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Evidence that central 5-HT_{2A} and 5-$HT_{2B/C}$ receptors regulate 5-HT cell firing in the dorsal raphe nucleus of the anaesthetised rat

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1 Systemic administration of phenethylamine-derived, 5-hydroxytryptamine$_2$ (5-HT_2) receptor agonists inhibits the firing of midbrain 5-HT neurones, but the 5-HT receptors involved are poorly defined, and the contribution of peripheral mechanisms is uncertain. This study addresses these issues using extracellular recordings of 5-HT neurones in the dorsal raphe nucleus of anaesthetised rats.

2 The 5-HT_2 receptor agonists DOI ((±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride) and DOB ((±)-2,5-dimethoxy-4-bromoamphetamine hydrobromide), caused a dose-related (10–100 $\mu g\,kg^{-1}$ i.v.) inhibition of 5-HT neuronal activity, with the highest dose reducing firing rates by >80%.

3 Pretreatment with the 5-HT_2 receptor antagonist ritanserin (1 $mg\,kg^{-1}$ i.v.) completely blocked the action of DOI. The 5-HT_{2A} receptor antagonist MDL 100,907 (0.2 $mg\,kg^{-1}$ i.v.) blocked the action of both DOI and DOB. In comparison, the 5-$HT_{2B/C}$ receptor antagonist SB 206553 (0.5 $mg\,kg^{-1}$ i.v.) caused a small, but statistically significant, shift to the right in the dose response to DOI and DOB.

4 Pretreatment with the peripherally acting 5-HT_2 receptor antagonist BW 501C67 (0.1 $mg\,kg^{-1}$ i.v.) had no effect on the DOI-induced inhibition of 5-HT cell firing, but completely blocked the DOI-induced rise in mean arterial blood pressure.

5 These data indicate that the inhibition of 5-HT cell firing induced by systemic administration of DOI and DOB is mediated predominantly by the 5-HT_{2A} receptor-subtype, but that 5-$HT_{2B/C}$ receptors also play a minor role. Moreover, central and not peripheral mechanisms are involved. Given evidence that 5-HT_2 receptors are not located on 5-HT neurones, postsynaptic 5-HT feedback mechanisms are implicated.
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Abbreviations: DRN, dorsal raphe nucleus; GABA, γ-aminobutyric acid; 5-HT, 5-hydroxytryptamine; ISI, inter-spike interval; MAP, mean arterial pressure; SSRI, selective serotonin reuptake inhibitor

Introduction

Feedback regulation is an essential aspect of the physiology of central 5-hydroxytryptamine (5-HT) neurones (Aghajanian, 1978). The role of presynaptic autoregulatory mechanisms in the control of 5-HT neurones is well recognised. Thus, somatodendritic 5-HT_{1A} autoreceptors regulate the firing of 5-HT neurones in the dorsal raphe nucleus (DRN), while 5-HT_{1B} autoreceptors regulate 5-HT release in terminal regions (Barnes & Sharp, 1999). Drug action at 5-HT autoreceptors has been linked to a range of behaviours including anxiolysis and changes in feeding and sexual behaviour (Barnes & Sharp, 1999). Moreover, knowledge of these autoreceptors has been fundamental to the development of novel antidepressant strategies. Specifically, evidence that 5-HT_{1A} autoreceptors are desensitised by selective serotonin reuptake inhibitors (Blier *et al*., 1990), has led to the use of 5-HT_{1A} receptor antagonists as antidepressant augmentation agents (Artigas *et al*., 2001).

In addition to 5-HT autoreceptors, recent reports indicate that 5-HT neurones are also regulated by postsynaptic 5-HT receptors. For example, experiments showing that cortical lesions attenuate the inhibitory effect of 5-HT_{1A} agonists on 5-HT cell firing suggest that postsynaptic 5-HT_{1A} receptors in cortical regions regulate the firing of DRN 5-HT neurones (Ceci *et al*., 1994; Hajós *et al*., 1999). A projection from the medial prefrontal cortex to the DRN has been proposed as the underlying anatomical substrate (Hajós *et al*., 1998; Varga *et al*., 2001).

5-HT_2 receptors are located postsynaptically (Palacios *et al*., 1991; Cornea-Hebert *et al*., 1999; Verge & Calas, 2000) and there is evidence that these receptors may also regulate 5-HT neurotransmission. Early experiments indicated that the phenethylamine derivative, DOM (1-(2,5-di-methoxy-4-methylphenyl)-2-aminopropane), inhibits the firing of DRN 5-HT neurones *in vivo* (Aghajanian *et al*., 1970). Later work found that DOI ((±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride) has a similar effect and causes an associated decrease in 5-HT release (Wright *et al*., 1990; Garratt *et al*., 1991). Although DOM and DOI are 5-HT_2 receptor agonists, the role of 5-HT_2 receptors in the inhibition of 5-HT cell firing is uncertain. Thus, one detailed study found that the nonselective 5-HT_2 receptor antagonists, ritanserin and ketanserin, did not block the effects of DOI (Garratt *et al*., 1991). On the other hand, a recent paper reported two instances in which the firing of a 5-HT neurone was inhibited by DOI and then completely restored by the 5-HT_{2A} selective antagonist

