 5-HT measured *in vitro* using the sucrose-gap technique is blocked by 5-HT_3 receptor antagonists (Bradley *et al.*, 1986), such as MDL 72222 and ICS 205-930, and by quipazine and metoclopramide (Azami *et al.*, 1985; Round & Wallis, 1987), but not by methysergide or cyproheptadine (Nash *et al.*, 1984).

On the other hand, the responses to g-pcg cells to 5-HT applied by superfusion are reduced in amplitude by cyproheptadine (20–50 μM) and methysergide (10 μM) (Dunn *et al.*, 1984), suggesting that a different kind of 5-HT receptor from that mediating the 5-HT-induced depolarization of the rabbit scg may be involved. The experiments presented here re-investigated the response of g-pcg cells to 5-HT, applied either by superfusion or by pressure ejection from a micropipette in the vicinity of the impaled cell. We demonstrated that two kinds of depolarizing response are evoked in these cells and we attempted to establish whether they are generated by different mechanisms and are mediated via different types of 5-HT receptors.

Methods

Young adult male guinea-pigs (200–300 g) were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.p.). The coeliac-superior mesenteric plexus together with the left greater splanchnic nerves were excised rapidly and transferred to the recording chamber (volume 0.5 ml, see Dun & Ma, 1984). The ganglia were superfused continuously with a Krebs solution of the following composition (mM): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25, NaH_2PO_4 1.2 and glucose 11.5, equilibrated with 95% O_2 and 5% CO_2, and prewarmed to 34°C (see Dun & Ma, 1984; Dun *et al.*, 1984). In experiments in which Na-free medium was used, NaCl, $NaHCO_3$ and NaH_2PO_4 were replaced with an isomolar amount of tris-(hydroxymethyl) aminomethane (Sigma), which was converted to Tris-HCl by titration with 5 N HCl to pH 7.4–7.5. For K-free medium, KCl was omitted from Krebs solution without compensation and for low Cl medium, NaCl was replaced with an isomolar amount of Na isethionate.

Intracellular measurements were obtained from neurones of the left coeliac ganglia by means of fibre-containing glass microelectrodes filled with 3 M potassium acetate, with an impedance of 30–50 MΩ. Depolarizing or hyperpolarizing currents were injected through the recording microelectrode via a bridge circuit of the pre-amplifier (WPI-707A). The left splanchnic nerves were desheathed carefully under a stage microscope and drawn into a suction electrode for orthodromic stimulation. Membrane potential changes were recorded on a Gould Brush pen recorder. The figures were reproduced from tracings of the pen recorder.

5-Hydroxytryptamine (5-HT) was applied to 122 cells (20 ganglia) by pressure ejection (Picospritzer, General Valve Co.) from a micropipette containing 10 mM 5-HT creatinine sulphate and, in a few experiments, 10 mM 5-HT hydrochloride or 10 mM acetylcholine chloride, using a constant pressure (300 kPa) but variable pulse duration. No noticeable difference in 5-HT responses was observed between the two 5-HT salts. In a few experiments, 5-HT in known concentrations dissolved in Krebs solution, was applied to the ganglion by superfusion. Other drugs were dissolved in Krebs solution and applied to the ganglion by superfusion only. The following compounds were used: hexamethonium bromide (May & Baker), tubocurarine chloride (Sigma), MDL 72222 (1αH, 3α,5αH-tropan-3-yl-3,5-dichlorobenzoate methanesulphonate, Merrell Dow), methysergide maleate (Sandoz), metoclopramide monochloride (Beecham), ICS 205-930 hydrochloride ([3α-tropanyl] 1H-indole-3-carboxylic acid ester, Sandoz) and quipazine maleate (Miles Laboratories).

Results

Fast and slow responses to 5-HT

The response of g-pcg to 5-HT was examined only in cells which had a resting membrane potential of at least −45 mV and an action potential, on direct stimulation of the cell, larger than 55 mV. Spontaneously discharging cells were not observed in this ganglion. Around 90% of cells of a total of 122 neurones examined responded to 5-HT with a depolarization. Contrary to the earlier observations with superfusion, g-pcg cells responded frequently to 5-HT, applied by pressure ejection, with a fast, phasic depolarization. In general, the depolarization response was either: (a) fast and transient, (b) fast and transient and followed by a slower, sustained component, or (c) slow and sustained. The criteria on which this categorization of the response was based are explained below.

Figure 1 shows typical fast and slow responses elicited in three different ganglion cells. If the response displayed a rapid rate of depolarization with a rise time of less than 1 s and a duration of 1 to 10 s, it was termed a 'fast' response. If the depolarization displayed a rise time of more than 10 s and a duration of 20 to 100 s., it was termed a 'slow' response.

Some cells depolarized by 5-HT fell into a third category in that the responses were clearly biphasic. These comprised an initial transient depolarization followed by a slower, sustained depolarization,

usually of smaller amplitude (Figure 8). Responses of this kind were termed 'biphasic'.

Examination of the responses to 5-HT in 122 g-pcg cells showed that, in all but 2 cells, the responses could be clearly categorized as fast, slow or biphasic. Thus, 52 of 122 (43%) cells displayed a fast response with no slower component, 31 of 122 (25%) cells displayed a slow response, while 37 of 122 (30%) cells displayed a biphasic response.

