5-Hydoxytryptamine evokes depolarizations and membrane potential oscillations in rat sympathetic preganglionic neurones

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1. Whole-cell recordings were made from seventy-seven identified rat sympathetic preganglionic neurones (SPN) in spinal cord slices. Perfusion of 5-HT (0·5–30 μM) strongly depolarized 90% of neurones. The response was slow in onset, could last over 10 min and was associated with an increase in input resistance. 5-HT could also evoke rhythmical membrane potential oscillations in a population of previously quiescent neurones.

2. The 5-HT response persisted in TTX and also in low-Ca^{2+}–high-Mg^{2+} artificial cerebrospinal fluid (ACSF), suggesting that the receptors are on SPN. The 5-HT uptake inhibitor 6-nitroquipazine potentiated the 5-HT-induced depolarization.

3. The 5-HT-induced depolarization was reduced and then abolished by membrane hyperpolarization to potentials of about −100 mV, but was not reversed in sign by further hyperpolarization. In voltage clamp, 5-HT evoked inward currents associated with the reduction of an outwardly rectifying potassium conductance.

4. The 5-HT_2 receptor agonist α-methyl-5-HT mimicked the 5-HT response on all neurones, as did the 5-HT_1 receptor agonist 5-carboxamidotryptamine (5-CT) on 71% of SPN. The responses to 5-HT, α-methyl-5-HT and 5-CT were inhibited by the 5-HT_2 antagonists ketanserin and ritanserin.

5. Pressure ejection of 5-HT over the central canal region could evoke a biphasic inhibitory–excitatory response. This response persisted in TTX, suggesting that an inhibitory 5-HT receptor may be located on the medial dendrites.

6. SPN are powerfully depolarized by 5-HT acting at 5-HT_2 receptors, via the closure of an outwardly rectifying potassium conductance. The long duration of the response and the ability of 5-HT to induce rhythmical oscillations suggest that 5-HT may have an important role in regulating SPN excitability.

The somata of sympathetic preganglionic neurones (SPN) are located primarily in the intermediolateral cell column (IML) of the spinal cord, which has been shown to receive a dense innervation of 5-HT terminals (Dahlstrom & Fuxe, 1965; Bowker, Westlund, Sullivan & Coulter, 1982). These terminals arise from brainstem 5-HT-containing neurones located predominantly in the caudal raphe nuclei and the rostroventrolateral medulla (Loewy, 1981; Loewy & Mckellar, 1981). 5-HT-containing synaptic terminals have been demonstrated on the dendrites of retrogradely labelled SPN (Bacon & Smith, 1988; Vera, Holets & Miller, 1990) and a monosynaptic pathway from raphe neurones to SPN has been described (Bacon, Zagon & Smith, 1990). Stimulation of the raphe nuclei *in vivo* has been shown to release 5-HT in the spinal cord, to cause sympathoexcitation and to evoke pressor responses (McCall, 1984; Pilowsky, Kapoor, Minson, West & Chalmers, 1986). However, other studies have reported conflicting data showing inhibitory responses following stimulation of the same nuclei (see review: Coote, 1988).

Ionophoretic application of 5-HT has been shown to excite SPN *in vivo* in the cat (De Groat & Ryall, 1967; Coote, Macleod, Fleetwood-Walker & Gilbey, 1981; Kadzielawa, 1983; McCall, 1983) and the rat (Lewis & Coote, 1990). A biphasic response showing an initial inhibition followed by an excitation has also been reported (Coote *et al.* 1981; Kadzielawa, 1983; Lewis & Coote, 1990). Intracellular recordings in the adult cat *in vitro* slice preparation have shown 5-HT to produce a prolonged depolarization in 68% of SPN, which was associated with an increase in input resistance (Yoshimura & Nishi, 1982; Inokuchi, Yoshimura, Polosa & Nishi, 1990). A similar depolarizing response has been described in neonatal rat lateral horn neurones (Ma & Dun, 1986).

We report here a study using the whole-cell patch-clamp recording technique (Pickering, Spanswick & Logan,

1991) that is addressed to determining the nature of the receptor(s) and the mechanisms underlying the responses of identified SPN to 5-HT. A preliminary report of this study has been communicated to The Physiological Society (Pickering, Spanswick & Logan, 1992*b*).

METHODS

The methods were similar to those described previously (Pickering *et al.* 1991; Pickering, Spanswick & Logan, 1993*b*). Sprague–Dawley rats (7–18 days old) of either sex were deeply anaesthetized with ~6% enflurane in oxygen. The rat was decapitated and a dorsal laminectomy performed allowing the spinal cord to be freed from the lumbar region up to the cervical area. The cord was removed and placed in chilled (4 °C) oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF) of the following composition (mM): NaCl, 127; KCl, 1·9; KH_2PO_4, 1·2; $CaCl_2$, 2·4; $MgSO_4$, 1·3; $NaHCO_3$, 26; D-glucose, 10; pH 7·4. The dura mater and pia mater were removed and an 8–10 mm length of cord taken from the thoracic and lumbar regions.

The cord was placed in a rectangular-shaped mould and then immersed in 1·5–2% agar in ACSF (35–40 °C), which was then set by chilling in cold ACSF. The embedded cord was immersed in cold (4 °C) ACSF and transverse slices (400–600 μm) were cut using a vibratome. The slices were transferred to an incubation chamber where they remained until they were used (up to 24 h later). For recording, the slice was held in a submersion chamber and perfused with ACSF at 2–6 ml min^{-1} at room temperature.

Drugs were added to the ACSF from a series of 20 ml syringes attached to three-way taps arranged in series with the main perfusion line. Focal pressure applications of 5-HT (50 μM to 1 mM) were made from a puffer pipette (5–10 μm tip diameter) positioned above the slice. Fast Green (0·005%) was sometimes added to the drug solution to visualize the puff.

Patch electrodes were fabricated on a Sutter P87 horizontal puller (Clark Electromedical Instruments, Pangbourne, Berks, UK). They were pulled in two stages from borosilicate filamented thin-walled tubing (GC150-TF10, Clark Electromedical Instruments). The electrodes were back-filled immediately before use with a solution containing (mM): potassium gluconate, 130; KCl, 10; $CaCl_2$, 1; Na_3-EGTA, 11; Hepes, 10; $MgCl_2$, 2; Na_2-ATP, 2; dipotassium Lucifer Yellow, 2 (or biocytin, 10 mM); pH adjusted to 7·4 with NaOH. When filled they had resistances of 5–10 MΩ. Recordings were made from neurones located in the IML of slices using the 'blind' method of obtaining whole-cell recordings (Blanton, LoTurco & Kriegstein, 1989). Typical series access resistances ranged between 15 and 40 MΩ. Recordings lasted for periods of between 30 min and 5 h.

Signals were recorded using an EPC-7 patch clamp amplifier (List Medical Electronic, Darmstadt, Germany). Output was observed on a digital oscilloscope (model 1602; Gould Instrument Systems, Essex, UK) and a two-channel chart recorder (Gould 2400S). All recordings were stored on videotape for off-line analysis (using a Sony PCM 701, modified after Lamb, 1985). The voltage-clamp ramp commands were generated using the PATCH software (Cambridge Electronic Design, Cambridge, UK). Data were digitized into a PC-AT class computer using Sigavg software to drive a 1401 interface (Cambridge Electronic Design).

To allow certain identification, recordings were made from only one neurone per slice. Slices were fixed after recording in 10% formaldehyde in 0·1 M phosphate buffered saline (PBS). Slices containing Lucifer Yellow-filled neurones were cleared by immersion in dimethyl sulphoxide (DMSO; Grace & Llinas, 1985). Neurones were viewed on an epifluorescence microscope with appropriate filters for Lucifer Yellow. The slices containing biocytin-filled neurones were resliced to a thickness of 75–100 μm. These were then incubated in a 1% Triton X-100 solution in PBS with avidin–HRP conjugate for 4 h (Horikawa & Armstrong, 1988) before being reacted with diamino benzidine–H_2O_2 for 3–4 min (ABC Vectastain kit, Vector Labs). The sections were dried on slides and mounted under coverslips for viewing.