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MDL 100,907 (Martin-Ruiz *et al*., 2001). The latter result is seemingly at odds with *in vitro* electrophysiological data that implicates roles for both 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors in the regulation of 5-HT cell firing (Liu *et al*., 2000). A final complicating factor is the proposal that peripheral and not central 5-HT$_2$ receptors are involved in the DOI-induced inhibition of 5-HT neuronal firing (Penington & Reiffenstein, 1986).

The present study tested the effect of certain phenethylamine-derived 5-HT$_2$ agonists on the firing of 5-HT neurones in the rat DRN *in vivo*, and used recently available selective antagonists to characterise the 5-HT receptor subtypes involved. The peripheral antagonist, BW 501C67 (Mawson & Whittington, 1970; Fuller *et al*., 1986), was used to establish whether the effect of 5-HT$_2$ agonists is mediated centrally or *via* peripheral mechanisms. Preliminary accounts of some of these experiments were presented to the British Pharmacological Society (Boothman *et al*., 2001, 2003).

Methods

Animals

All procedures were carried out in accordance with the U.K. Home Office Animals (Scientific Procedures) Act (1986) and associated Home Office guidelines. Male Sprague–Dawley rats (240–320 g; Harlan Olac, Bicester, U.K.) were housed in groups under conditions of constant temperature ($21 \pm 1^{\circ}$C) and humidity under a 24 h light–dark cycle (lights on 08:00–20:00 h) with food and water freely available. In all experiments, rats were anaesthetised with chloral hydrate (460 mg kg^{-1} i.p.) with additional doses (60–120 mg kg^{-1} i.p.) administered as required, supplemented with a single dose of saffan (1.2 mg kg^{-1} i.v.) during surgery. A lateral tail vein was cannulated for drug administration. Body temperature was maintained at 36°C using a thermoregulated heating pad.

Electrophysiological recording of 5-HT neuronal activity

Extracellular single-unit recordings of 5-HT neurones were made essentially as described previously (Hajós *et al*., 1998). Single barrel glass electrodes (filled with 2 M NaCl containing 2% pontamine sky blue, *in vitro* resistance 6–20 MΩ) were lowered under stereotaxic control into the DRN (coordinates of A/P −7.5 mm, L/M 0.0 mm D/V −4.5 mm to −5.5 mm; Paxinos and Watson, 1986). Single-unit potentials were amplified and filtered (Gain 1 k; 500 Hz to 1.5 kHz band pass; Neurolog system, Digitimer Ltd., Welwyn Garden City, U.K.), captured using a 1401*plus* interface system and analysed off-line using Spike2 software (Version 4.01) (Cambridge Electronic Design, Cambridge, U.K.).

5-HT neurones were identified on the basis of their electrophysiological characteristics (Hajós *et al*., 1995). All cells included in this study fulfilled at least three of the following criteria: slow firing rate (<2 Hz), regular firing pattern (typical coefficient of variation <0.5), triphasic extracellular waveform with a wide action potential duration (>1.5 ms) and an inhibitory response to administration of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (10 μg kg^{-1} i.v.). Most 5-HT neurones discharged action potentials in single spikes, but some 5-HT neurones discharging both single spikes and spikes in very short bursts (Hajós *et al*., 1995) were included.

Following a 5 min period of baseline recording, agonists were administered in increasing doses (10, 20, 40, 80, 100 μg kg^{-1} i.v. at 2 min intervals), either alone or in animals pretreated for 5 min with an antagonist. In one set of experiments, DOI was administered alone or in animals pretreated with 1 mg kg^{-1} i.v. ritanserin (5-HT$_2$ receptor antagonist), 0.2 mg kg^{-1} i.v. MDL 100,907 (5-HT$_{2A}$ receptor antagonist) or 0.5 mg kg^{-1} i.v. SB 206553 (5-HT$_{2B/C}$ receptor antagonist). In a second set of experiments, (±)-2,5-dimethoxy-4-bromoamphetamine hydrobromide (DOB) was administered alone or in animals pretreated with 0.2 mg kg^{-1} i.v. MDL 100,907 or 0.5 mg kg^{-1} i.v. SB 206553. In a third set of experiments, DOI was administered alone or in animals pretreated with 0.1 mg kg^{-1} i.v. BW 501C67 (peripheral 5-HT$_2$ receptor antagonist).

In animals pretreated with ritanserin and MDL 100,907, a vehicle (5% glucose solution with 20 μl 100% acetic acid) was injected 3 min prior to the antagonist. When neurones were 'lost' during recording, an alternative neurone was sought if no more than the first dose of agonist had been given. At the end of every experiment, a small amount of dye was expelled by iontophoresis (−3.6 mA, pulses of 200 ms duration with 21 ms interpulse interval for 30 min) and the location of the electrode was confirmed histologically.