Characteristics of the fast response to 5-HT

Figure 1a,b shows fast responses elicited in two different cells. The duration of the ejection pulse required to elicit a fast response was often short (< 50 ms). In cells which were depolarized by 5-HT but which did not discharge action potentials, the amplitude of the evoked depolarization was clearly related to the duration of the ejection pulse; an ejection pulse of

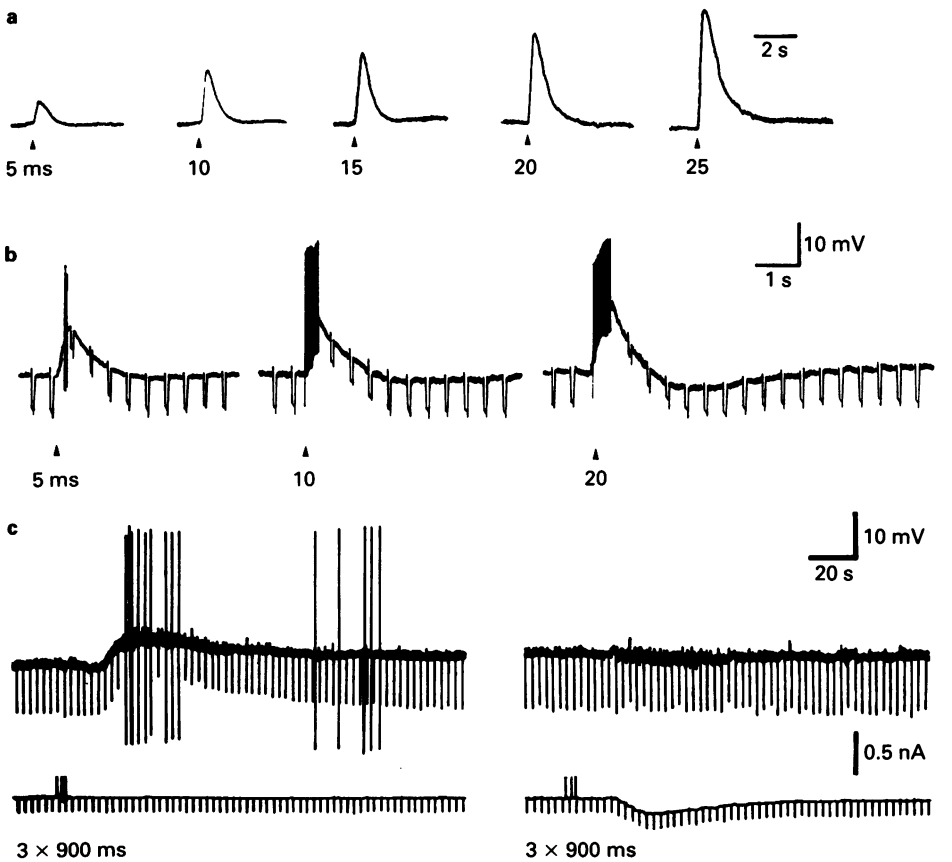

Figure 1 Responses of guinea-pig coeliac ganglion cells to 5-hydroxytryptamine (5-HT) applied by pressure ejection. Chart records of responses from three different cells. Solid triangles (▲) indicate onset of ejection of 5-HT. In this and subsequent figures, the numbers indicate the duration in ms of the ejection. (a) Increased duration of the ejections caused graded depolarizations. (b) The main effect of increasing the duration of the ejection pulse in this cell was to increase the intensity of spike discharge. Note that the depolarization evoked by a 20 ms pulse of 5-HT was followed by a clear after-hyperpolarization. In (b) and (c), the downward deflections in the traces are electrotonic potentials evoked by brief pulses of inward current. The amplitude of the induced hyperpolarization allowed an estimation of the input resistance. (c) Ejection of 5-HT failed to evoke a fast response. Ejection pulses, 900 ms in duration in quick succession, evoked a slow, low amplitude depolarization accompanied by cell discharge (left-hand trace). Note the slower chart speed. When the membrane was voltage-clamped under manual control during application of 5-HT (right-hand trace), the amplitude of the electrotonic potentials was slightly reduced, indicating a small fall in membrane input resistance. The lower trace represents a record of the current. Note that action potentials were attenuated by the frequency response of the chart recorder.

25 ms elicited a maximum response in the experiment shown in Figure 1a. In cells which fired repetitively during depolarization, the main effect of increasing the duration of the ejection pulse was to increase the intensity and duration of the spike discharge. (Figure 1b).

The duration of ejection pulse required to elicit a maximum depolarization was determined for each cell and pooled data were derived from maximal responses (average pulse duration 70 ms). Fast responses had a mean amplitude of 12 ± 0.8 mV (mean ± s.e.mean, here and below, $n = 51$), a duration of 6.4 ± 1.0 s and a latency of 0.4 ± 0.1 s. The transient depolarization was followed, in certain cells, by a small after-hyperpolarization (Figures 1b and 5a). Fast responses were associated with a fall in input membrane resistance, as judged from the decline in the amplitude of the electrotonic potentials evoked by brief pulses of inward current (Figures 1b and 5a). The amplitude of the fast response was decreased on depolarizing the