The following drugs were used: α-methyl-5-HT, 2-methyl-5-HT, 5-carboxyamidotryptamine (5-CT) and 8-hydroxy-2-(di-*N*-propylamino)tetralin (8-OH-DPAT, Cookson Chemicals, Southampton, UK); 1-(4-iodo-2,5-dimethoxyphenyl)-2-amino-propane (DOI), ketanserin, ritanserin and 6-nitroquipazine (6-NQ, Research Biochemicals Inc., St Albans, UK); 5-HT, dipotassium Lucifer Yellow, noradrenaline and carbachol (Sigma, UK); quisqualate (Tocris Neuramin, Bristol, UK).

RESULTS

Properties of SPN

The response to 5-HT was examined in seventy-seven morphologically identified neurones. These neurones were identified as SPN on the basis of the following characteristics: (i) their somata were located in the IML; (ii) their axon exited or clearly coursed towards the ventral root; and (iii) they possessed a dendritic organization characteristic of SPN (see Pickering *et al.* 1991 and Fig. 10). These neurones had similar electrophysiological properties to those previously reported by us for SPN (Pickering *et al.* 1991) with mean resting potential of $-58 \pm 1·3$ mV (± S.E.M.) and mean input resistance of 923 ± 117 MΩ. The neurones were classified into quiescent, active and oscillating groups on the basis of their ability to fire action potentials from rest and on the presence of subthreshold membrane potential oscillations (see Spanswick & Logan, 1990).

Actions of 5-HT

The indolamine was applied to neurones either by perfusion ($n = 62$) or by focal pressure ejection ($n = 15$). Perfusion of 5-HT (0·5–30 μM) elicited a depolarizing response in fifty-six neurones (Fig. 1) and no response in the remaining six. The pressure ejection of 5-HT (50 μM to 1 mM) over the IML elicited a similar depolarizing response in fourteen of the fifteen neurones to which it was applied (Fig. 2). In three of these cells 5-HT ejected over the central canal region evoked a biphasic response with an initial hyperpolarization followed by depolarization (Fig. 10).

The depolarizing response was slow in onset, taking over 1 min to peak with focal pressure applications (Fig. 2). Both pressure ejection and perfusion of 5-HT produced prolonged responses, which could last in excess of 10 min

Figure 1. Typical depolarizing response to perfused 5-HT
In response to the application of a brief bolus of 5-HT (20 μM, 5 s, arrowhead) the neurone depolarized by 18 mV and reached threshold for spike discharge.

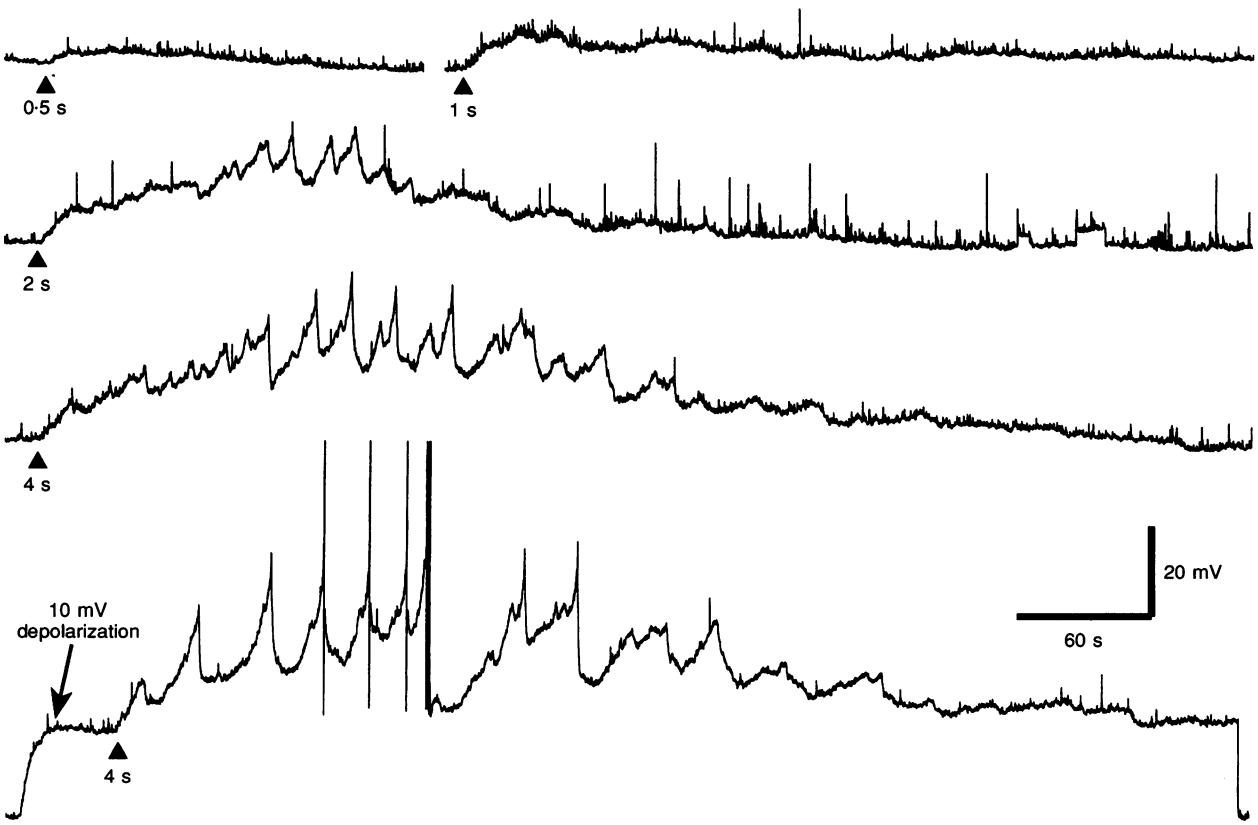

Figure 2. Responses to pressure-ejected 5-HT
Depolarizations evoked with increasing ejection durations of 5-HT (50 μM) applied over the IML (arrowheads). The depolarizing response was slow to peak and had a long duration. Subthreshold membrane potential oscillations were seen during the response. The amplitude of the response was increased by injecting current to depolarize the neurone by 10 mV (bottom trace).

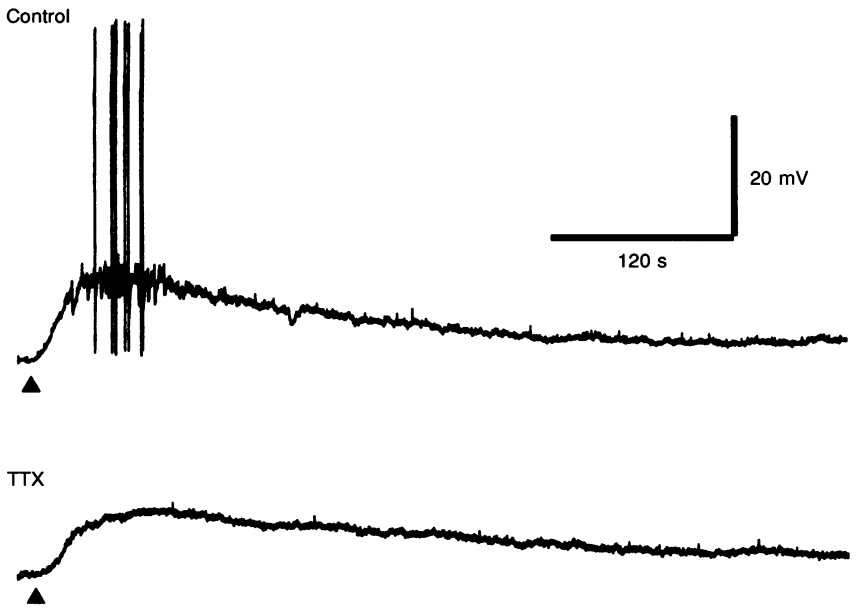

Figure 3. 5-HT acts directly on SPN
Pressure ejection of 5-HT (50 μM, 2 s, arrowheads) evoked a typical prolonged depolarizing response crested by oscillations and spikes. After 30 min of perfusion of TTX (500 nM) the same dose of 5-HT evoked a similar depolarization. Note that the TTX inhibited both spike firing and the induction of oscillations. The second response was smaller, as the neurone showed some desensitization to 5-HT.