Firing rates were quantified in the final 1 min of each baseline and post-vehicle/drug interval. The regularity of cell firing was calculated over the same period using the coefficient of variation analysis (standard deviation of interspike interval/interspike interval mean). Neurones discharging spikes in short bursts were analysed using the first spike of each burst.

Recordings of arterial blood pressure

In additional experiments arterial blood pressure was measured in chloral hydrate-anaesthetised rats. A carotid artery was cannulated, connected to a pressure transducer and the signal was captured (1401*plus* C.E.D. interface) and analysed off-line (Spike2 version 4.01 software, Cambridge Electronic Design, U.K.). Following a 5 min baseline period of stable blood pressure, either saline (5 × 0.1 ml kg^{-1}) or DOI (10, 20, 40, 80, 100 μg kg^{-1} i.v.) was injected at 2 min intervals. In some experiments, 0.1 mg kg^{-1} i.v. BW 501C67 was injected 5 min prior to DOI.

Mean arterial pressure (MAP) was measured over the final 1 min of each baseline and post-saline/drug interval. Systolic (S) and diastolic pressure (D) were discriminated and MAP was calculated using the formula, $D + 1/3(S-D)$, with values averaged over each 1 min period.

Statistical analysis

For electrophysiological experiments, the effect of agonist alone was tested by comparing the firing rate at each dose with pre-drug values using one-way ANOVA and Dunnett's *post hoc* test (Graph Pad Prism Software, San Diego, CA, USA). The effect of antagonist was tested by comparing the firing rate after each agonist dose in the presence and absence of

antagonist using two-way ANOVA and Bonferroni's *post hoc* test across all drug conditions in each set of experiments. The effect of antagonist and vehicle treatments on 5-HT neurone firing rate, and of all drugs on the regularity of 5-HT neurone firing were tested by comparing pre- and post-drug values using paired two-tailed Student's *t*-test.

Data from blood pressure experiments, testing the effect of DOI against saline or pretreatment with BW 501C67, were analysed across all drug conditions using two-way ANOVA and Bonferroni's *post hoc* test. The effect of BW 501C67 alone was tested by comparing pre- and post-drug values using a two-tailed Student's *t*-test. For all experiments, mean ± s.e.m. values are presented. *P*-values of 0.05 or less were considered statistically significant.

Drugs

The drugs used (from the sources indicated) were: DOI (Sigma-Aldrich, U.K.), DOB (Sigma-Aldrich, U.K.), (±)-8-hydroxy-2-(dipropylamino)-tetralin (8-OH-DPAT; Sigma-Aldrich, U.K.), *n*-3-pyridinyl-3,5-dihydro-5-methyl-benzo (1,2-b:4,5-b′)dipyrrole-1(2H)carboxamide (SB 206553; Sigma-Aldrich, U.K.), ritanserin (gift, Janssen Phamaceuticals, Beerse, Belgium), *R*-(+)-α-(2,3-dimethoxyphenil)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol (MDL 100,907; gift, Eli Lilly & Co., Windlesham, U.K.) and α-anilino-*N*-2-m-chlorophenoxypropylacetamidine (BW 501C67; generous gift from Dr A. Ramage). All drugs were dissolved in 0.9% saline, except ritanserin and MDL 100,907 which were dissolved in 5% glucose solution with 20 μl 100% acetic acid and brought to pH 5 with 5 M NaOH.

Results

Electrophysiological characteristics of 5-HT neurones

A total of 83 presumed 5-HT neurones in the DRN were recorded. Of these, 9 were excluded from the analysis due to an atypical response to 5-HT$_2$ agonist administration (see later). The 74 neurones analysed fired broad triphasic spikes (waveform length 3.29 ± 0.10 ms) in a slow and regular firing pattern (baseline firing rate, 0.96 ± 0.06 Hz; baseline coefficient of variation, 0.31 ± 0.01).

Effect of DOI and DOB on 5-HT neuronal activity

Systemic administration of DOI (10–100 μg kg^{-1} i.v.) caused a dose-related inhibition of 5-HT cell firing compared to predrug values (Figures 1 and 2a). This effect was statistically significant at 20 μg kg^{-1}, and the highest dose tested reduced firing by 83% (range 57–100%) of pre-drug values (one-way ANOVA: [$F_{5,40} = 22.1$] $P < 0.0001$; Dunnett's *post hoc* test $P < 0.05$ 20 μg kg^{-1}, $P < 0.001$ 40–80 μg kg^{-1}) (Figure 3a). Similarly, DOB (10–100 μg kg^{-1} i.v.) caused a marked and dose-related inhibition in 5-HT cell firing to 84% (range 59–100%) of pre-drug values (one-way ANOVA: [$F_{5,24} = 15.5$] $P < 0.0001$; Dunnett's *post hoc* test $P < 0.01$ 20–100 μg kg^{-1}) (Figure 3b). Neither DOI nor DOB altered the regularity of 5-HT cell firing (Figure 5b).