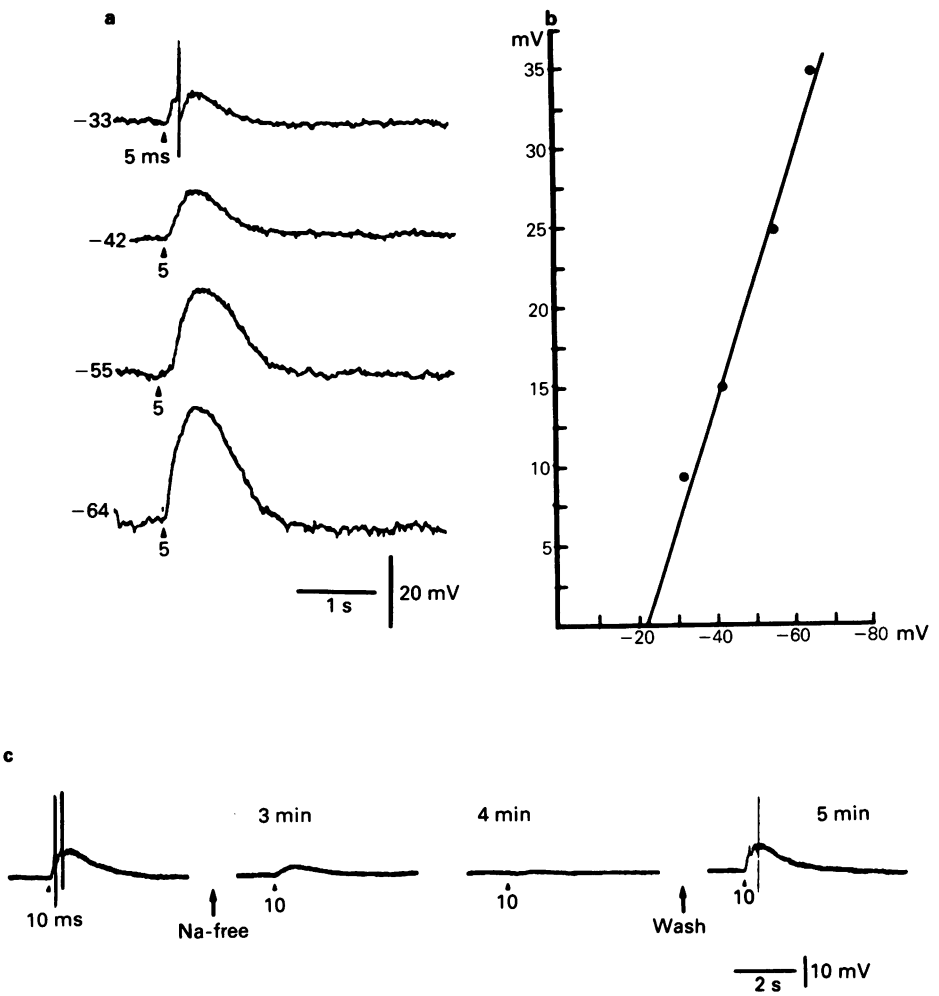

Figure 2 Effect of altering the membrane potential and reducing the external Na ion concentration on fast responses of guinea-pig coelic ganglion cells evoked by 5-hydroxytryptamine (5-HT) applied by pressure ejection (▲). (a) Effect of depolarizing or hyperpolarizing the membrane (membrane potential (mV) indicated to left of traces) on responses. (b) Graph of depolarization amplitude against membrane potential (abscissa scale). Extrapolation of a line fitted by eye indicated a reversal potential of −22 mV. Resting membrane potential was −55 mV. (c) In another cell superfusion of a sodium-free medium for 3 min reduced, and superfusion for 4 min abolished, the response. Washing for 5 min with Krebs solution led to recovery of the response.

membrane by current passage through the recording electrode and increased on hyperpolarizing the membrane (Figure 2). There was a linear relationship between response amplitude and membrane potential (Figure 2b); the reversal potential estimated by extrapolating the line fitted to the points was −22 mV. In 14 cells the mean reversal potential for the fast response was -22.8 ± 2.4 mV. Fast responses were abolished (Figure 2c) or greatly reduced in amplitude in a solution in which all sodium salts had been replaced by Tris HCl. The mean reduction in 4 experiments was $89 \pm 4\%$. A contribution by K ions to the fast response was suggested by the findings that K-free media caused a $25 \pm 10\%$ reduction in fast response amplitude ($n = 5$) and the reversal potential was shifted to a more negative value (not illustrated). A low chloride medium, in which NaCl was replaced by Na isethionate, did not reduce fast response amplitude ($n = 3$).

The fast response in cells without a slower phase of depolarization (category (a)) and that in cells with a slow depolarization (category (b), biphasic response), showed no significant differences. Thus, the initial depolarization of the biphasic response had an amplitude of 15.8 ± 1.2 mV, a duration of 4.0 ± 0.5 s and a latency of 0.4 ± 0.2 s. It was associated with cell discharge in many cells (e.g. Figures 7 and 8), and also with a fall in input membrane resistance (not illustrated). In pooling results from experiments with putative antagonists, responses were categorized as either fast or slow irrespective of whether there was a second potential change in a particular cell.

5-HT receptor mediating the fast response

The fast 5-HT response in g-pcg cells showed marked tachyphylaxis (Figure 3). Repeated applications of 5-HT, using ejection pulses of 10 ms at 1 s intervals did not evoke a sequence of responses (Figure 3a), even when the second and third ejections were delivered at intervals of 4 to 6 s after a preceding pulse (Figure 3b). However, with a longer (approximately 30 s) interval, responses of comparable amplitude to controls were elicited. Superfusion of the ganglion with 5-HT (100 μM) completely blocked the response elicited by an ejection pulse of 10 ms. The response recovered after washing out the 5-HT (Figure 3c). On average, 5-HT (100 μM) depressed response amplitude by $95 \pm 3\%$ ($n = 3$).