(Fig. 2). The depolarization was dose dependent, showing increased amplitude and duration with larger 5-HT applications (Fig. 2). The threshold for action potential discharge was commonly exceeded at the peak of the response to 5-HT. The response persisted in the presence of TTX (0·2–1 μM, $n = 6$), which blocked spike firing (Fig. 3). It was also unaffected by a low-Ca^{2+} (0·6 mM)–high-Mg^{2+} (3·1 mM) ACSF ($n = 3$), which reduced the level of synaptic activity in the slice. These results suggest that 5-HT acts directly upon receptors located on SPN. Interestingly, the application of the low-Ca^{2+}–high-Mg^{2+} ACSF to neurones resulted in a reversible depolarization.

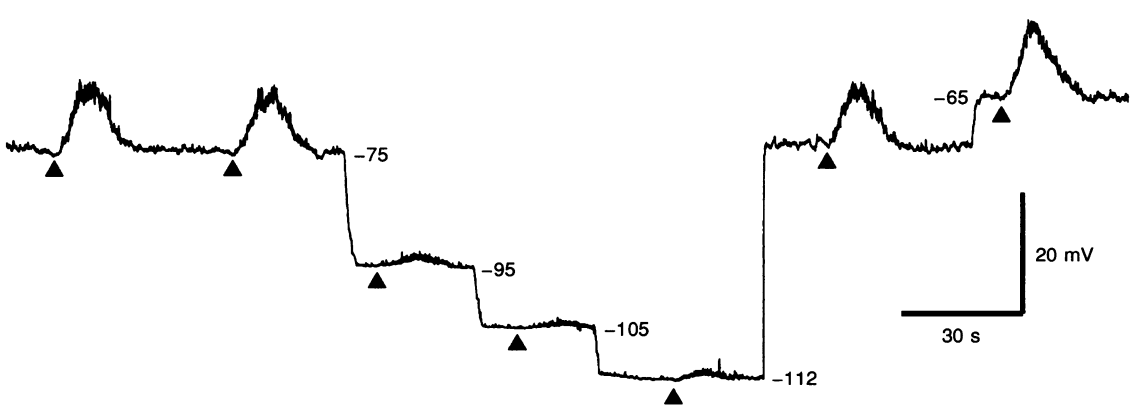

Figure 4. Voltage sensitivity of the 5-HT depolarization
This voltage trace shows a continuous record from a neurone responding to pressure-ejected 5-HT (100 μM, 50 ms, arrowheads). At rest (−75 mV) the ejection of 5-HT reproducibly evoked an 11 mV depolarization. By injecting current through the patch electrode the membrane potential was altered and the 5-HT response examined. The response was greatly reduced in amplitude by hyperpolarization to −95 mV. No reversal of the response was seen with hyperpolarization to −112 mV. Depolarization to −65 mV potentiated the response to 5-HT.

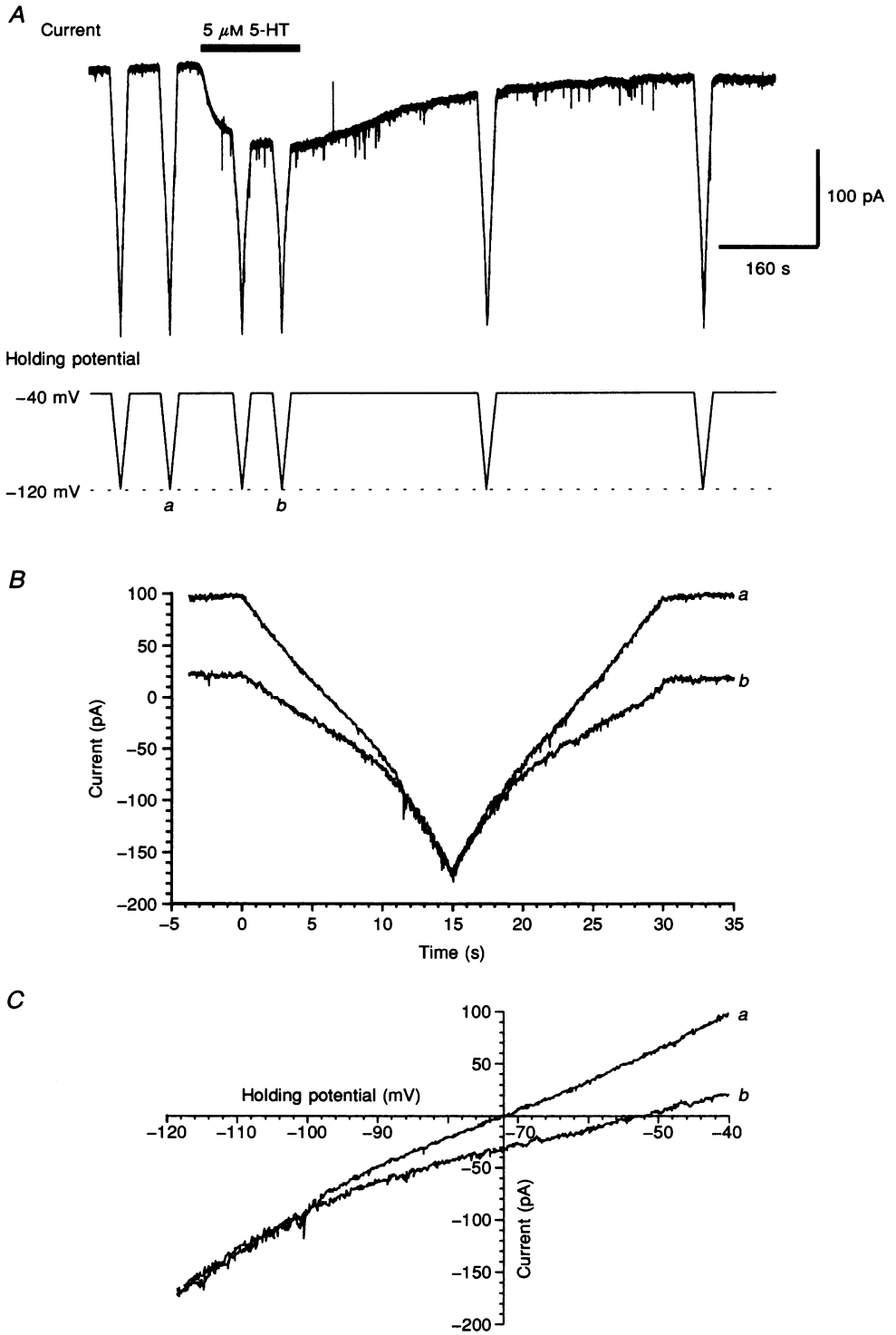

Figure 5. Characteristics of the 5-HT inward current
A, the application of 5-HT to this neurone voltage clamped at a holding potential of −40 mV (rest, −72 mV) evoked an inward current of maximum amplitude 80 pA. A series of 6 voltage command ramps to −120 mV were used to examine the effect of 5-HT on the current–voltage relationship. *B*, superimposition of the current responses to the voltage command ramps shown above before (*a*) and during (*b*) the application of 5-HT. *C*, the current–voltage relationship shows that 5-HT decreased the slope conductance of the neurone at potentials close to rest from 2·9 to 1·7 nS. However, 5-HT had no effect upon the slope conductance at potentials negative to −100 mV.

In about 60% of neurones the response to 5-HT desensitized. In these neurones the initial depolarizing response to 5-HT was characteristically prolonged in duration and the membrane potential failed to completely repolarize following the wash-out of the agonist (see for example Fig. 3). Subsequent applications of 5-HT evoked smaller responses that also failed to repolarize to baseline. The 5-HT response did not recover once it had desensitized. Efforts to avoid desensitization by leaving long periods between applications or by using prolonged low doses or brief high doses of 5-HT were unsuccessful. The desensitization was specific to 5-HT, since responses to other agonists such as noradrenaline or quisqualate showed no change in amplitude or duration with repeated exposures. In the other 40% of neurones 5-HT produced depolarizations which fully repolarized and were reproducible over many hours.