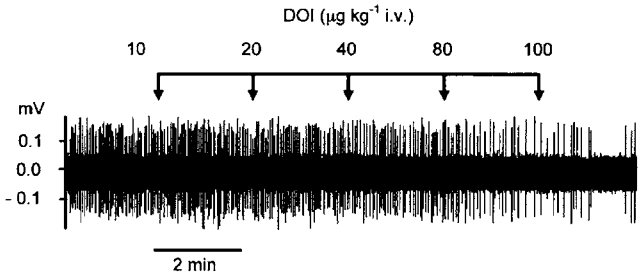

Figure 1 Effect of the 5-HT$_2$ agonist DOI on the firing of a 5-HT neurone in the DRN of an anaesthetised rat. Each vertical line in the spike train represents a single action potential. DOI was administered in increasing doses at 2 min intervals as indicated.

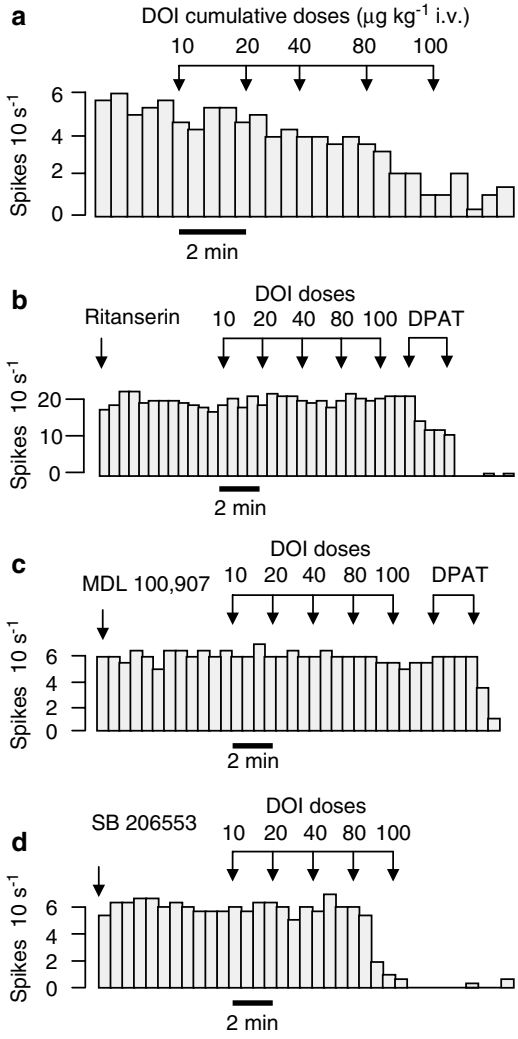

Figure 2 Rate metre recordings demonstrating the effect of DOI on 5-HT cell firing either (a) alone or in rats pretreated with (b) the 5-HT$_2$ receptor antagonist ritanserin (1 mg kg^{-1} i.v.), (c) the 5-HT$_{2A}$ receptor antagonist MDL 100,907 (0.2 mg kg^{-1} i.v.) or (d) the 5-HT$_{2B/C}$ receptor antagonist SB 206553 (0.5 mg kg^{-1} i.v.). Antagonists were administered 5 min prior to DOI, which was given in increasing doses at 2 min intervals. Note also the characteristic inhibitory response of the 5-HT neurones to the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (10 μg kg^{-1} i.v.).

