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Effects of 5-HT and 5-HT_{1A} receptor agonists and antagonists on dorsal vagal preganglionic neurones in anaesthetized rats: an ionophoretic study

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1 Effects of ionophoretic administration of 5-hydroxytryptamine (5-HT) and selective 5-HT_{1A} receptor agonists and antagonists on identified dorsal vagal preganglionic and dorsal raphe neurones were studied in pentobarbitone sodium or chloral hydrate-anaesthetized rats, respectively.

2 Extracellular recordings were made from 176 preganglionic neurones in the dorsal vagal nucleus (DVN). Application of 5-HT at low currents ($\leq$10 nA) increased the activity of these neurones. However, at increased currents (10-60 nA), it had a predominantly depressant effect. Application of selective 5-HT_{1A} receptor antagonists, (±)-pindolol or WAY-100635, attenuated the excitatory responses evoked by 5-HT.

3 Ionophoresis of the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (5-30 nA) increased the firing rate of 19 and decreased that of 67 of the 104 vagal neurones tested. Other 5-HT_{1A} receptor agonists, flesinoxan and N,N-di-n-propyl-5-carboxamidotryptamine (DP-5-CT) also had predominantly depressant effects.

4 (±)-Pindolol attenuated excitations but not inhibitions evoked by 8-OH-DPAT. Surprisingly, WAY-100635 and 8-OH-DPAT produced the same effect on these neurones and when applied together, WAY-100635 failed to attenuate the 8-OH-DPAT responses.

5 Dorsal raphe neurones were identified by their low, regular firing rate and their subsequent histological localization. 8-OH-DPAT reversibly reduced the activity in all 7 neurones tested and this was antagonized by WAY-100635 in all 3 neurones tested.

6 In conclusion, 5-HT applied to vagal preganglionic neurones evokes excitatory and inhibitory responses. The excitatory, but not the inhibitory responses may be mediated, at least in part, by activation of 5-HT_{1A} receptors.

Keywords: 5-Hydroxytryptamine; dorsal raphe neurone; vagal neurone; 5-HT_{1A} receptor

Introduction

Intravenous administration in cats of the 5-HT_{1A} agonists, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), ipsapirone and flesinoxan evokes a vagally mediated bradycardia of central origin (Ramage & Fozard, 1987; Ramage *et al.*, 1988). This led to the hypothesis that central 5-HT pathways, acting via 5-HT_{1A} receptors, control parasympathetic outflow to the heart and possibly other visceral organs. Consistent with this, administration of 8-OH-DPAT into the fourth ventricle (Shepheard *et al.*, 1994) or directly into the nucleus ambiguus (a site of cardiac vagal preganglionic neurones) evokes a bradycardia (Izzo *et al.*, 1988). In addition, 8-OH-DPAT binding sites have been demonstrated in the nucleus ambiguus (Dashwood *et al.*, 1988) and identified cardiac vagal preganglionic neurones receive synaptic contracts from 5-HT immunoreactive boutons (Izzo *et al.*, 1988).

In rats, there is similar evidence for a 5-HT-containing pathway modulating cardiac activity. Administering 8-OH-DPAT or flesinoxan intravenously (Gradin *et al.*, 1985; Dreteler *et al.*, 1990) or directly into the dorsal raphe nucleus (Connor & Higgins, 1990) and i.c.v. 5-HT (Dalton, 1986) produce a bradycardia due at least in part to an increase in cardiac vagal drive. Cardiac vagal preganglionic neurones are located in the nucleus ambiguus and the dorsal vagal nucleus (DVN) in rats (Nosaka *et al.*, 1982; Jones *et al.*, 1995) and both these regions contain 5-HT immunoreactive fibres (Steinbusch, 1981) and 8-OH-DPAT binding sites (Pazos & Palacios, 1985; Thor *et al.*, 1992). Indeed; 5-HT immunoreactive boutons make synaptic contact with cardiac vagal preganglionic neurones in the rat nucleus ambiguus (Izzo *et al.*, 1993). Finally, microinjections of 8-OH-DPAT into the DVN evokes a bradycardia (Sporton *et al.*, 1991) whilst i.c.v. (±)-pindolol attenuates the bradycardia evoked during the von Bezold-Jarisch reflex (Bogle *et al.*, 1990). Although this provides strong evidence for a role for 5-HT-containing neurones, acting via 5-HT_{1A} receptors, in control of cardiac vagal outflow, the experiments are unable to distinguish between direct effects on the preganglionic neurones and actions on antecedent neurones. The present experiments address this by applying 5-HT and 5-HT_{1A} receptor agonists by microionophoresis. To determine that effects on neuronal firing are due to actions on 5-HT_{1A} receptors, we have examined whether the responses are antagonized by the 5-HT_{1A} receptor antagonist, (±)-pindolol (Schoeffter & Hoyer, 1988; Wouters *et al.*, 1988) and the newer and highly selective antagonist, WAY-100635 (Forster *et al.*, 1995).