Ionic mechanisms

The ionic mechanism mediating the depolarization was examined by injecting current to change the membrane potential of the cell ($n = 5$). The 5-HT-induced depolarization was reduced by hyperpolarization and increased by depolarization of the cell from rest (Fig. 4). The 5-HT-induced depolarization was abolished at potentials between −90 and −100 mV, but was not reversed in polarity at potentials more negative than −110 mV ($n = 3$). The change in neurone input resistance during the 5-HT response was examined by injecting small hyperpolarizing current pulses (5–20 pA) and recording the voltage transients (see Fig. 7). In all cells examined 5-HT produced an increase in the cell input resistance ($n = 9$). The increase in input resistance was dose dependent, ranging from 10 to 50%, and was still seen if

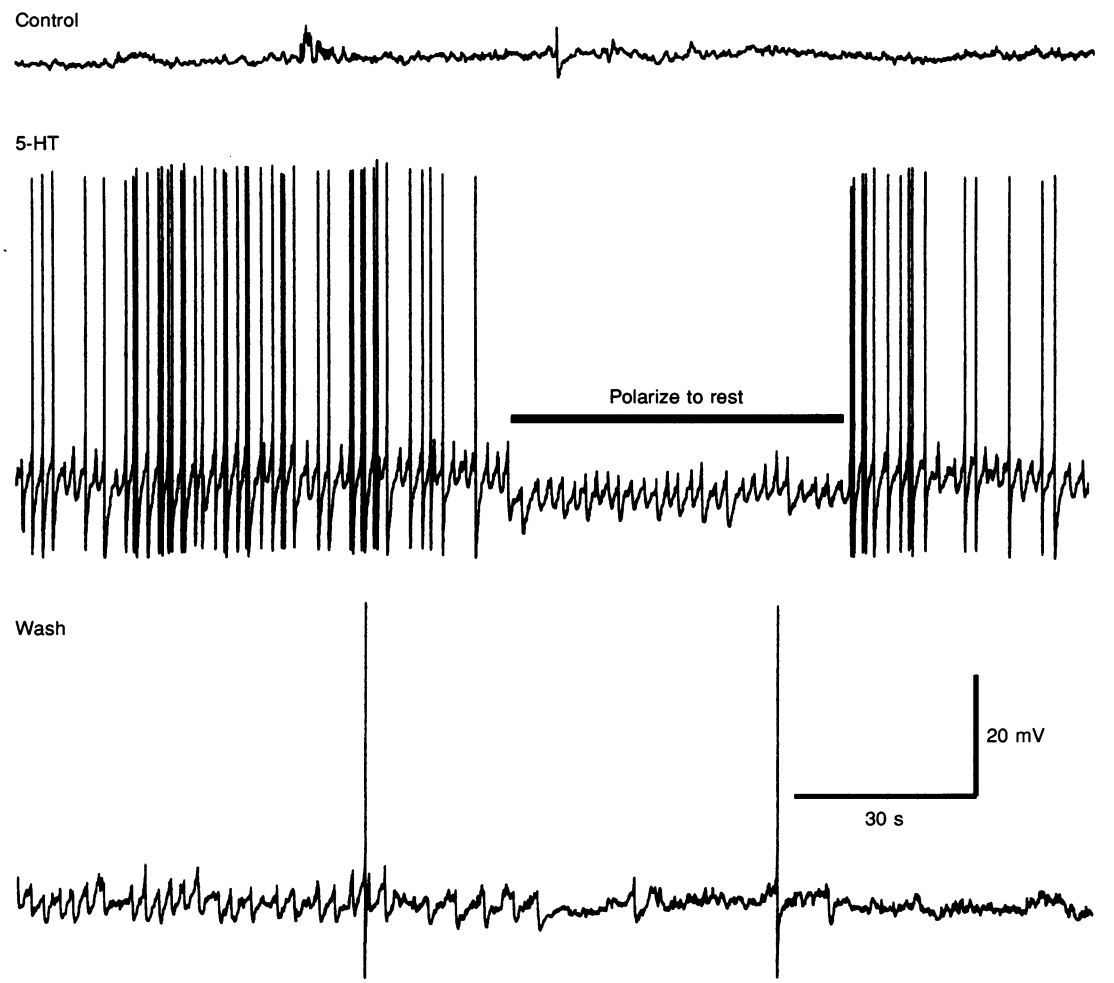

Figure 6. Induction of oscillations by 5-HT
This previously quiescent neurone responded with a 6 mV depolarization to the application of 5-HT (30 μM, 45 s) and discharged action potentials in bursts. The injection of a small hyperpolarizing current returned the neurone to its original resting potential and revealed subthreshold membrane potential oscillations. These oscillations ceased as the 5-HT response decayed.

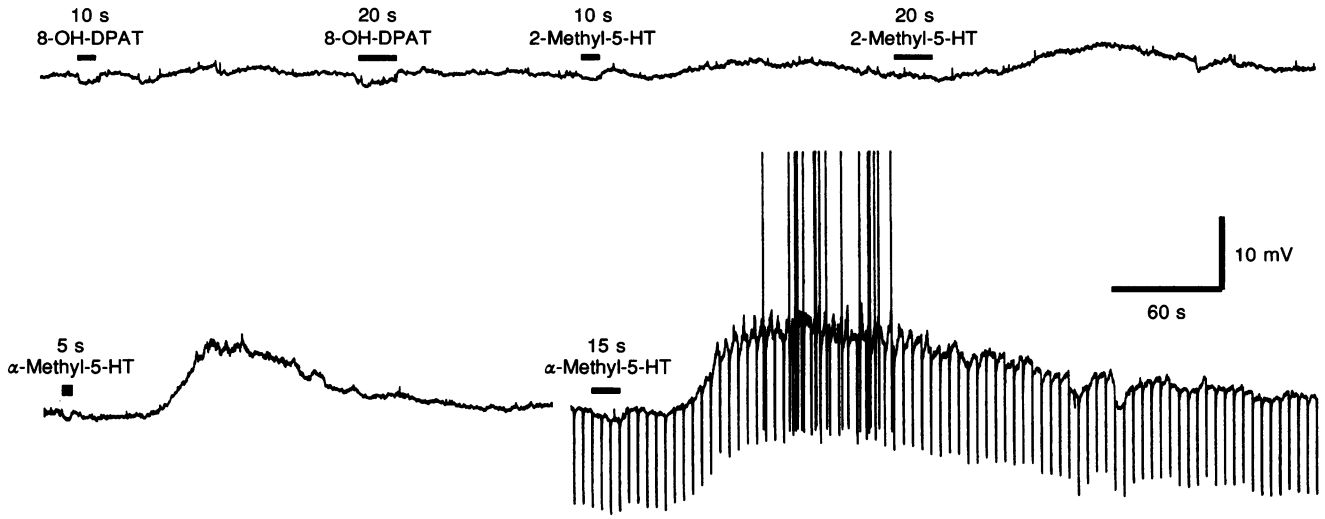

Figure 7. Action of selective 5-HT receptor agonists
The effects of three selective 5-HT receptor agonists were studied on this neurone, which was excited by 5-HT (same cell as Fig. 10). Perfusion of 8-OH-DPAT evoked no response (10 μM). The application of 2-methyl-5-HT (10 μM) evoked weak depolarizations. α-Methyl-5-HT, by comparison, evoked large depolarizing responses. During the second α-methyl-5-HT response the input resistance was observed to increase by 25% as measured from the hyperpolarizing response to injected current pulses.

the neurone was repolarized to rest (by injecting current) at the peak of the 5-HT response.