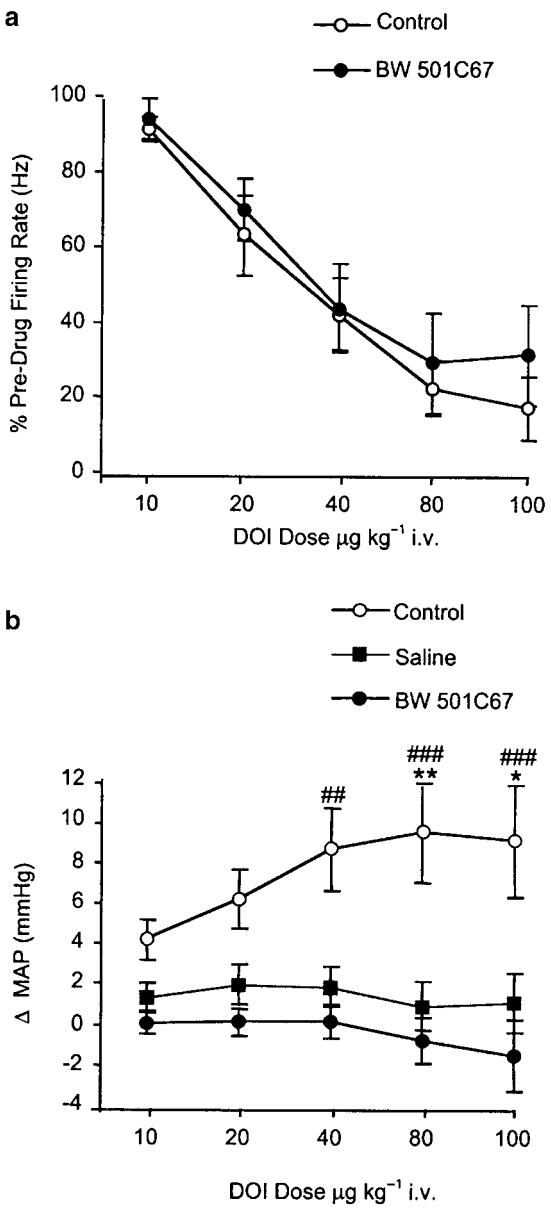

Figure 3 Effect of DOI (a) and DOB (b) in the presence of the 5-HT$_2$ receptor antagonist ritanserin (1 mg kg^{-1} i.v.), the 5-HT$_{2A}$ receptor antagonist MDL 100,907 (0.2 mg kg^{-1} i.v.) or the 5-HT$_{2B/C}$ receptor antagonist SB 206553 (0.5 mg kg^{-1} i.v.). Antagonists were administered 5 min prior to either DOI or DOB, which was given in increasing doses at 2 min intervals. Controls received DOI or DOB alone. Data points are mean ± s.e.m. of n observations at agonist doses of 10, 20, 40, 80, 100 μg kg^{-1} respectively: (a) control $n=8,8,8,8,6$; ritanserin $n=6,6,5,4,4$; MDL 100,907 $n=7,6,4,4,4$; SB 206553 $n=7,6,6,5,5$; (b) control $n=5,5,5,5,5$; MDL 100907 $n=3,3,3,3,3$; SB 205663 $n=8,8,8,6,6$. $^{**}P<0.01$, $^{***}P<0.001$ for control *versus* ritanserin, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ for control *versus* MDL 100907, $^{+}P<0.05$, $^{++}P<0.01$ for control *versus* SB 206553 (two-way ANOVA with Bonferroni's *post hoc* test).

Effect of DOI and DOB on 5-HT neuronal activity in the presence of 5-HT$_2$ receptor antagonists

Pretreatment with the nonselective 5-HT$_2$ receptor antagonist ritanserin (1 mg kg^{-1} i.v.) completely blocked the inhibition of 5-HT cell firing induced by DOI (10–100 μg kg^{-1} i.v.) (Figures 2b and 3a). Pretreatment with the selective 5-HT$_{2A}$ antagonist MDL 100,907 (0.2 mg kg^{-1} i.v.) also blocked the inhibition of 5-HT cell firing induced by DOI (10–100 μg kg^{-1} i.v.) (Figures 2c and 3a). Pretreatment with the 5-HT$_{2B/C}$ antagonist SB 206553 (0.5 mg kg^{-1} i.v.) caused a moderate attenuation of the effect of DOI as indicated by a rightward shift in the dose response (two-way ANOVA: interaction [$F_{12,97}=5.0$] $P<0.0001$, treatment [$F_{3,97}=32.9$] $P<0.0001$, dose [$F_{4,97}=17.3$] $P<0.0001$ (Figures 2d and 3a).

Pretreatment with MDL 100,907 (0.2 mg kg^{-1} i.v.) also blocked the inhibitory effect of DOB (10–100 μg kg^{-1} i.v.). In comparison, SB 206553 caused a small but statistically significant shift to the right in the dose response to DOB although this was not as clearcut as with DOI (two-way ANOVA: interaction [$F_{8,61}=1.2$] $P>0.05$, treatment [$F_{2,61}=21.4$] $P<0.0001$, dose [$F_{4,61}=7.3$] $P<0.0001$; subsequent two-way ANOVA for control *versus* SB 206553 revealed a significant effect of treatment [$F_{1,51}=4.3$] $P<0.05$) (Figure 3b).

Effects of DOI on 5-HT neuronal activity and blood pressure in the presence of peripheral 5-HT$_2$ antagonists

Pretreatment with the peripheral 5-HT$_2$ antagonist BW 501C67 (0.1 mg kg^{-1} i.v.) had no effect on the DOI-induced inhibition of 5-HT cell firing (two-way ANOVA: interaction [$F_{4,60}=0.1$] $P>0.05$, treatment [$F_{1,60}=1.2$] $P>0.05$, dose [$F_{4,60}=20.0$] $P<0.0001$) (Figure 4a).