Preliminary accounts of some of these data have been published (Wang *et al.*, 1994; 1995).

Methods

Experiments were performed on male Sprague-Dawley rats (300-440 g body weight) anaesthetized with pentobarbitone sodium (60 mg kg^{-1} i.p., $n=69$) or chloral hydrate (400 mg kg^{-1} i.p., $n=6$). Depth of anaesthesia was assessed by monitoring the stability of the arterial blood pressure and heart rate and the cardiovascular responses to pinching the paws. Anaesthesia was supplemented when necessary. A tracheotomy was performed low in the neck and a femoral artery and vein cannulated for measurement of blood pressure and

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administration of supplemental anaesthesia and drugs. Tracheal and arterial blood pressures were measured with pressure transducers (Statham P23Db) and a lead II ECG was recorded, amplified and filtered (Neurolog NL100, 104A and 125 modules). Animals were ventilated with oxygen-enriched room air using a positive pressure ventilator (Harvard rodent ventilator, model 683) with 1 cmH_2O positive end expiratory pressure. End-tidal CO_2 was continuously measured with a fast response CO_2 meter (ADC Ltd., Model FM1) and maintained at 4%. Arterial blood samples were regularly taken and blood gases and pH monitored with a Corning pH/blood gas analyser (Model 238). Blood gases were maintained between 90–130 mmHg Po_2, 40–50 mmHg PCo_2 and pH 7.3–7.4 by slow i.v. infusions of sodium bicarbonate (1.0 M) or adjustments of the respiratory pump.

The animals were placed in a stereotaxic frame and the right vagus nerve dissected free from the sympathetic trunk and placed on bipolar silver electrodes for electrical stimulation (1 Hz, 2–5 V, 1.0 ms) with a digital programmer (Master 8, AMPI) and isolated stimulator (Digitimer DS2). To expose the caudal brainstem in the region of the DVN the nuchal muscles were removed from the back of the neck, the occipital bone opened and the dura overlying the brainstem cut and reflected laterally. To access the DVN, in some experiments it was necessary to displace the cerebellum rostrally with a small retractor. To access the dorsal raphe nuclei a burr hole was drilled in the midline of the skull around the Lambda suture. The saggital sinus was tied and removed and any bleeding controlled with gelfoam. The nuclei were approached vertically at stereotaxic coordinates A = 0.2–1.5, L = 0.0, V = 5.0–6.0 from the dorsal surface of the brain.

Protocol

Extracellular recordings were made from brainstem neurones using 5- or 7-barrelled microelectrodes (tip diameter 3–5 μm) made from borosilicate glass (Clarke Electromedical, GC 150F-10). The recording barrel contained 4 M sodium chloride, and the other barrels contained Pontamine Sky Blue dye, DL-homocysteic acid (DLH), and a selection of 5-HT and its receptor agonists and antagonists. With the exception of DLH, drugs were ejected with positive currents, a retaining current of 10 nA being applied between ejection periods. Neuronal recordings were amplified × 5000 (Dagan 2400) and filtered (0.1–3 kHz). Vagal preganglionic neurones in the DVN were identified by standard criteria of antidromic activation, including constant latency of the responses to electrical stimulation of the cervical vagus nerve (Figure 1a) and collision of the antidromically-evoked response with appropriately timed ongoing activity (Figure 1b). The characteristics of the ongoing activity of these neurones, their synaptic inputs and location have been described previously (Nosaka *et al.*, 1982; Jones *et al.*, 1995). Recordings from dorsal raphe neurones were identified by their regular, low discharge rate (Sprouse & Aghajanian, 1987) which was confirmed by constructing autocorrelograms of neuronal firing (Figure 7a) and subsequently by their histological localization.

Drugs were applied to the vicinity of identified neurones by ionophoresis (Neurophore, Medical Systems). Neurones with no ongoing activity were induced to fire by application of low currents (0–20 nA) of the excitant amino acid DLH. When the neuronal firing rate was steady, the effects of agonist and/or antagonist drugs given alone and/or together were then tested. In all experiments possible current artefacts were overcome using the automatic current balancing available on the Neurophore. In some experiments, the possibility of current and/or pH artefacts was tested directly by passing current through saline of the same pH as the ejected drug. A significant artefact was never seen with this test.

In 21 of the DVN and all dorsal raphe experiments, recording sites were marked by deposition of Pontamine Sky Blue dye. At the end of these experiments the brain was removed and fixed in 10% formal saline. Frozen sections (80 μm) were cut, the marked sites visualized and mapped onto standard sections of the brain (Paxinos & Watson, 1986).

Analysis of data

Arterial blood pressure, tracheal pressure, ECG and neuronal activity were recorded on video tape via a digital interface (Instrutech, VR-100B). Off-line analysis of the recorded data was made with commercially available software (CED Spike 2 and Signal Averager) on a computer (Viglen 486 DX2 66) accessed via an A–D interface (CED 1401plus). Single unit activity was discriminated on a window discriminator (Digitimer D130) and displayed as a rate histogram. Drugs were classed as evoking excitation or inhibition if, during the ejection period, activity was increased or decreased by at least 20% of baseline.