Voltage clamp

The current underlying the depolarizing response to 5-HT was examined using voltage clamp of the cells at potentials close to rest ($n = 12$). Perfusion of 5-HT induced prolonged inward currents (10–80 pA) and was associated with increased frequency of postsynaptic currents (Fig. 5). The amplitude of the current was reduced at holding potentials below rest and was abolished at potentials between −90 and −100 mV but was not reversed. The nature of the

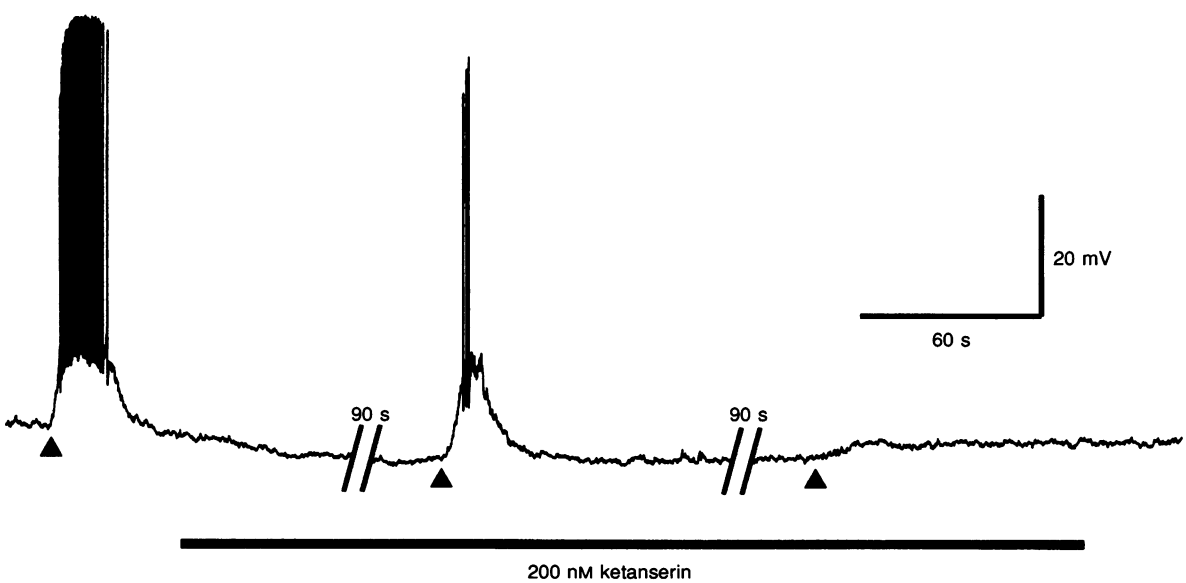

Figure 8. Antagonism of 5-HT response by ketanserin
The pressure ejection of 5-HT (100 μM, 100 ms, arrowheads) reproducibly evoked a strong depolarizing response crested by spikes. The perfusion of ketanserin (200 nM, bar) abolished the response after 7 min of exposure to the antagonist. The 5-HT depolarization did not recover after 30 min of wash.

conductance regulated by 5-HT was investigated by ramping the holding potential from −40 to potentials between −110 and −140 mV (Fig. 5, $n = 5$). The application of 5-HT reduced the slope conductance of the neurones at potentials close to rest. However, it had no effect upon the conductance at potentials negative to −100 mV. At about the same potential the slope conductance of the neurone was seen to increase dramatically due to the activation of an instantaneous anomalous rectification.

Membrane potential oscillations

The application of 5-HT to quiescent neurones could induce rhythmical subthreshold membrane potential oscillations ($n = 16/22$, Figs 2 and 6). These quiescent neurones typically had low input resistances, large resting potentials, high firing thresholds and did not fire action potentials spontaneously. The application of 5-HT caused a depolarization and induced subthreshold oscillations composed of a fast excitatory component followed by a slower hyperpolarization. The presence of these oscillations at the peak of the 5-HT response could result in bursting spike discharge. The oscillations could persist for over an hour following the application of 5-HT. It was possible to slow the frequency of the oscillations by hyperpolarization to potentials negative to −120 mV, but their polarity was never reversed. Oscillations could not be induced in quiescent neurones by injecting depolarizing current in the absence of 5-HT. The addition of TTX (0·2–1 μM) to the ACSF prevented 5-HT from inducing oscillations, although the depolarization remained (Fig. 3). The 5-HT-induced oscillations were identical to those observed to occur spontaneously in approximately 25% of SPN (Spanswick & Logan, 1990). 5-HT also depolarized and increased the cycle frequency in spontaneously oscillating neurones ($n = 6$).

Selective agonists

Selective 5-HT agonists were applied to neurones to characterize further the receptor mediating the depolarizing response (Fig. 7). All of the neurones to which the selective agonists were applied were potently excited by 5-HT. The application of α-methyl-5-HT (a 5-HT_2 agonist) mimicked the 5-HT depolarization in all neurones tested ($n = 16$). It was approximately equipotent with 5-HT, being effective in the dose range 5–20 μM. The 5-HT_1 receptor agonist 5-CT (10–20 μM) excited ten of fourteen neurones to which it was applied with equal potency to 5-HT and α-methyl-5-HT.

The application of a 5-HT_3 receptor agonist 2-methyl-5-HT (10–50 μM) weakly excited two of seven neurones and had no effect on the remainder. DOI, a putative 5-HT_2 receptor agonist, (10–50 μM) was an unreliable agonist, producing weak excitations in three of eight neurones to which it was applied. The DOI excitations

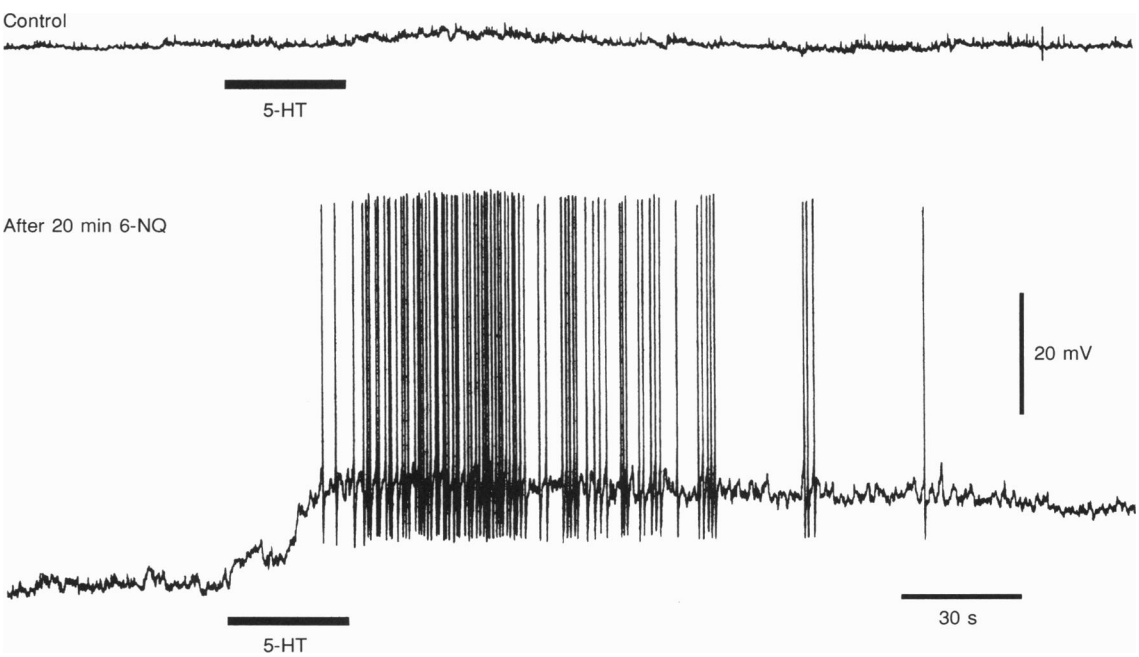

Figure 9. 6-Nitroquipazine potentiates the 5-HT response
The upper trace shows the small response to 1 μM 5-HT perfused for 30 s. The selective 5-HT uptake blocker 6-NQ was then included in the ACSF at a concentration of 10 μM. This had no discernible effect upon the resting membrane properties of the neurone. After 20 min the same dose of 5-HT evoked a large depolarizing response that lasted in excess of 20 min.