In separate experiments, DOI induced a dose-related (10–100 μg kg^{-1} i.v.) increase in MAP compared to saline controls. BW 501C67 (0.1 mg kg^{-1} i.v.) abolished this effect of DOI (two-way ANOVA: interaction [$F_{8,65}=1.0$] $P>0.05$, treatment [$F_{2,65}=38.2$] $P<0.0001$, dose [$F_{4,65}=0.5$] $P>0.05$) (Figure 4b). BW 501C67 (0.1 mg kg^{-1} i.v.) alone did not alter MAP (two-tailed Student's t-test, $P>0.05$).

Effect of 5-HT$_2$ antagonists and vehicles alone on 5-HT neuronal activity

None of the 5-HT receptor antagonists tested (1 mg kg^{-1} i.v. ritanserin, 0.2 mg kg^{-1} i.v. MDL 100,907, 0.5 mg kg^{-1} i.v. SB 206553 or 0.1 mg kg^{-1} i.v. BW 501C67) altered either the rate or regularity of 5-HT cell firing (paired two-tailed Student's t-tests $P>0.05$). Similarly, these parameters were not altered by saline or 5% glucose solution with acetic acid vehicles (paired two-tailed Student's t-tests, $P>0.05$) (Figure 5).

Excitation of 5-HT neurones by DOI and DOB

A small number of 5-HT neurones (9/83) were found to increase in firing rate in response to DOI or DOB administration (10–100 μg kg^{-1} i.v.). This increase ranged between 112 and 195% of predrug levels, and was detected both in the presence and absence of antagonist pretreatment (DOI alone $n=1$ cell, ritanserin/DOI $n=1$, MDL 100,907/DOI $n=1$, MDL 100,907/DOB $n=1$, SB 206553/DOB $n=2$, BW 501C67/DOI $n=3$).

Discussion

Systemic administration of phenethylamine-derived 5-HT$_2$ agonists inhibits the firing of 5-HT neurones, but the role of

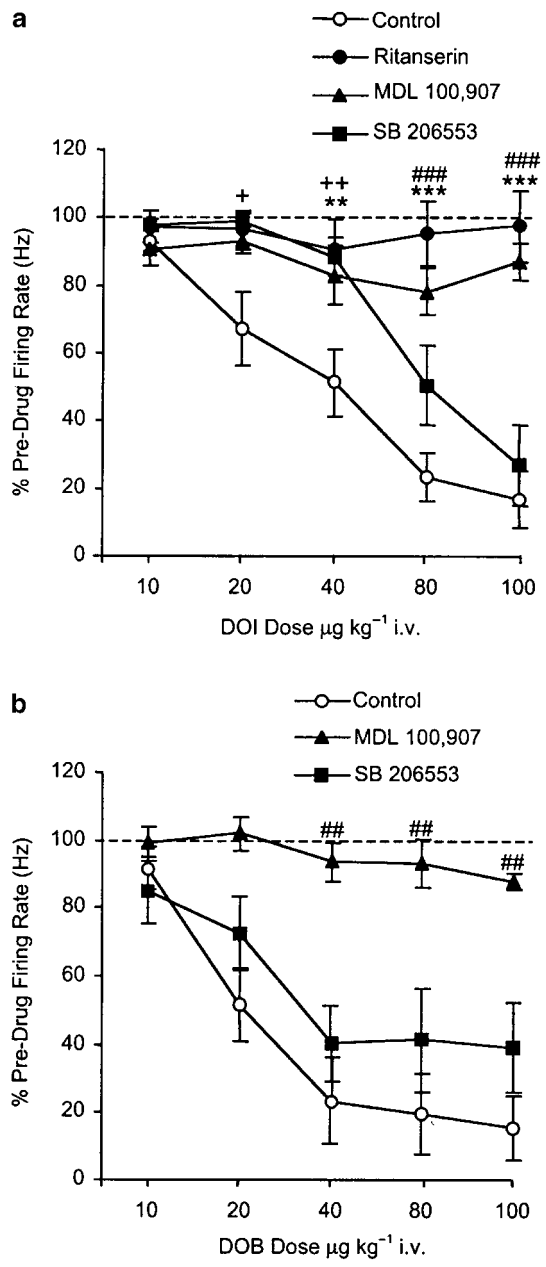

Figure 4 Effect of DOI on 5-HT neuronal activity (a) and mean arterial blood pressure (b) in the presence and absence of the peripheral 5-HT$_2$ receptor antagonist BW 501C67 (0.1 mg kg^{-1} i.v.). DOI was given in increasing doses at 2 min intervals. When tested, BW 501C67 was administered 5 min prior to DOI. Controls received DOI alone and the saline condition received five sequential injections of saline. Data points are mean ± s.e.m. of n observations at agonist doses of 10, 20, 40, 80,100 μg kg^{-1} respectively: (a) control n = 8,8,8,8,6; BW 501C67 n = 8,7,6,6,5; (b) control n = 6,6,6,6,6; saline n = 4,4,4,4,4; BW 501C67 n = 6,6,6,6,6. $^{*}P<0.05$, $^{**}P<0.01$ for control *versus* saline, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ for control *versus* BW 501C67 (two-way ANOVA with Bonferroni's *post hoc* test).