Drugs and solutions

The following drugs were freshly dissolved in 1 mM saline and their pH adjusted by addition of drops of either 0.1 M HCl or 0.1 M NaOH:- 5-hydroxytryptamine maleate (5-HT; 50 mM, pH 5), 8-hydroxy-2-(di-n-propylamino)tetralin HBr (8-OH-DPAT; 20 mM, pH 4). N,N-di-n-propyl-5-carboxamidotryptamine maleate (DP-5-CT; 10 mM, pH 4) and (±)-pindolol HCl (20 mM, pH 4), all from Research Biochemicals; Semat Technical Ltd, St. Albans; Flesinoxan (20 mM, pH 5; a gift from Duphar-Solvay, Weesp, Netherlands), N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridyl)cyclohexanecarboxamide trichloride (WAY-100635; 0.5 μM–10 mM, pH 4; a gift from Wyeth Research U.K., Maidenhead). Pon-

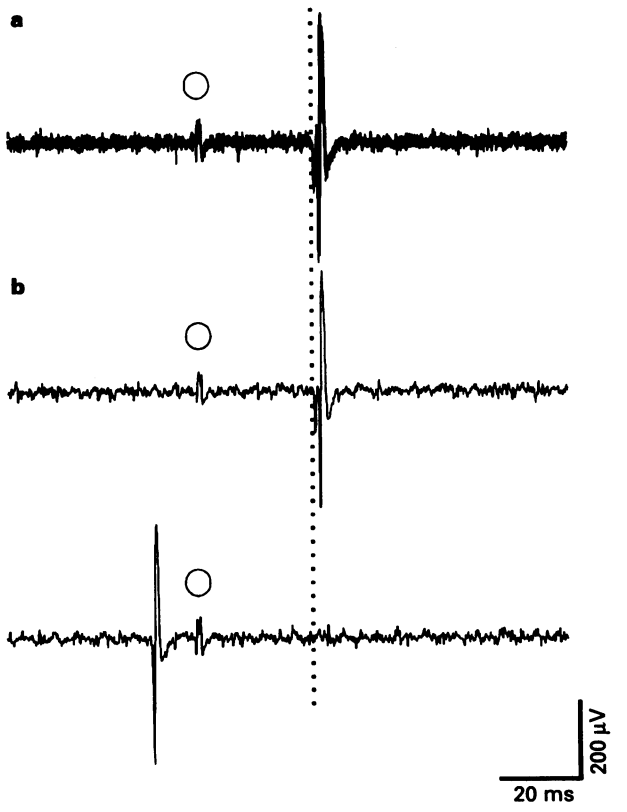

Figure 1 Extracellular recording from a vagal preganglionic neurone in the dorsal vagal nucleus of the rat. (a) Five superimposed single sweeps showing the constant latency of the antidromic spike evoked by stimulation of the cervical vagus at (○) (1 Hz, 1 ms, 4 V). Estimated axonal conduction velocity = 0.68 m s^{-1}. (b) Two consecutive oscilloscope sweeps showing the antidromically evoked spike in the top trace, and below, collision of this with a spontaneous action potential.

tamine sky blue dye (20 mg ml^{-1}; BDH, Poole) was dissolved in 0.5 M sodium acetate and DL-homocysteic acid (DLH, 100 mM, pH 8.5; Sigma, Poole) in distilled water.

Results

Extracellular activity was recorded from 176 antidromically identified dorsal vagal motoneurones (Figure 1). Calculated axonal conduction velocities (0.69 ± 0.02 ms^{-1}, mean ± s.e.mean) indicated that they had non-myelinated axons.

Effects of 5-HT on vagal preganglionic neurones

Ionophoretic application of 5-HT (0–10 nA) increased activity in 53 of the 59 dorsal vagal preganglionic neurones tested (Figures 2a, b, 3, 4). Firing rate rapidly increased to a stable level and gradually returned to baseline following removal of the current. The response was repeatable (Figures 2, 3). In the