That the fast depolarization was not the result of activation of nicotinic acetylcholine receptors was indicated by the effects of hexamethonium. At the high concentration of 1 mM, hexamethonium very quickly eliminated the e.p.s.p. elicited by stimulating the preganglionic nerve, but left the depolarization evoked by 5-HT unaltered (Figure 4b). Tubocurarine (50 μM), on the other hand, which is known to block 5-HT responses at certain sites (Wallis, 1981), reduced both the amplitude of the e.p.s.p. and that of the fast response (Figure 4a). Tubocurarine (1 and 10 μM) reduced the amplitude of fast responses to 5-HT by about 7% and 30%, respectively, while 50 μM reduced responses by $50 \pm 9\%$ ($n = 6$) and 100 μM reduced them by $75 \pm 6\%$ ($n = 6$). The pIC_{50} measured from 5 experiments was about 4.4

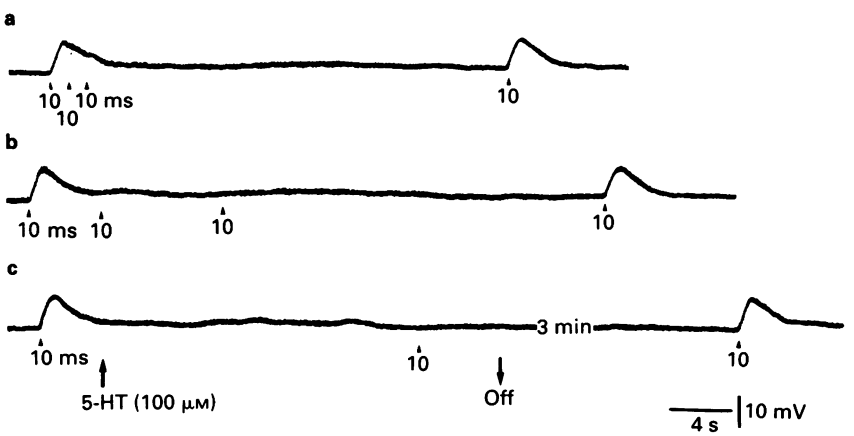

Figure 3 Effect of 5-hydroxytryptamine (5-HT) on the fast responses of a guinea-pig coeliac ganglion cell to 5-HT applied by pressure ejection (▲). In (a) and (b) ejections of 5-HT were made at intervals after the initial application. Note that tachyphylaxis was pronounced; further applications of 5-HT at 1 s intervals (a) had virtually no effect, although the response had recovered after an interval of about 30 s. A second or third application 4–6 s after the initial ejection (b) was ineffective. In (c), 5-HT (100 μM) was applied by superfusion for the period indicated by the arrows following an initial ejection of the indoleamine. Several minutes washing was required before the response recovered to control values.

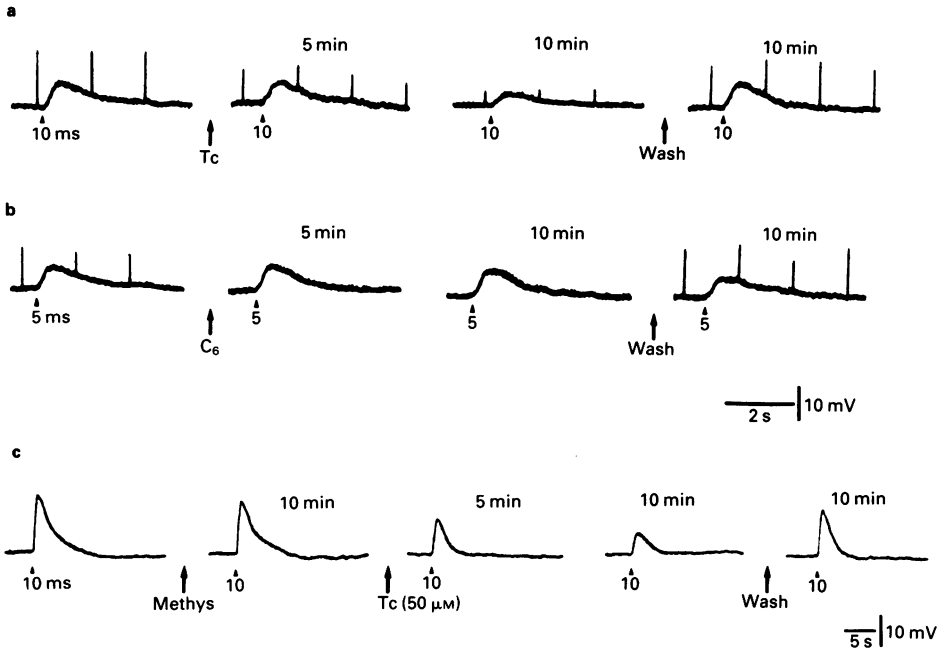

Figure 4 Effect of tubocurarine, hexamethonium and methysergide on e.p.s.ps evoked orthodromically in response to stimulation of the splanchnic nerve and fast depolarizations obtained in response to ejection of 5-hydroxytryptamine (5-HT) (▲) in guinea-pig coeliac ganglion cells. Chart records from intracellular measurements in two different cells. The fast upward pen deflections are e.p.s.ps evoked at a frequency of 0.8 Hz. (a) Tubocurarine (Tc, 50 μM) reduced the amplitude of both the e.p.s.ps and the fast depolarization to 5-HT. These effects were rapidly reversed on washing out the drug. (b) In the same cells, hexamethonium (C_6, 1 mM) rapidly abolished the e.p.s.p. but not the fast depolarization to 5-HT. (c) In another cell, methysergide (Methys 1 μM) was without substantial effect on the fast depolarization evoked by 5-HT, but the response was subsequently reduced by tubocurarine (50 μM).