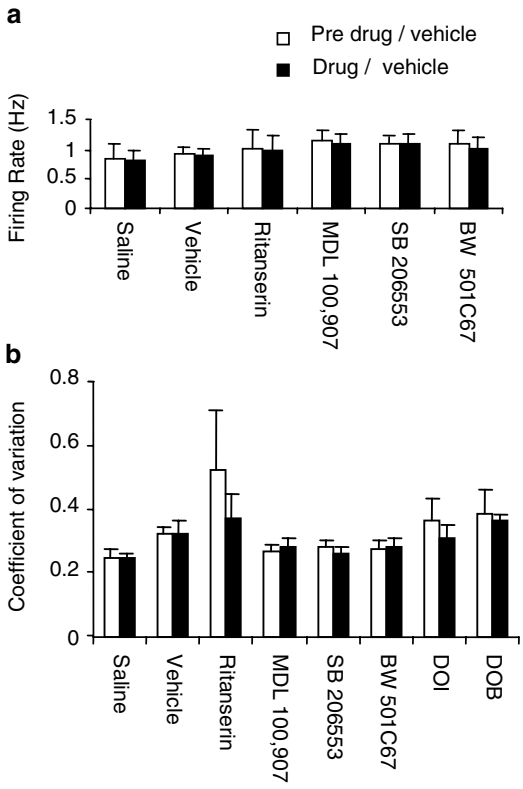

Figure 5 Effect of 5-HT receptor antagonists and vehicle alone on the firing rate (a), and firing regularity (b) of 5-HT neurones. Data for 5-HT$_2$ receptor agonists are also included in (b). Measurements were made during the final 1 min of a 5 min pretreatment for antagonists or a 2 min period for agonist or vehicle. Doses were ritanserin (1.0 mg kg^{-1} i.v.), MDL 100,907 (0.2 mg kg^{-1} i.v.), SB 206553 (0.5 mg kg^{-1} i.v.), BW 501C67 (0.1 mg kg^{-1} i.v.), DOI (40 μg kg^{-1} i.v.), DOB (40 μg kg^{-1} i.v.). Data are mean ± s.e.m. from groups of 3–10 rats. $P>0.05$ *versus* predrug values (Student's two-tailed paired t-tests).

specific 5-HT$_2$ receptor subtypes is uncertain, and a peripheral site of action has been suggested (see Introduction). The present study addresses these issues using extracellular recordings of 5-HT neurones in the DRN of anaesthetised rats.

It was found that the phenethylamine-derived 5-HT$_2$ agonists DOI or DOB inhibit the firing of 5-HT neurones, in confirmation of earlier studies testing DOI and DOM (Aghajanian *et al*., 1970; Wright *et al*., 1990). Pretreatment with the 5-HT$_2$ receptor-selective antagonist ritanserin completely blocked the inhibitory action of DOI, implicating the involvement of 5-HT$_2$ receptors in this effect. Although an earlier study found that ritanserin did not block this effect of DOI (Garratt *et al*., 1991), the dose of ritanserin was lower than that used here (0.5 *versus* 1 mg kg^{-1} in current experiments), and the pretreatment period was longer (20 *versus* 5 min).

Pretreatment with the 5-HT$_{2A}$ receptor-selective antagonist MDL 100,907 (Kehne *et al*., 1996), at a dose (0.5 mg kg^{-1}) that causes full occupancy of 5-HT$_2$ receptors in animal PET studies (Hirani *et al*., 2003), also blocked the inhibition of 5-HT cell firing induced by DOI and DOB. These findings are in accordance with a recent paper describing two instances in which the firing of a DRN 5-HT neurone was inhibited by DOI and then restored by MDL 100,907 (Martin-Ruiz *et al*., 2001). These observations suggest that 5-HT$_{2A}$ receptors play a major role in the 5-HT$_2$ agonist-induced inhibition of 5-HT cell firing; however, other data in the present study also suggest the involvement, albeit minor, of 5-HT$_{2B/C}$ receptors. Thus, the 5-HT$_{2B/C}$ receptor-selective antagonist SB 206553 (0.5 mg kg^{-1} i.v.) caused a small, but statistically significant, shift to the right in the dose response to both DOI and DOB.