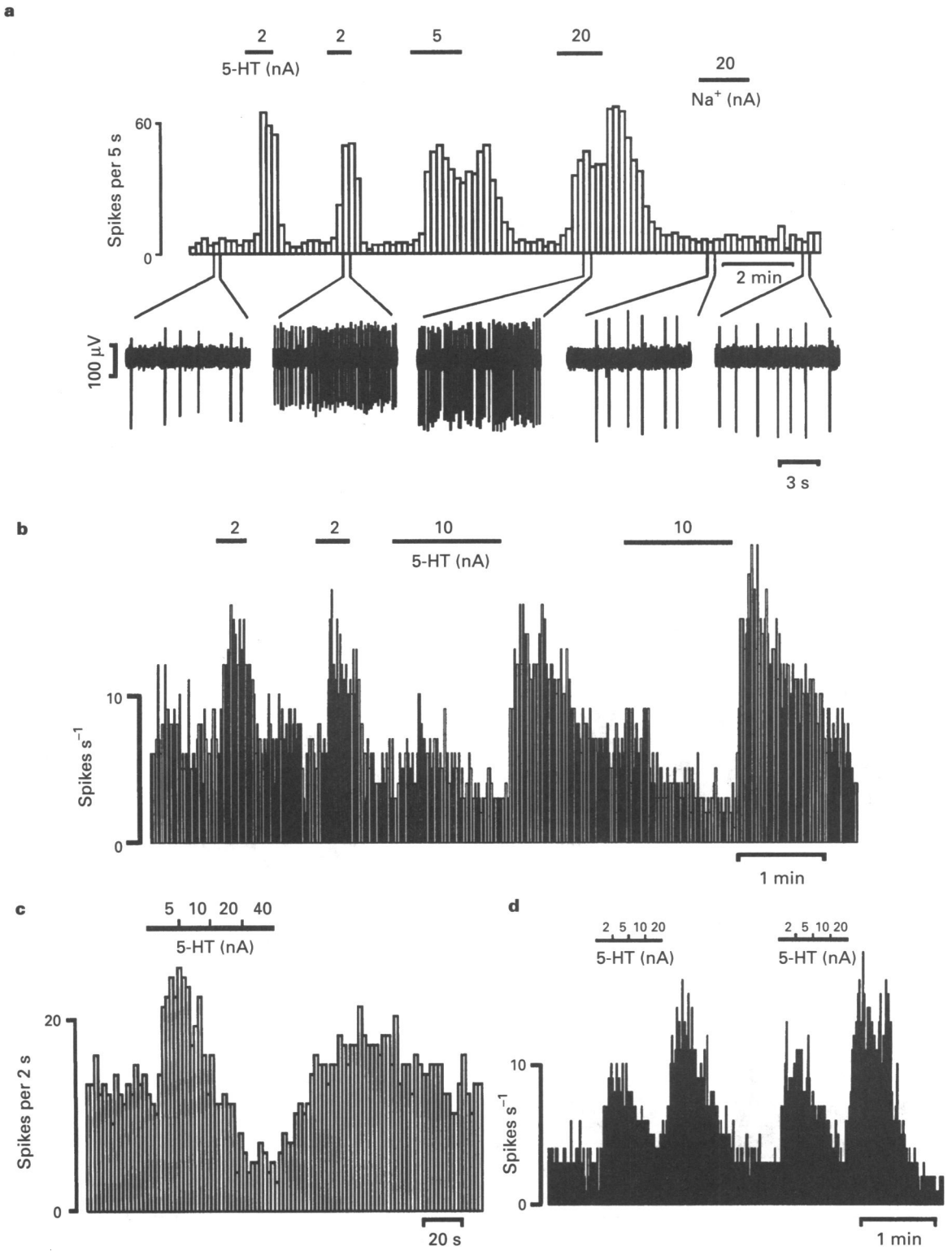

Figure 2 Ratemeter records of vagal preganglionic neurone activity during application of 5-HT or current (Na^+) with the stated ionophoretic ejection currents during the bars. (a) Shows a continuous ratemeter record and representative original spike records during pulse application of 5-HT. (b) Shows a continuous ratementer record during pulse application of 5-HT. (c and d) Show continuous ratemeter records during a stepped increase in ejection current.

7 neurones tested excitation was maintained when 5-HT application was prolonged (2–10 min); there was no indication of desensitization. In 3 neurones the 5-HT evoked excitation was further increased when the current was increased (10–60 nA) but in 21 of the 30 neurones tested at these higher currents, the magnitude of the excitatory response was reduced (Figures 2b, c, d) and in 11 cases activity fell below the baseline firing rate (Figures 2b, c). Three neurones showed only inhibition to 5-HT whatever the current, and 2 of the 3 cells unaffected by low currents were inhibited at the higher currents. Following removal of the 5-HT, activity of the inhibited cells rapidly returned to baseline (within 30 s) before increasing above this and then declining back to baseline. This 'off excitation' was seen in 23 of 30 neurones tested with the higher currents (Figure 2).

Effects of (±)-pindolol and WAY-100635 on the excitatory effects of 5-HT

In 11 of 14 neurones tested, (±)-pindolol (0–30 nA, 3–10 min) attenuated, but did not abolish, the 5-HT excitation (Figure 3a). WAY-100635 (5–30 nA, 3–10 min) similarly attenuated the 5-HT evoked excitation in 21 of 33 neurones. In these cells effects of the antagonist on baseline firing rate was minimal (Figures 3b, 4). In 3 neurones, the 5-HT excitation was potentiated by WAY-100635 whilst in the other 9 neurones the compound was without effect.