The fast response to 5-HT was not significantly reduced in the presence of methysergide (1 μM, Figure 4c), although tubocurarine subsequently reduced it considerably. Methysergide (1 μM) had no unequivocal effect on the fast response elicited in 8 out of 9 cells, each from a different ganglion. The depression seen in one cell may have been unrelated to the action of the antagonist, since the response amplitude did not return to control values during washout.

The fast response was, however, considerably reduced in amplitude by the 5-HT_3 receptor antagonist MDL 72222 (Bradley *et al.*, 1986). This action was reversed on washing (Figure 5a). MDL 72222 (1 and 5 μM) reduced the amplitude of the fast response in 6 cells by 33 ± 12% and in 4 cells by 50 ± 14%, respectively. The pIC_{50} measured in 3 cells was about 5.8. The nicotinic receptor-mediated e.p.s.p. evoked by orthodromic stimulated was unaffected (not illustrated).

Fast responses to 5-HT were also substantially reduced by quipazine (1 μM) (Figures 5b and 6a) and by ICS 205-930 (1 μM, Figure 6b). In the cell the responses of which are shown in Figure 5b, quipazine itself appeared to produce some membrane depolarization. In 3 cells, quipazine (1 μM) depressed the amplitude of the fast response by 65 ± 15% and ICS 205-930 (1 μM) reduced it by 64 ± 14% ($n = 7$). The selectivity of the 5-HT_3 receptor antagonists was established by testing their effect on the depolarization evoked by ejection of acetylcholine. Acetylcholine potentials were unaffected by quipazine (1 μM), ICS 205-930 (1 μM) (Figure 6a,b) or MDL 72222 (1 μM). Metoclopramide antagonized the fast response to 5-HT, but was less effective than MDL 72222 or quipazine. At 1 μM, it reduced the amplitude of the fast response by about 15% (2 cells) and at 10 μM by about 45%.

Characteristics of the slow response to 5-HT

Other cells, as illustrated in Figure 1c, responded to 5-HT with a much slower, more sustained depolarization. These slow depolarizations were often accom-

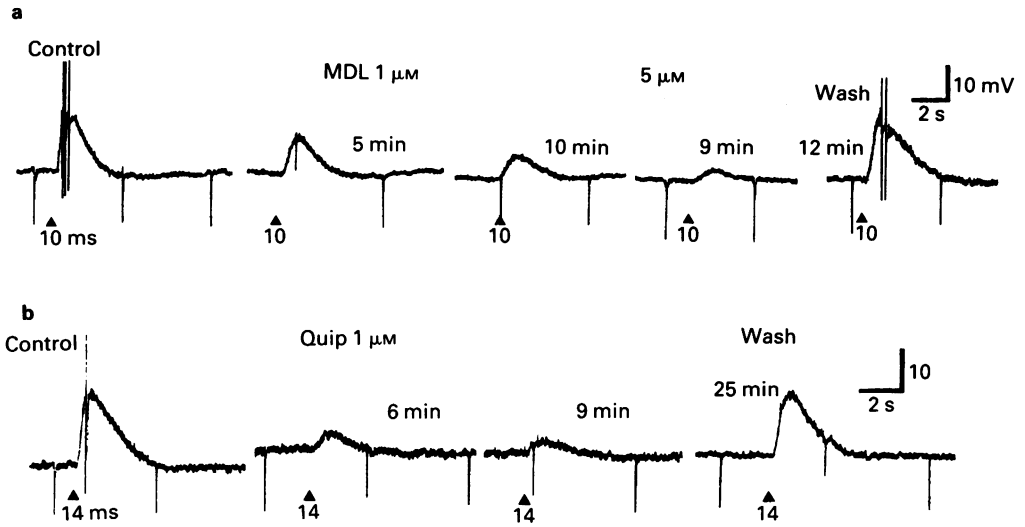

Figure 5 Effect of MDL 72222 and quipazine on the fast depolarization evoked by 5-hydroxytryptamine (5-HT) (▲) in guinea-pig coeliac ganglion cells. Chart records from two different cells. Downward deflections of the trace are electrotonic potentials evoked by brief pulses of inward current. (a) MDL 72222 (MDL, 1 μM) substantially reduced the response to 5-HT over the course of 10 min. A higher concentration (5 μM) further reduced the amplitude of the response. Recovery was seen on washing out the antagonist. (b) Quipazine (Quip, 1 μM) also caused a substantial reduction in responses to 5-HT, an effect that could be largely reversed on washing.

panied by sporadic action potential discharge. Among 122 g-pcg cells, 31 showed slow depolarizations to 5-HT with no sign of a fast transient response (category (c)). In a small minority of cells (<10%), repeated ejection pulses of 900 ms were required to evoke a maximum slow response (Figure 1c).