SB 206553 has over a 100-fold higher affinity for 5-HT$_{2B/C}$ binding sites *versus* 5-HT$_{2A}$ and other 5-HT receptor subtypes *in vitro* (pK_I 5-HT$_{2A}$ = 5.8; pA_2 5-HT$_{2B}$ = 8.9; pK_I 5-HT$_{2C}$ = 7.9; Kennett *et al*., 1996). Moreover, SB 206553 acts as a potent antagonist in *in vivo* models of 5-HT$_{2C}$ function with an ID_{50} of 0.3 mg kg^{-1} i.v. (Kennett *et al*., 1996; Millan *et al*., 1997) and completely reverses 5-HT$_2$ receptor agonist-induced decreases in dopamine neurone firing at the dose used in the present study (Gobert *et al*., 2000). The low levels of 5-HT$_{2B}$ receptors in the rat brain (Barnes & Sharp, 1999) make it more likely that 5-HT$_{2C}$ receptors are involved. It is possible that the role of 5-HT$_{2C}$ receptors may be underestimated in the present experiments, as both DOI and DOB have a marginally higher affinity for 5-HT$_{2A}$ compared to 5-HT$_{2C}$ receptor subtypes (K_i values–DOI: 5-HT$_{2A}$ = 19 nM, 5-HT$_{2C}$ = 30 nM; DOB: 5-HT$_{2A}$ = 41 nM, 5-HT$_{2C}$ = 70 nM; Glennon *et al*., 1992).

In this study, a small number of 5-HT neurones increased in activity in response to administration of DOI or DOB. An excitatory response of a subpopulation of 5-HT neurones to 5-HT$_2$ agonists has been reported in previous studies both *in vivo* (Trulson *et al*., 1981; Martin-Ruiz *et al*., 2001) and *in vitro* (Liu *et al*., 2000), and has been likened to the excitatory action of the unsubstituted phenethylamine, amphetamine, on DRN 5-HT neurones (Aghajanian *et al*., 1970). In this study, DOI or DOB excited cells even in animals pretreated with ritanserin, MDL 100,907 or SB 206553 indicating that this effect is not 5-HT$_2$ receptor mediated.

On the basis of observations that the decrease in 5-HT cell firing induced by DOI occurs together with a rise in blood pressure, it has been suggested that these effects are linked and that the site of action of DOI is not central but peripheral vascular 5-HT$_2$ receptors (Penington & Reiffenstein, 1986). Importantly, in the present study it was found that pretreatment with the peripherally acting 5-HT$_2$ antagonist BW 501C67 (Mawson & Whittington, 1970; Knowles & Ramage, 1999) had no effect on the DOI-induced inhibition of 5-HT cell firing, even although the drug completely blocked the peripheral 5-HT$_2$ receptor-mediated pressor response as reported previously (Fuller *et al*., 1986). These results not only implicate a central site of action of DOI, but provide further evidence that 5-HT cell firing in the DRN can change independently of alterations in blood pressure (Foote *et al*., 1969; Fornal *et al*., 1990).

The present data suggest that the inhibition of 5-HT neuronal activity by phenethylamine 5-HT$_2$ agonists is mediated centrally, and there are a number of putative neuroanatomical substrates that might be involved. Although 5-HT$_2$ receptors are not located on 5-HT neurones (Cornea-Hebert *et al*., 1999), a direct action of phenethylamines within the DRN is supported by the presence of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor mRNA in this region (Wright *et al*., 1995), and a report that DOI inhibits 5-HT cell firing when locally applied (Garratt *et al*., 1991). Also, a recent *in vitro* electrophysiological study found evidence that 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors located on GABA interneurones within the DRN mediate a local inhibitory feedback onto the adjacent 5-HT neurones (Liu *et al*., 2000). Alternative or additional sites of action include the 5-HT$_2$ receptor-mediated regulation of DRN afferents such as inputs from the medial prefrontal cortex (Hajós *et al*., 1998; Martin-Ruiz *et al*., 2001), lateral habenula (Aghajanian & Wang, 1977) and locus coeruleus (Peyron *et al*., 1996).

The putative 5-HT$_2$ receptor feedback system does not appear to be tonically active, as the 5-HT$_2$ receptor antagonists alone had no effect on 5-HT cell firing rate, and previous studies indicate that these drugs do not alter brain extracellular 5-HT (Ichikawa *et al*., 1998). Endogenous 5-HT might, however, activate 5-HT$_2$ receptor-mediated feedback pathways under conditions of elevated 5-HT such as in the presence of a 5-HT uptake inhibitor. It is noteworthy that although the inhibition of 5-HT cell firing by 5-HT uptake inhibitors can be reversed by WAY 100635, this effect is often short lasting and is not detected in all neurones (Gartside *et al*., 1995).

In conclusion, the present data suggest that the inhibition of DRN 5-HT neurones by the phenethylamine 5-HT$_2$ agonists, DOI and DOB, involves activation of central 5-HT$_2$ receptors of the 5-HT$_{2A}$ subtype, with the 5-HT$_{2B/C}$ subtype also playing a minor role. As evidence suggests that 5-HT$_2$ receptors are not located on 5-HT neurones, a postsynaptic 5-HT$_2$ receptor-mediated feedback loop is implicated. Since antagonists of other 5-HT feedback pathways (5-HT autoreceptors) have SSRI augmentation properties (see Introduction), 5-HT$_2$ receptor antagonists may also have this potential.

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