Applied alone, (±)-pindolol (0–30 nA, 3–10 min) had variable effects on baseline firing rate. Activity decreased in 25 neurones, increased in 5 and was unchanged in 20. Similarly, WAY-100635 (0–40 nA) increased the baseline firing rate in 8 of 70 neurones whilst the activity of 31 neurones was reduced (Figures 4, 6b).

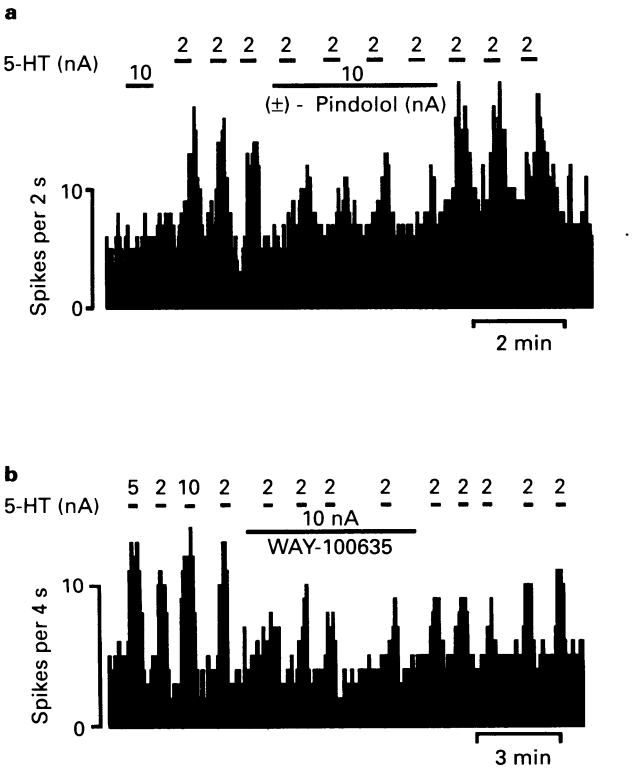

Figure 3 Effects of (±)-pindolol and WAY-100635 on the excitatory effects of 5-HT applied ionophoretically with the stated ejection currents during the time shown by the bars. (a) Shows a continuous ratemeter record during pulse application of 5-HT before, during and following ionophoretic application of (±)-pindolol. (b) Shows a continuous ratemeter record during pulse application of 5-HT before, during and following ionophoretic application of WAY-100635.

Effect of WAY-100635 on the DLH evoked excitation of DVN neurones

WAY-100635 had no effect on the DLH response in 7 neurones, it attenuated the response in 4 and potentiated the response in another 4. In a further 6 neurones the effects of WAY-100635 on the excitatory effects of 5-HT and DLH were compared. In 5 of these WAY-100635 attenuated the 5-HT but not the DLH excitations (Figure 4).

Effect of 8-OH-DPAT, flesinoxan and DP-5-CT on dorsal vagal neurones

Application of 8-OH-DPAT (5–60 nA, 20–120 s) increased ongoing activity of 19 neurones (Figures 5c, 6a) and reduced that of 67 neurones (Figures 5a, 6b), the remaining 18 neurones being unaffected. Flesinoxan (5–30 nA) (Figure 5a) and DP-5-CT (0–5 nA) (Figure 5b) also decreased activity in 3 of 4, and 4 of the 5 neurones tested, respectively.

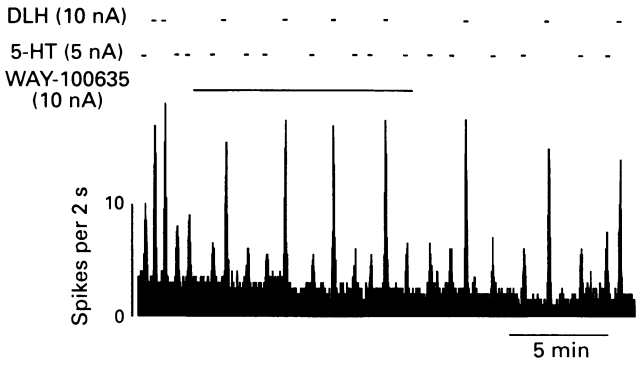

Figure 4 Effects of WAY-100635 on the excitatory effects of 5-HT and the excitatory amino acid DLH applied ionophoretically to a vagal preganglionic neurone with the stated ejection currents at the time shown by the bars. The trace shows a continuous ratemeter record during pulse application of 5-HT and DLH before, during and following ionophoretic application of WAY-100635.