The duration of the ejection pulse required to elicit a maximum depolarization was determined for each cell and pooled data derived from maximal responses (average pulse duration 550 ms). Maximal slow responses had a mean amplitude of 6.4 ± 0.7 mV, a mean duration of 62 ± 6 s, and a mean latency of 3.5 ± 0.8 s (n = 31). Slow responses seemed more labile than fast responses and it was more difficult to elicit responses of constant amplitude. Consequently, quantitative information on slow responses was obtained less readily than on fast responses. The slow component of depolarization in 37 g-pcg cells showing a biphasic response (category (b)) was similar in magnitude (5.8 ± 0.8 mV) and duration (47 ± 6 s) to the slow response recorded in category (c) cells.

Slow responses varied in amplitude with the duration of the ejection pulse of 5-HT. Cell membrane input resistance as judged by the amplitude of the electrotonic potential evoked by brief pulses of inward current was little changed in 3 of 5 cells (Figure 7) or slightly reduced (2 of 5 cells, e.g. Figure 1c). The prolonged time-course of the slow response allowed the membrane voltage to be clamped manually, preventing cell discharge and any effect of membrane depolarization *per se* on input resistance. Under voltage clamp (Figure 1c), the amplitude of the electrotonic potentials was slightly reduced.

Membrane hyperpolarization by current passage through the recording electrode had somewhat variable effects on the amplitude of the slow response. In 6 of 8 cells, there was a definite but variable increase in amplitude, while in 2 cells there was a reduction or no clear change.

The slow response of g-pcg cells to 5-HT was reduced by methysergide; 0.1 and 1 μM methysergide reduced slow response amplitude by 20 ± 12% (n = 4) and 76 ± 15% (n = 5, pIC_{50} = 6.5), respectively. MDL 72222 (1 μM), on the other hand, had no effect on slow response amplitude. Methysergide (1 μM) also reduced the slow component of the biphasic response, which was often associated with vigorous cell discharge (Figure 7). In this cell, the precise amplitude of the fast component was difficult to assess because of action potential discharge, but it appeared little affected by 12 min exposure to methysergide (Figure 7). Spike discharge and the amplitude of the slow depolarization were greatly reduced in the presence of methysergide. These changes were reversed on washing. However, in 2 of 6 biphasically-responding cells, methysergide (1 to 5 μM) did not reduce the amplitude

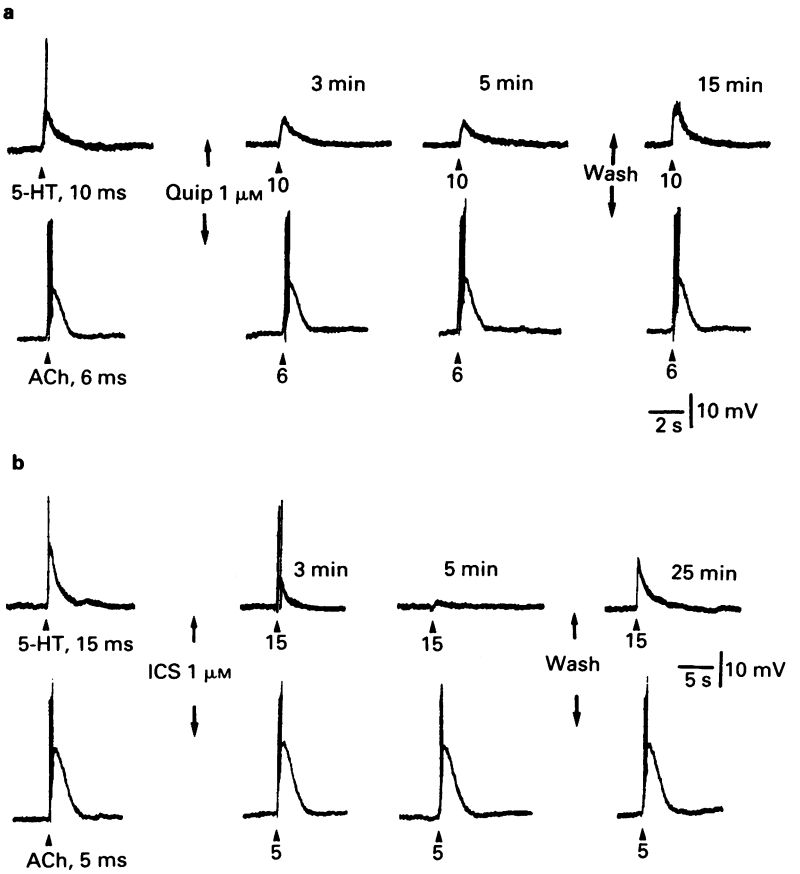

Figure 6 Effect of quipazine and ICS 205-930 on the fast depolarization evoked by 5-hydroxytryptamine (5-HT) (▲) and on the depolarization evoked by acetylcholine (ACh) (▲) in guinea-pig coeliac ganglion cells. Chart records from four different cells. (a) Quipazine (Quip 1 μM) reduced the response to 5-HT but had no effect on responses to ACh. (b) ICS 205-930 (ICS 1 μM) reduced the response to 5-HT, substantial recovery being seen on washing out the antagonist. ACh potentials were unaffected by ICS 205-930.

of the slow depolarization. Tubocurarine (50 μM) did not reduce the amplitude of the slow component of the biphasic response but enhanced it, while attenuating the fast response (Figure 8). In this cell, the effect of superfusing the antagonist for 5 min was to suppress action potential discharge during the fast response and enhance slow response amplitude (Figure 8). After 10 min superfusion, the initial component had largely disappeared and the residual response was a large, slow depolarization. These changes were reversed on washing. In 3 cells, the slow component of the biphasic response was potentiated by 46 ± 18% in the presence of tubocurarine (50 μM). A higher concentration (100 μM) reduced the amplitude of the slow component in 3 of 4 cells, the mean reduction in these 3 cells being 35 ± 17%.