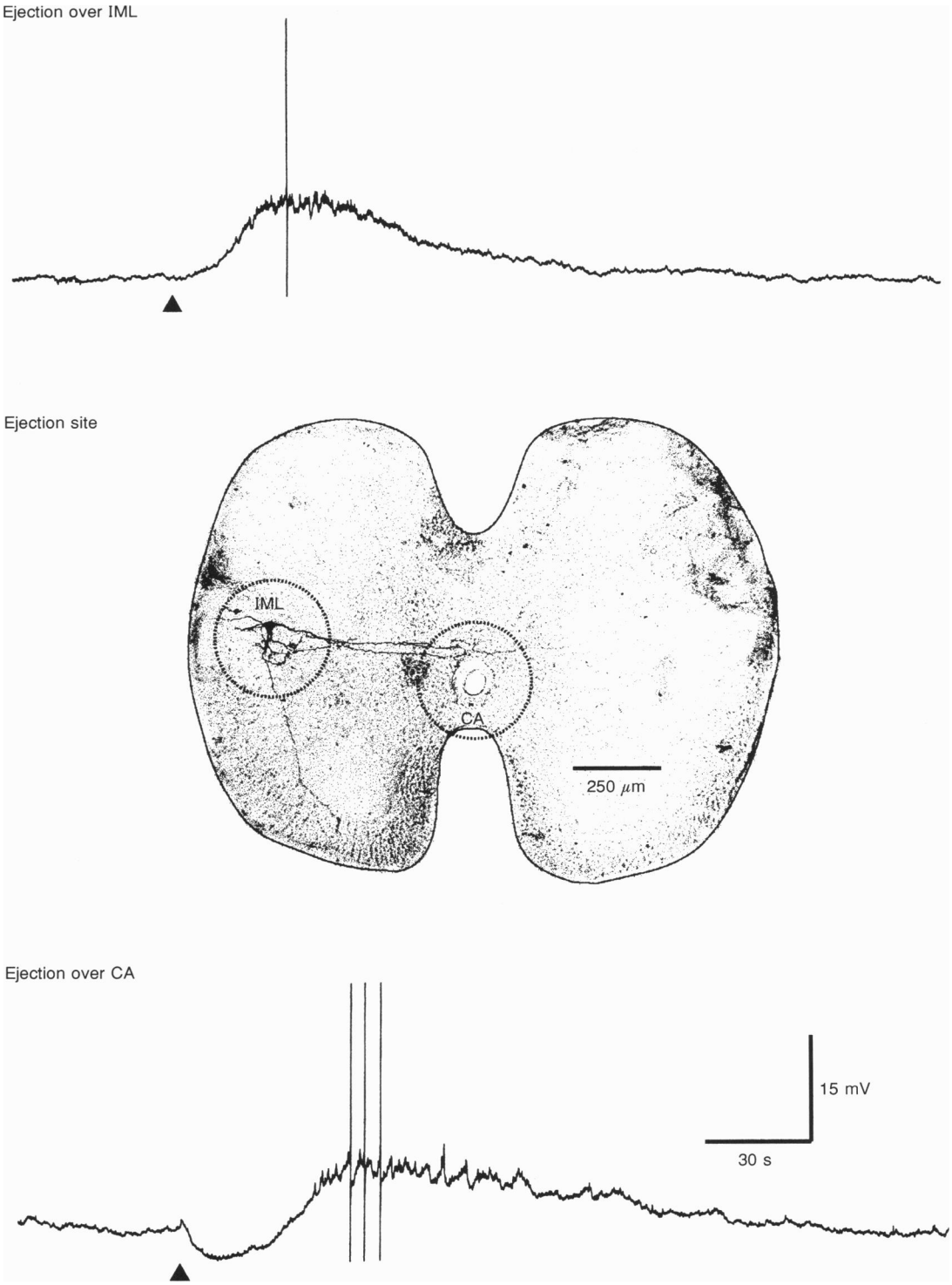

Figure 10. Biphasic response to pressure-ejected 5-HT
Pressure ejection of 5-HT over the IML (100 μM, 4 s, arrowheads) evoked a typical depolarizing response in this neurone. However, when 5-HT was ejected over the central autonomic region (CA) there was an initial hyperpolarization followed by a depolarization. The approximate sites of both ejections are shown superimposed on the spinal cord containing the biocytin-filled neurone. The initial hyperpolarization seemed to be specific to the CA region, since ejection over other sites, including the ventral and dorsal horns, evoked either a weak depolarization or no response.

were typically of small amplitude and prolonged in duration. It was noted that following the application of DOI subsequent responses to 5-HT were attenuated. The 5-HT_{1A} agonist 8-OH-DPAT (10–50 μM) was without effect ($n = 6$).

Antagonism

Ketanserin (a 5-HT_2 antagonist) was applied to neurones which showed no desensitization to control applications of 5-HT ($n = 14$). In all cases ketanserin (40 nM to 2 μM, $n = 9$) and ritanserin (2 μM, $n = 2$) inhibited the 5-HT depolarization (Fig. 8). The response to 5-HT was reduced after 5–15 min of incubation in ketanserin (depending on the concentration of antagonist). The antagonism produced by doses of ketanserin greater than 100 nM was complete, but essentially irreversible, even after prolonged periods of wash-out ($n = 9$, Fig. 8). With lower concentrations of ketanserin the antagonism was partial but more readily reversed ($n = 5$). The responses to α-methyl-5-HT ($n = 4$) and 5-CT ($n = 3$) were also blocked by ketanserin. The antagonism was selective for 5-HT, since responses to other agonists, such as noradrenaline (acting via α_2-adrenoceptors), carbachol or quisqualate, were unaffected. The perfusion of ketanserin alone had no effect on the resting activity of any neurones.

Uptake blockade

A selective 5-HT uptake blocker 6-nitroquipazine (6-NQ) was used to examine the influence of uptake on the 5-HT response. The application of 6-NQ (10 μM) to neurones caused a marked potentiation of the response to 5-HT ($n = 4$, Fig. 9). The potentiation developed following a period of incubation of between 15 and 30 min and was essentially irreversible. Pre-incubation of slices in 1 μM 6-NQ for more than 1 h before their transfer to the slice chamber produced an order of magnitude increase in the potency of 5-HT. Maximal 5-HT responses were seen with doses of 0·5–1 μM compared with the control slices, in which doses of 5–10 μM were needed.

Biphasic responses

In several neurones the pressure application of 5-HT produced biphasic responses that were not seen when 5-HT was perfused onto the same cells ($n = 3$, Fig. 10). Focal applications over different regions of the slice could selectively evoke either a depolarization alone, a biphasic response or no response at all. The biphasic responses were elicited by pressure ejection over the central canal, whereas ejections over the IML produced only a depolarization. Of seven SPN which were depolarized by ejection over the IML, three showed biphasic responses to ejection over the central canal. The biphasic response consisted of a small hyperpolarization of less than 5 mV lasting up to 30 s followed by a delayed excitation similar to that described previously. Both phases of the response were dose dependent and remained in the presence of TTX. The amplitude of both components of the biphasic response were increased by depolarizing the cell with injected current. The delay between ejection and the onset of the depolarization increased with the distance of the pipette from the IML, perhaps suggesting that 5-HT was diffusing from the ejection site to the IML to evoke the depolarization. The perfusion of either 5-HT or any of the selective agonists used in this study onto neurones exhibiting these biphasic responses failed to mimic the inhibitory response.

DISCUSSION

We have used whole-cell recording to examine the action of 5-HT upon sympathetic preganglionic neurones *in vitro*. Our results have shown 5-HT to depolarize 90% of SPN by acting through a 5-HT_2 receptor to close a potassium conductance. In contrast to previous *in vitro* studies (Yoshimura & Nishi, 1982; Ma & Dun, 1986; Inokuchi *et al.* 1990), we found that 5-HT commonly depolarized SPN beyond the threshold for spike firing, resulting in action potential discharge. The response was slow in onset and prolonged in duration, often lasting over 10 min, which would be consistent with it being mediated via second messengers rather than directly through an ionophore coupled directly to the 5-HT receptor. Similar response time courses have been reported in previous extracellular *in vivo* studies (De Groat & Ryall, 1967; Coote *et al.* 1981; Kadzielawa, 1983; McCall, 1983; Lewis & Coote, 1990).

We have seen that the response to 5-HT desensitizes in about 60% of SPN. This is in agreement with the tachyphylaxis of the response to ionophoresed 5-HT seen in cat SPN *in vivo* (Kadzielawa, 1983). Our observation that the membrane potential fails to return to baseline following 5-HT responses suggests that there is long-term inactivation of a proportion of the potassium channels, which are therefore unavailable for subsequent applications of 5-HT. The long duration of the depolarizing response makes it well suited to the long-term regulation of neuronal excitability.