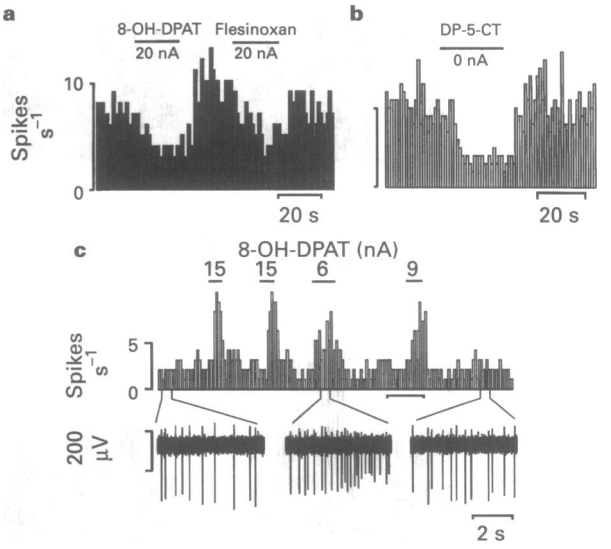

Figure 5 Ratemeter records of vagal preganglionic neurone activity during application of 5-HT$_{1A}$ receptor agonists with the stated ionophoretic ejection currents during the bars. (a) A continuous ratemeter record showing the inhibition of activity produced during application of 8-OH-DPAT and flesinoxan. (b) A continuous ratemeter record showing the inhibition of activity produced by application of DP-5-CT. (c) A continuous ratemeter record and representative original spike records showing the increase in activity produced by application of 8-OH-DPAT.

Both 8-OH-DPAT (10 nA) and 5-HT (10 nA) were tested on 4 neurones. All were excited by the 5-HT but inhibited by 8-OH-DPAT.

Effect of (±)-pindolol and WAY-100635 on the responses of dorsal vagal neurones to 8-OH-DPAT and DP-5-CT

In 5 neurones, (±)-pindolol application had minimal effect on baseline firing rate yet it attenuated the excitatory actions of 8-OH-DPAT (Figure 6a). In contrast, it never attenuated the inhibitory effects of 8-OH-DPAT. When applied to 6 neurones inhibited by 8-OH-DPAT, it potentiated the inhibition in 3 and was without effect in the remainder. Similarly, (±)-pindolol potentiated inhibitory effects of DP-5-CT in 2 of the 4 cells tested.

Applied alone, WAY-100635 (0–40 nA) always had the same effect as 8-OH-DPAT. Both drugs increased ongoing activity in 4 cells but reduced it in 16. When applied together, WAY-100635 failed to attenuate the effects of 8-OH-DPAT and in 8 of the 12 neurones the effects of 8-OH-DPAT were actually potentiated by WAY-100635 (Figure 6b).

Effects of 8-OH-DPAT and WAY-100635 on dorsal raphe neurones

8-OH-DPAT (5–20 nA) inhibited ongoing activity in all 7 neurones. This inhibition was antagonized by WAY-100635 (0–10 nA, $n=3$) (Figure 7b).

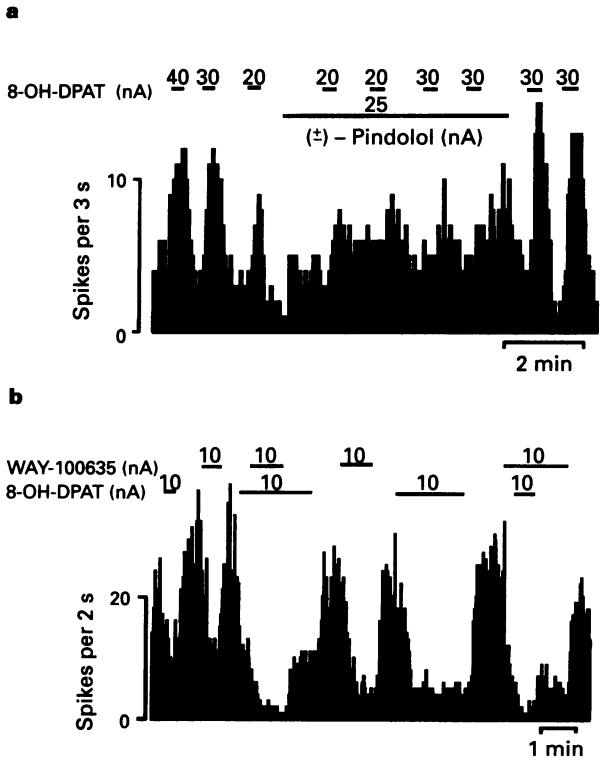

Figure 6 Effects of (±)-pindolol and WAY-100635 on the effects of 8-OH-DPAT applied ionophoretically to 2 vagal preganglionic neurones with the stated ejection currents during the bars. (a) A continuous ratemeter record showing the excitation produced by pulse application of 8-OH-DPAT before, during and following ionophoretic application of (±)-pindolol. (b) A continuous ratemeter record showing the inhibition of activity produced by pulse application of 8-OH-DPAT and WAY-100635 applied individually, or in parallel.