The effect of superfusing 5-HT (100 μM) was tested in 15 cells. In 10 cells, 5-HT evoked a sustained, low amplitude depolarization which ranged in amplitude from 2.5–9 mV, but no unequivocal phasic response. In 4 cells, 5-HT evoked an apparent hyperpolarization of low amplitude (2.5–7 mV), while 1 cell showed no response. There was no clear relationship between the nature of the response to superfused 5-HT evoked from a particular cell and the nature of the response to 5-HT applied by pressure ejection, whether a fast, slow or biphasic response. Responses to superfusion of 5-HT were not investigated systematically as this had been the object of a study by Dun *et al.* (1984).

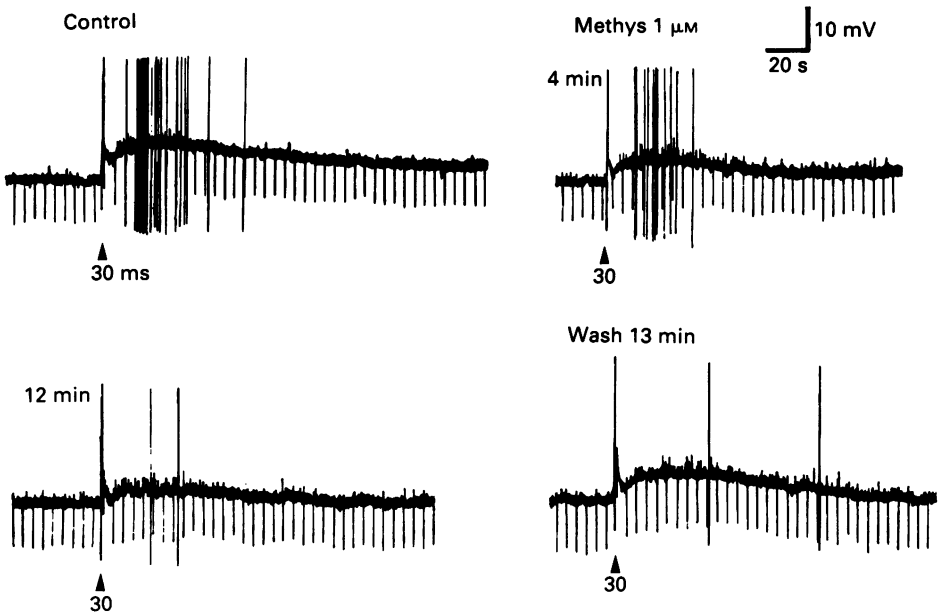

Figure 7 Effect of methysergide (Methys) on the fast and slow depolarizations of a guinea-pig coeliac ganglion cell evoked by 5-hydroxytryptamine (5-HT) (▲). Downward deflections of the trace are electrotonic potentials evoked by brief pulses of inward current. In this cell, an ejection of 5-HT for 30 ms evoked a fast depolarization with cell discharge which was followed by a slow and sustained depolarization also accompanied by cell discharge. Methysergide (1 μM) reduced the slow depolarization substantially; the fast component was difficult to measure because of spike discharge, but after 12 min exposure to methysergide there was no unequivocal reduction of the fast component. Washing out the antagonist led to a slow recovery in amplitude of the slow response.

Discussion

In this study, 89 of 122 cells (72%) responded to 5-HT applied by pressure ejection with fast, phasic responses, including 37 cells which responded in a biphasic manner. This kind of response was not observed in a previous study (Dun *et al.*, 1984), nor in this study when the ganglion was superfused with 5-HT, possibly because of the rapid and pronounced tachyphylaxis displayed by the fast response. Thus, g-pcg cells displayed a fast depolarization under experimental conditions where the indoleamine was delivered rapidly by pressure pulse, as did rabbit scg cells to iontophoretic application of 5-HT (Wallis & North,

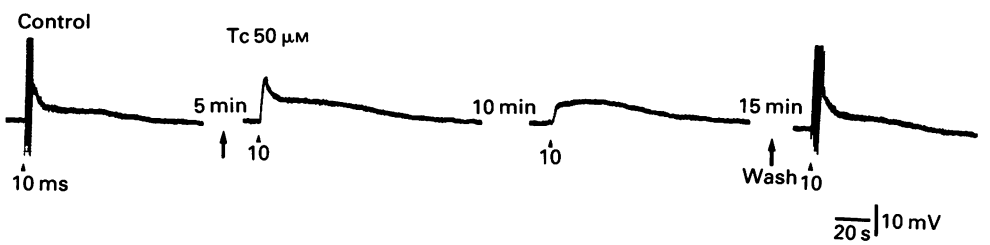

Figure 8 Effect of tubocurarine on a guinea-pig coeliac ganglion cell whose response to 5-hydroxytryptamine (5-HT) (▲) was biphasic. Pressure ejection of 5-HT 10 ms evoked an initial fast depolarization with cell discharge which was followed by a sustained, low amplitude depolarization. Tubocurarine (Tc, 50 μM) reduced the fast component, but enhanced the amplitude of the slow response. The effect of tubocurarine was reversed on washing.