5-HT evoked subthreshold membrane potential oscillations in a population of previously quiescent SPN. The presence of these oscillations results in a bursting action potential discharge pattern at the peak of the 5-HT response. These oscillations were relatively voltage independent but were blocked by the application of TTX. The presence of these oscillations may underlie the bursting discharge patterns which have occasionally been seen in response to ionophoretic applications of 5-HT *in vivo* (De Groat & Ryall, 1967; Coote *et al.* 1981). It should be noted, however, that several of the anaesthetics commonly used in such *in vivo* experiments (including halothane and α-chloralose) have been shown to inhibit oscillations *in vitro* (D. Spanswick, M. Nolan & I. C. Gibson, unpublished observations).

We have previously described identical membrane potential oscillations, which occur spontaneously in about 25% of SPN (Spanswick & Logan, 1990). A similar oscillatory behaviour has been shown to result from electrotonic coupling between neurones of the locus coeruleus (Christie, Williams & North, 1989). We have demonstrated short latency depolarizations and biocytin dye coupling between spontaneously oscillating SPN, strongly suggesting that the oscillations result from gap junctional coupling (Pickering, Spanswick, Gibson & Logan, 1992*a*; Pickering, Spanswick, Gibson & Logan, 1993*a*). There are two possible mechanisms by which 5-HT could evoke oscillations in quiescent SPN. The quiescent SPN may be gap junction coupled to other quiescent SPN. The application of 5-HT may then induce oscillations by simply bringing some of these coupled quiescent neurones to the threshold for action potential discharge. Alternatively, 5-HT may be acting via a second messenger cascade to open gap junctions between SPN that were closed at rest, so enabling electrotonic transmission of the oscillations. At present we have not been able to distinguish between these two possibilities.

Biphasic responses to pressure-ejected 5-HT were seen in some SPN when the ejection pipette was positioned over the central canal area of the spinal cord. On the same neurones focal application over the lateral horn evoked a depolarization without the preceding inhibitory phase. This observation suggests that the inhibitory component of the response is mediated by receptors located in the region of the central canal. Since the response persists in the presence of TTX, it seems likely that these receptors are located upon the medial dendrites of SPN, which form a plexus in the central autonomic area. The identity of the 5-HT receptor mediating this response is uncertain, since none of the selective agonists used in this study evoked a hyperpolarizing response. Biphasic responses with an initial inhibition followed by an excitation have been reported in a minority of SPN in ionophoretic studies *in vivo* (Coote *et al.* 1981; Kadzielawa, 1983; Lewis & Coote, 1990).

The whole-cell conductance was decreased during the depolarizing 5-HT response, indicating that ion channels are closed in the cell membrane. The voltage sensitivity of the response suggests that a potassium channel is being closed. Surprisingly, the response failed to reverse polarity at potentials more negative than the potassium equilibrium potential (−98 mV with our pipette solution). The lack of a reversal for the 5-HT response has been reported previously in lateral horn neurones (Ma & Dun, 1986) and SPN (Inokuchi *et al.* 1990).

We have shown 5-HT to evoke inward currents in SPN voltage clamped at rest. It reduced the slope conductance of the neurones at potentials positive to the potassium reversal potential, but had no effect on the conductance at more hyperpolarized potentials. This indicates that the potassium conductance regulated by 5-HT is inactive at potentials below about −100 mV. This may be because the conductance is an outward rectifier or alternatively it may be inactivated at potentials below −100 mV. It has previously been suggested that 5-HT modulates a calcium-activated potassium conductance in SPN (Inokuchi *et al.* 1990), although our finding that 5-HT evokes depolarizations in low-Ca^{2+}–high-Mg^{2+} saline argues against this conductance being involved. Further work is needed to characterize the nature of the conductance involved.

Similar excitatory 5-HT_2 responses mediated via closure of potassium conductances have previously been seen with intracellular recordings in other CNS neurones *in vitro*, for example in the rat nucleus accumbens (North & Uchimura, 1989), in guinea-pig and cat nucleus reticularis thalami (McCormick & Wang, 1991), in rat piriform cortex (Sheldon & Aghajanian, 1991) and in rat motoneurones (Wang & Dun, 1990; Elliott & Wallis, 1992).

The mimicry of the depolarizing response by α-methyl-5-HT and its selective antagonism by ketanserin and ritanserin suggest that it is mediated by 5-HT_2 receptors. This is in agreement with previous extracellular ionophoretic studies *in vivo* which have shown the response to be mimicked by α-methyl-5-HT (Lewis & Coote, 1990) and to be antagonized by methysergide and metergoline (Kadzielawa, 1983; McCall, 1983). Similarly, intracellular recordings from SPN *in vitro* have shown antagonism with methysergide, mianserin and cyproheptadine (Ma & Dun, 1986; Inokuchi *et al.* 1990). This also corresponds well with the autoradiographic demonstration of high levels of ketanserin binding in the rat IML (Marlier, Teilhac, Cerruti & Privat, 1991).

It is therefore interesting that DOI, whose binding sites are present in the IML (Helke, Thor & Phillips, 1991), was found by us to provide a poor mimic of the 5-HT response. A similar finding has been reported in an *in vivo* study where ionophoresis of DOI failed to excite cat SPN (Clement & McCall, 1990). Our observation that, following the application of DOI, the response to 5-HT was attenuated may suggest that DOI is acting as a weak partial agonist at the 5-HT_2 receptor, as has been reported previously (Wallis, Connell & Kvaltinova, 1991).

It was surprising that the 5-HT_1 receptor agonist 5-CT mimicked the 5-HT response in SPN. The finding that 5-CT was equipotent with 5-HT and α-methyl-5-HT suggests that it is not acting at a 5-HT_1 receptor. This is supported by the observation that the 5-CT response was blocked by ketanserin. A similar excitation of SPN by 5-CT has been described *in vivo* (Lewis & Coote, 1990). A 5-HT response with a very similar agonist profile has been seen with ventral root recordings in the hemisected neonatal rat spinal cord preparation (Connell & Wallis, 1988; Wallis *et al.* 1991; Elliott & Wallis, 1992). These authors concluded that this 5-HT response was likely to be mediated by a novel class of 5-HT_2 receptors.

Currently two subtypes of 5-HT_2 receptor have been described in the mammalian CNS, the 5-HT_{2A} and 5-HT_{2C} receptors (see recent 5-HT receptor classification:

Humphrey, Hartig & Hoyer, 1993). Both receptors are members of the G protein-coupled receptor family, they exhibit a close sequence homology and are coupled to the phosphoinositide second messenger system. Not surprisingly they have very similar pharmacological profiles with few agents showing good selectivity. On the basis of the results presented in this study we feel confident that 5-HT is acting at a 5-HT_2 receptor but are unable to specify which subtype.

In summary we have shown 5-HT to exert a powerful excitatory influence on sympathetic preganglionic neurones. The long duration of the 5-HT response described here makes it well suited for long-term regulation of neuronal excitability. In this respect it is of interest that *in vivo* studies have demonstrated that the application of 5-HT antagonists decreases the spontaneous activity of SPN in intact but not spinal animals (Kadzielawa, 1983; McCall, 1983). This may suggest that a descending 5-HT pathway from the brainstem can exert a tonic excitatory influence on SPN. Our observation that 5-HT can induce synchronous bursting discharges in groups of SPN provides a novel mechanism for the integration of sympathetic discharge. These results support the notion that 5-HT plays an important role in the regulation of output from the sympathetic nervous system.

REFERENCES

Bacon, S. J. & Smith, A. D. (1988). Preganglionic sympathetic neurons innervating the rat adrenal-medulla: immunocytochemical evidence of synaptic input from nerve-terminals containing substance-P, GABA or 5-hydroxytryptamine. *Journal of the Autonomic Nervous System* **24**, 97–122.

Bacon, S. J., Zagon, A. & Smith, A. D. (1990). Electron-microscopic evidence of a monosynaptic pathway between cells in the caudal raphe nuclei and sympathetic preganglionic neurons in the rat spinal-cord. *Experimental Brain Research* **79**, 589–602.

Blanton, M. G., LoTurco, J. J. & Kriegstein, A. R. (1989). Whole-cell recording from neurons in slices of reptilian and mammalian cerebral cortex. *Journal of Neuroscience Methods* **30**, 203–210.

Bowker, R. M., Westlund, K. N., Sullivan, M. C. & Coulter, J. D. (1982). Organization of descending serotoninergic projections to the spinal cord. *Progress in Brain Research* **57**, 239–265.