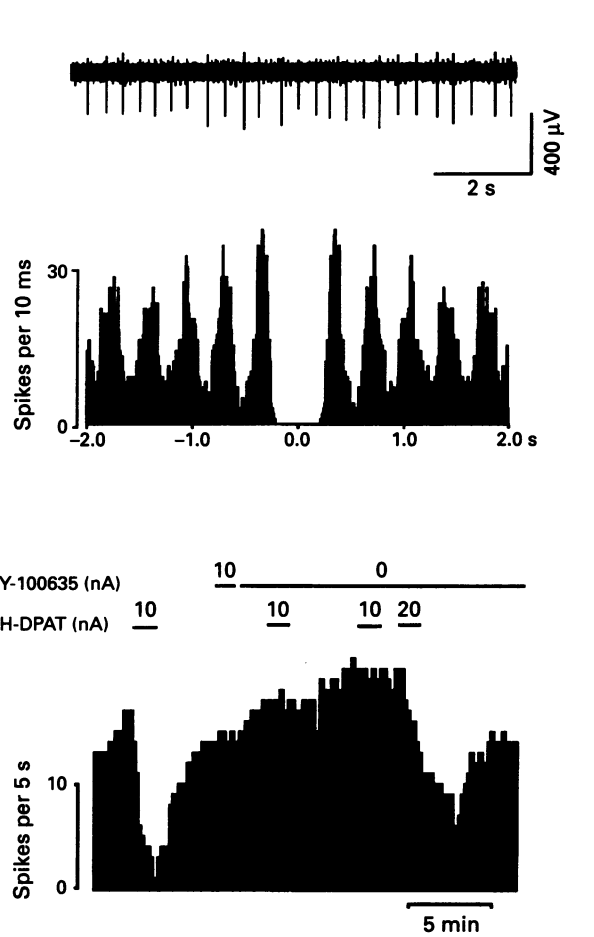

Figure 7 Effects of 8-OH-DPAT and WAY-100635 ionophoretically applied to dorsal raphe neurones. (a) Traces show a continuous original record (above) and autocorrelogram (120 sweeps, 10 ms bins) of ongoing activity in a presumed 5-HT containing dorsal raphe neurone. (b) The inhibitory effects of ionophoretic application of 8-OH-DPAT to a dorsal raphe neurone is antagonized by application of WAY-100635.

Discussion

The hypothesis that vagal motoneurones receive inputs from 5-HT-containing pathways was tested in the present experiments which demonstrated for the first time *in vivo* that 5-HT can have both excitatory and inhibitory actions when applied in the vicinity of identified vagal motoneurones in the DVN of rats. The effects are current-dependent and reversible. These results accord with studies performed *in vitro* on rat DVN neurones where 5-HT increased neurone excitability, the frequency of spontaneous postsynaptic potentials and the firing rate of spontaneously active neurones (Brooks, 1991; Albert & Brooks, 1994; Travagii & Gillis, 1995) suggesting both pre- and postsynaptic effects.

In the present experiments the excitatory action of 5-HT may be mediated, in part, by activation of 5-HT$_{1A}$ receptors. It was attenuated by both (±)-pindolol and WAY-100635, two structurally very different 5-HT$_{1A}$ receptor antagonists. Whilst (±)-pindolol has been shown to antagonize the discriminative stimulus properties of 8-OH-DPAT (Tricklebank *et al.*, 1987) and the central depressor effects of flesinoxan and 8-OH-DPAT (Wouters *et al.*, 1988), it would also block some of the actions of noradrenaline. WAY-100635, a more selective 5-HT$_{1A}$ receptor antagonist (Forster *et al.*, 1995), blocks the hypothermia and inhibition of dorsal raphe neurones evoked by 8-OH-DPAT (Forster *et al.*, 1995) and the forebrain effects of DP-5-CT on blood pressure (Gallagher & Ramage, 1995) in rats. Although it binds weakly to α_1-adrenoceptors, it has little

affinity for β-adrenoceptors (Forster *et al.*, 1995). In some cells the antagonist drugs did alter baseline activity and have non-specific effects on cell excitability but in at least some neurones 5-HT excitations could be attenuated without effecting similar reduction in DLH excitations. In the brainstem 5-HT also excites midline neurones, by activation of 5-HT$_2$ receptors (Davies *et al.*, 1988a) and facial motoneurones, by reducing resting K^+ conductance and enhancing I_h. This latter effect is mimicked by 5-CT, but not by 8-OH-DPAT, DP-5-CT or 2-methyl-5-HT (Larkman & Kelly, 1991; 1992). Similarly, sympathetic preganglionic neurones are excited by 5-HT, 5-HT$_1$ and 5-HT$_2$ receptor agonists *in vivo* (Lewis & Coote, 1990) and *in vitro* (Lewis *et al.*, 1993; Pickering *et al.*, 1994).