1978) where this response was the characteristic one.

Further, when 5-HT was applied by pressure ejection to guinea-pig scg or inferior mesenteric ganglion cells both fast and slow depolarization components were seen (Dun & Wallis, unpublished). Recent data indicate that both guinea-pig submucous ganglion cells (Surprenant & Crist, 1986) and myenteric ganglion cells (Mawe *et al.*, 1986) display a fast phasic depolarization, associated with an increased membrane conductance and a slow depolarization accompanied by a decreased membrane conductance in response to 5-HT.

Sixty-eight of 122 cells (55%) responded to 5-HT ejections with a slow depolarization, usually of low amplitude; this included 37 cells which showed a biphasic response. A quarter of all cells showed no sign of the fast, phasic response. Dun *et al.* (1984) had shown in an earlier study that the characteristic response of g-pcg cells to 5-HT applied by superfusion was a sustained, low amplitude depolarization.

In g-pcg cells, fast and slow depolarizations evoked by pressure ejected 5-HT differed in a number of ways, apart from the short latency, phasic nature and relatively large amplitude of the former.

(i) Fast responses were accompanied by a substantial increase (Figure 1) and slow responses by little change (Figure 7) or a slight increase in membrane conductance. This suggests at least a quantitative difference in the conductance mechanisms underlying the responses.

(ii) Fast responses were greatly potentiated by hyperpolarizing current (Figure 2), whereas slow responses were somewhat enhanced in the majority of cells sampled here. The non-cholinergic slow e.p.s.p. evoked in many of these cells by repetitive stimulation of the splanchic nerve (Dun & Ma, 1984) is probably mediated by 5-HT and was also increased in amplitude by conditioning hyperpolarization.

(iii) The ionic basis of the fast response is probably an increased conductance to Na (Figure 2) and K, similar to responses to 5-HT in rabbit scg (Wallis & Woodward, 1975; Wallis & North, 1978) and nodose ganglion cells (Higashi & Nishi, 1982). The conductance change underlying the slow response may be an opening of Na and simultaneous closure of K channels, a different admixture of these two components accounting for the variable change in membrane resistance during the slow response. This mechanism was suggested for the non-cholinergic slow e.p.s.p. which may be mediated by 5-HT (Dun & Ma, 1984).

(iv) Fast responses were sensitive to 5-HT_3 receptor antagonists (Fozard, 1984; Bradley *et al.*, 1986), such as MDL 72222, ICS 205-930, quipazine and metoclopramide. Tubocurarine was also an antagonist, although at higher concentrations. Fast responses were unaffected by methysergide. In contrast, slow responses were reduced in amplitude by methysergide but not by tubocurarine. The slow depolarization elicited by superfusion of g-pcg cells with 5-HT (Dun *et al.*, 1984) was reduced by cyproheptadine (20–50 μM). Thus, there is pharmacological evidence to indicate that fast and slow responses are mediated via different 5-HT receptors. Interestingly, the slow response in biphasic cells was less consistently reduced by methysergide; this finding has not been explored further. It is possible that a mixed population of 5-HT receptors might mediate this slow component.

(v) Slow responses could be evoked by superfusing the ganglion with 5-HT (see Dun & Ma, 1984; Dun *et al.* 1984), whereas fast responses could not. The reason for this may be the pronounced tachyphylaxis associated with the fast response. The fast responses of rabbit scg cells to 5-HT show pronounced tachyphylaxis (Wallis & North, 1978; Round & Wallis 1986), and 5-HT is one of the most effective agents in antagonizing 5-HT responses (Wallis, 1981; see Figure 3). Superfusion may desensitize cells so rapidly that the build up of amine around the cell is sufficiently slow to prevent a manifest fast response.

The receptor mediating the fast response has the characteristics of a 5-HT_3 receptor (Bradley *et al.*, 1986). The estimates of potency and the order of potency obtained with MDL 72222, quipazine and metoclopramide must be considered in the light of the difficulty with intracellular studies of allowing prolonged incubation with an antagonist. Thus, full equilibrium between drug and receptors may not have been achieved. Even after incubation for 1 h, equilibration of MDL 72222 with receptors may be incomplete (Round & Wallis, 1987).

5-HT_3 receptors mediate fast depolarizations of rabbit scg and nodose ganglion cells (Round & Wallis, 1987). In view of the existence of several subtypes of 5-HT_3 receptor (Fozard, 1984; Richardson *et al.*, 1985), a study to determine antagonist dissociation constants is required to establish which subtype is present on g-pcg cells.

The receptor mediating the slow depolarization cannot be identified at present. Its insensitivity to MDL 72222 and its sensitivity to methysergide suggest that it is not a 5-HT_3 receptor. It may be similar to the receptor which mediates slow depolarization to 5-HT in guinea-pig myenteric (Wood & Mayer, 1979; Johnson *et al.*, 1980; Mawe *et al.*, 1986) and submucous plexus (Surprenant & Crist, 1986) neurones. In this context, a dipeptide (N-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide) has recently been shown to block selectively the slow depolarization evoked by 5-HT in myenteric neurones (Mawe *et al.*, 1986). The question as to whether this dipeptide is effective against the 5-HT receptor mediating the slow depolarization in g-pcg neurones remains to be addressed.

In conclusion, fast and slow responses in g-pcg

neurones have been described. These have different modes of electrogenesis and are mediated, respectively, by 5-HT_3 and unidentified 5-HT receptors.

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