Christie, M. J., Williams, J. T. & North, R. A. (1989). Electrical coupling synchronizes subthreshold activity in locus coeruleus neurons *in vitro* from neonatal rats. *Journal of Neuroscience* **9**, 3584–3589.

Clement, M. E. & McCall, R. B. (1990). Studies on the site and mechanism of the sympathoexcitatory action of 5-HT agonists. *Brain Research* **515**, 299–302.

Connell, L. A. & Wallis, D. I. (1988). Responses to 5-hydroxytryptamine evoked in the hemisected spinal cord of the neonate rat. *British Journal of Pharmacology* **94**, 1101–1114.

Coote, J. H. (1988). The organization of cardiovascular neurons in the spinal cord. *Reviews of Physiology, Biochemistry and Pharmacology* **110**, 147–285.

Coote, J. H., Macleod, V. H., Fleetwood-Walker, S. & Gilbey, M. P. (1981). The response of individual sympathetic preganglionic neurones to microelectrophoretically applied endogenous monoamines. *Brain Research* **215**, 135–145.

Dahlstrom, A. & Fuxe, K. (1965). Evidence for the existence of monoamine containing neurons in the central nervous system. II. Experimentally induced changes in the intraneuronal amine levels of bulbospinal neuron systems. *Acta Physiologica Scandinavica* **247** (suppl.), 1–85.

De Groat, W. C. & Ryall, R. W. (1967). An excitatory action of 5-hydroxytryptamine on sympathetic preganglionic neurones. *Experimental Brain Research* **3**, 299–305.

Elliott, P. & Wallis, D. I. (1992). Serotonin and L-norepinephrine as mediators of altered excitability in neonatal rat motoneurons studied *in vitro*. *Neuroscience* **47**, 533–544.

Grace, A. A. & Llinas, R. (1985). Morphological artifacts induced in intracellularly stained neurons by dehydration: Circumvention using rapid dimethyl sulfoxide clearing. *Neuroscience* **16**, 461–475.

Helke, C. J., Thor, K. B. & Phillips, E. T. (1991). 5-Hydroxytryptamine 1C/2 agonists in the thoracic spinal cord: cardiovascular effects and binding sites in the intermediolateral cell column. *Journal of Pharmacology and Experimental Therapeutics* **259**, 1335–1343.

Horikawa, K. & Armstrong, W. E. (1988). A versatile means of intracellular labeling: injection of biocytin and its detection with avidin conjugates. *Journal of Neuroscience Methods* **25**, 1–11.

Humphrey, P. P. A., Hartig, P. & Hoyer, D. (1993). A proposed new nomenclature for 5-HT receptors. *Trends in Pharmacological Sciences* **14**, 233–236.

Inokuchi, H., Yoshimura, M., Polosa, C. & Nishi, S. (1990). The effects of 5-hydroxytryptamine on cat sympathetic preganglionic neurons *in vitro*. *Kurume Medical Journal* **37**, 309–312.

Kadzielawa, K. (1983). Antagonism of the excitatory effects of 5-hydroxytryptamine on sympathetic preganglionic neurones and neurones activated by visceral afferents. *Neuropharmacology* **22**, 19–27.

Lamb, T. D. (1985). An inexpensive digital tape recorder suitable for neurophysiological signals. *Journal of Neuroscience Methods* **15**, 1–13.

Lewis, D. I. & Coote, J. H. (1990). The influence of 5-hydroxytryptamine agonists and antagonists on identified sympathetic preganglionic neurones in the rat, *in vivo*. *British Journal of Pharmacology* **99**, 667–672.

Loewy, A. D. (1981). Raphe pallidus and raphe obscurus projections to the intermediolateral cell column in the rat. *Brain Research* **222**, 129–133.

Loewy, A. D. & Mckellar, S. (1981). Serotoninergic projections from the ventral medulla to the intermediolateral cell column in the rat. *Brain Research* **211**, 146–152.

Ma, R. C. & Dun, N. J. (1986). Excitation of lateral horn neurons of the neonatal rat spinal cord by 5-hydroxytryptamine. *Brain Research* **389**, 89–98.

McCall, R. B. (1983). Serotoninergic excitation of sympathetic preganglionic neurons: A microiontophoretic study. *Brain Research* **289**, 121–127.

McCall, R. B. (1984). Evidence for serotoninergically mediated sympatho-excitatory response to stimulation of medullary raphe nuclei. *Brain Research* **311**, 131–139.

McCormick, D. A. & Wang, Z. (1991). Serotonin and noradrenaline excite GABAergic neurones of the guinea-pig and cat nucleus reticularis thalami. *Journal of Physiology* **442**, 235–255.

Marlier, L., Teilhac, J.-R., Cerruti, C. & Privat, A. (1991). Autoradiographic mapping of 5-HT_1, 5-HT_{1A}, 5-HT_{1B} and 5-HT_2 receptors in the rat spinal cord. *Brain Research* **550**, 15–23.

North, R. A. & Uchimura, N. (1989). 5-Hydroxytryptamine acts at 5-HT_2 receptors to decrease potassium conductance in rat nucleus accumbens neurones. *Journal of Physiology* **417**, 1–12.

PICKERING, A. E., SPANSWICK, D., GIBSON, I. C. & LOGAN, S. D. (1992*a*). Electrotonic coupling and oscillations in rat sympathetic preganglionic neurones *in vitro*. *Neuroscience Letters* **42**, S15.

PICKERING, A. E., SPANSWICK, D., GIBSON, I. C. & LOGAN, S. D. (1993*a*). Sympathetic preganglionic neurones are electrotonically coupled. *XXXII Congress of the International Union of Physiological Sciences* 172.43/P.

PICKERING, A. E., SPANSWICK, D. & LOGAN, S. D. (1991). Whole-cell recordings from sympathetic preganglionic neurons in rat spinal cord slices. *Neuroscience Letters* **130**, 237–242.

PICKERING, A. E., SPANSWICK, D. & LOGAN, S. D. (1992*b*). 5-HT depolarizes and induces oscillations in rat sympathetic preganglionic neurones *in vitro*. *Journal of Physiology* **452**, 37*P*.

PICKERING, A. E., SPANSWICK, D. & LOGAN, S. D. (1993*b*). Patch-clamp recording from neurones in spinal cord slices *in vitro*. In *Electrophysiology: A Practical Approach*, ed. WALLIS, D. I., pp. 169–188. Oxford University Press, Oxford, UK.

PILOWSKY, P. M., KAPOOR, V., MINSON, J. B., WEST, J. B. & CHALMERS, J. P. (1986). Spinal cord serotonin release and raised blood pressure after brainstem kainic acid injection. *Brain Research* **366**, 354–357.

SHELDON, P. W. & AGHAJANIAN, G. K. (1991). Excitatory responses to serotonin (5-HT) in neurons of the rat piriform cortex: evidence for mediation by 5-HT_{1C} receptors in pyramidal cells and 5-HT_2 receptors in interneurons. *Synapse* **9**, 208–218.

SPANSWICK, D. & LOGAN, S. D. (1990). Spontaneous rhythmic activity in the intermediolateral cell nucleus of the neonate rat thoracolumbar spinal cord *in vitro*. *Neuroscience* **39**, 395–403.

VERA, P. L., HOLETS, V. R. & MILLER, K. E. (1990). Ultrastructural evidence of synaptic contacts between substance P-, enkephalin-, and serotonin-immunoreactive terminals and retrogradely labeled sympathetic preganglionic neurons in the rat: a study using a double-peroxidase procedure. *Synapse* **6**, 221–229.

WALLIS, D. I., CONNELL, L. A. & KVALTINOVA, Z. (1991). Further studies on the action of 5-hydroxytryptamine on lumbar motoneurones in the rat isolated spinal cord. *Naunyn-Schmiedeberg's Archives of Pharmacology* **343**, 344–352.

WANG, M. Y. & DUN, N. J. (1990). 5-Hydroxytryptamine responses in neonate rat motoneurones *in vitro*. *Journal of Physiology* **430**, 87–103.

YOSHIMURA, M. & NISHI, S. (1982). Intracellular recordings from lateral horn cells of the spinal cord *in vitro*. *Journal of the Autonomic Nervous System* **6**, 5–11.

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