Whilst 5-HT$_{1A}$ receptors may play a role in the excitatory actions of 5-HT on vagal neurones, application of 5-HT$_{1A}$ receptor antagonists never abolished them suggesting that other 5-HT receptors may also be involved. Indeed, there is now evidence from both *in vivo* and *in vitro* studies that 5-HT$_2$ receptors may be important. Albert & Brooks (1994) reported that 5-HT excitations *in vitro* were mimicked by application of α-methyl-5-HT and usually antagonized by ketanserin whilst in experiments similar to those in the present study, Wang *et al.* (1995) demonstrated that the 5-HT excitations could also be attenuated by both ketanserin and cinanserin.

Application of larger currents often reduced the magnitude of 5-HT excitations and in some cases activity fell below baseline. This suggests that other receptors have inhibitory actions which, to varying degrees, mask the excitation. In a minority of cells 5-HT had only depressant actions. As the inhibitory effects required larger currents, it may be argued that they are mediated either by somatic receptors which require a higher concentration of 5-HT to be effective, or that the receptors are located at more distal sites, requiring greater diffusion of transmitter. In the present experiments inhibitory responses were never antagonized by (±)-pindolol or WAY-100635. Thus, they may be mediated by a different subtype of 5-HT receptor on the vagal motoneurones. Alternatively 5-HT may be activating an antecedent inhibitory interneurone as suggested in sympathetic preganglionic neurones where 5-HT inhibitions are antagonized by strychnine (Lewis *et al.*, 1993).

Some cells excited by 5-HT could also be excited by 8-OH-DPAT, an effect that was blocked by (±)-pindolol which might suggest an action on 5-HT$_{1A}$ receptors. However, Davies *et al.* (1988a) argued that 8-OH-DPAT-induced excitation of midline brainstem neurones is unlikely to involve 5-HT$_1$-like receptors since the currents required to produce excitation were up to 10 times greater than those for 5-HT. Whilst this may also be the case in the present experiments. In some cells low currents of 5-HT and 8-OH-DPAT both evoked excitation. The predominant effects of 8-OH-DPAT, flesinoxan and DP-5-CT in the present experiments were inhibitory and these could not be blocked by (±)-pindolol or WAY-100635. These depressant actions, like those of 5-HT, are unlikely to be due to non-specific local anaesthetic or membrane stabilizing actions of the drugs since the amplitude of the action potential were either unchanged or increased during such inhibitions. 5-HT and 5-HT$_{1A}$ agonists also have similar inhibitory actions on neurones in other brainstem regions including the RVLM (Wang & Lovick, 1992) and midline (Davies *et al.*, 1988b; McCall & Clement, 1989) and in those studies selective antagonism of the effects could not be observed.

When applied alone, (±)-pindolol and WAY-100635 had variable effects on baseline neuronal activity, some neurones were excited, some unaffected and others inhibited. The reasons for this are unclear. Whilst in some cases the effects may reflect inhibition of the effects of endogenously released 5-HT, this cannot explain all of the observed responses. In several cases application of these antagonists and the selective agonists to the same neurone evoked responses of the same sign. For example, when WAY-100635 and 8-OH-DPAT were applied to the same neurones they always produced the same effects on baseline firing rate. However, when applied to presumed 5-HT containing neurones in the dorsal raphe nucleus, then as expected, WAY-100635 antagonized the inhibitory effects of ionophoretically applied 8-OH-DPAT, confirming that at least at this site it appears to act as an antagonist of the 5-HT$_{1A}$ receptor agonist actions of 8-OH-DPAT (Forster *et al.*, 1995). The failure to uncover a similar antagonist effect on vagal motoneurones was surprising. However, there are reports in the literature that there are interactions between 5-HT and excitatory amino acid receptors. 5-HT can facilitate (Nedergaard *et al.*, 1986; Eaton & Salt, 1989; Charléty *et al.*, 1993) or attenuate (Sprouse & Aghajanian, 1988; Eaton & Salt, 1989; Charléty *et al.*, 1993) excitatory responses of both glutamate and excitatory amino acid receptor agonists. As many of the vagal neurones in our experiments were activated by DLH, it is possible that an interference with this excitation might account for the inhibitory effects of WAY-100635. This cannot be the entire explanation, however, since in the majority of neurones tested, WAY-100635 was able to attenuate the excitatory effects of 5-HT without altering the excitation produced by pulses of DLH.

In the present experiments vagal neurones were identified by antidromic activation from the cervical vagus nerve. The population of cells is unlikely to be functionally homogeneous. It is likely to include neurones innervating the gut, heart and airways (see Loewy & Spyer, 1990). Since 8-OH-DPAT had both excitatory and inhibitory actions on vagal motoneurones it is possible that each of the effects was related to different functional groups but this awaits further experiments. With respect to cardiac control, it is possible that the vagal neurones with chronotropic action are among those excited by 5-HT since previous experiments in rats have demonstrated that 5-HT$_{1A}$ receptor antagonists applied i.c. block the bradycardias evoked during cardiopulmonary afferent stimulation (Bogle *et al.*, 1990) and stimulation of the upper airways with smoke (Dando *et al.*, 1994).

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