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Modulation of the vagal bradycardia evoked by stimulation of upper airway receptors by central 5-HT_1 receptors in anaesthetized rabbits

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1 The effects of central application of 5-HT_{1A} and 5-$HT_{1B/1D}$ receptor ligands on the reflex bradycardia, apnoea, renal sympathoexcitation and pressor response evoked by stimulating upper airway receptors with smoke in atenolol-pretreated anaesthetized rabbits were studied.

2 Intracisternal administration of the 5-HT_{1A} receptor antagonists WAY-100635 (100 μg kg^{-1}) and (−)pindolol (100 μg kg^{-1}) significantly reduced the smoke-induced bradycardia, attenuated the pressor response and in the case of (−)pindolol, sympathetic nerve activity. The same dose of WAY-100635 i.v. was without effect.

3 Buspirone (200 μg kg^{-1}, i.c.) potentiated the reflex bradycardia. This action was prevented if the animals were pretreated with WAY-100635 (100 μg kg^{-1}, i.v.)

4 (+)8-OH-DPAT (25 μg kg^{-1}, i.c.) attenuated the evoked bradycardia, pressor response, apnoea and renal sympathoexcitation. The attenuation of the apnoea and renal sympathoexcitation, but not the bradycardia or pressor response was prevented in animals pretreated with WAY-100635 (100 μg kg^{-1}, i.v.). The attenuation of the reflex bradycardia and the reduction in the renal sympathoexcitation were reduced by pretreatment with the 5-$HT_{1B/1D}$ receptor antagonist GR127935 (100 μg kg^{-1}, i.v.).

5 In WAY-100635 (100 μg kg^{-1}, i.v.) pretreated animals, sumatriptan (a 5-$HT_{1B/1D}$ receptor agonist) reduced the reflex bradycardia and the pressor response. The 5-$HT_{1B/1D}$ receptor antagonist GR127935 (20 μg kg^{-1}, i.c. or 100 μg kg^{-1}, i.v.) had no effect on the reflex responses.

6 In conclusion, the present data are consistent with the hypothesis that activation of central 5-HT_{1A} receptors potentiate whilst activation of 5-$HT_{1B/1D}$ receptors attenuate the reflex activation of cardiac preganglionic vagal motoneurones evoked by stimulation of upper airway receptors with smoke in rabbits.

Keywords: 5-HT_{1A} receptors; 5-$HT_{1B/1D}$ receptors; upper airways; preganglionic cardiac vagal motoneurones; buspirone; sumatriptan, (+)8-OH-DPAT; GR127935; WAY-100635

Introduction

Injections of 5-HT_{1A} receptor agonists intravenously (Ramage & Fozard, 1987; McCall *et al*., 1994), into the fourth ventricle (Shepheard *et al*., 1994) or into brain nuclei containing cardiac vagal preganglionic neurones, the nucleus ambiguus (Izzo *et al*., 1988; Chitravanshi & Calaresu, 1992) and dorsal motor vagal nucleus (Sporton *et al*., 1991) cause an increase in cardiac vagal tone. In addition, ionophoretic application of the 5-HT_{1A} receptor agonist (+)8-OH-DPAT activates a small population of vagal preganglionic neurones in the dorsal motor vagal nucleus and this is blocked by the application of 5-HT_{1A} receptor antagonists (Wang *et al*., 1995, 1996). Binding sites for 5-HT_{1A} receptors have been localized in both the nucleus ambiguus and the dorsal vagal nucleus in cats (Dashwood *et al*., 1988), rats (Pazos & Palacios, 1985; Thor *et al*., 1992) and humans (Pazos *et al*., 1987). In addition, 5-HT immunoreactive nerve fibres innervate these regions (Steinbusch, 1981) and make synaptic contact with cardiac vagal motoneurones (Izzo *et al*., 1988, 1993). The physiological function of this pathway is unknown. However, 5-HT_{1A} receptor antagonists acting centrally have been shown to block the increase in vagal drive to the heart caused by activation of cardiopulmonary afferents (Bogle *et al*., 1990), suggesting that this pathway is important in the reflex excitation of cardiac preganglionic vagal motoneurones. In this respect, in anaesthetized rabbits, central 5-HT_{1A} receptors have been implicated in the increased cardiac vagal tone caused by activation of upper airway receptors (Futuro-Neto *et al*., 1993). However, in those experiments 8-OH-DPAT attenuated whilst buspirone potentiated the reflex increase in cardiac vagal tone. This observation was somewhat unexpected, since, if it is assumed that 8-OH-DPAT is acting as an agonist and buspirone as a partial agonist (Schoeffter & Hoyer, 1988; Boddeke *et al*., 1992), it would suggest that activation of 5-HT_{1A} receptors reduces the excitability of cardiac vagal preganglionic neurones. Such a conclusion is opposite to that indicated by the previous data, which suggest that activation of central 5-HT_{1A} receptors should cause excitation of cardiac preganglionic vagal neurones. Therefore the present experiments were carried out to investigate further this effect of buspirone and (+)8-OH-DPAT on the reflex bradycardia evoked by stimulating upper airway receptors with smoke in atenolol-pretreated anaesthetized rabbits. Atenolol is a selective β_1-adrenoceptor antagonist which does not bind to 5-HT receptors (Middlemiss *et al*., 1977) and poorly penetrates the central nervous system (Street *et al*., 1979). Therefore, changes in cardiac preganglionic vagal neuronal activity can be inferred from changes in heart rate and/or R-R interval (Bogle *et al*.,

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1990; Futuro-Neto *et al*., 1993). Preliminary accounts of some of these observations have been published (Dando *et al*., 1994a, b; 1996)

Methods

Experiments were performed on male New Zealand White rabbits (1.8–3.0 kg body weight) anaesthetized with urethane (1.5 g kg^{-1} given *via* an ear vein) and supplemented with 0.15 g kg^{-1}, i.v. when necessary. The level of anaesthesia was assessed by continuously monitoring the stability of arterial blood pressure, heart rate, phrenic nerve discharge, and the absence of limb withdrawal in response to paw pinch. A bi-directional tracheotomy was performed below the larynx. This allowed ventilation of the lungs with room air whilst also providing access to the upper airways. The mouth was blocked with a small piece of cotton wool soaked in saline to ensure that smoke passed rostrally from the tracheal cannula, exited *via* the nostrils. The right and left axillary arteries were cannulated for measurement of arterial blood pressure using a pressure transducer (Gould Statham) and to allow arterial blood samples to be taken. Arterial blood gases were measured (Corning 238 pH/blood gas analyser) and kept within the ranges P_{O2} 100–140 mmHg, P_{CO2} 30–40 mmHg, $[HCO_3^-]$ 12–20 mMol l^{-1} and pH 7.30–7.45. The left and right axillary veins were cannulated for administration of drugs and an infusion respectively. The infusion solution, which maintained blood volume and prevented non-respiratory acidosis, consisted of 50 ml each of distilled water and gelofusin containing 0.84% (w/v) sodium bicarbonate and 0.2% glucose and was infused at 6 ml kg^{-1} h^{-1}. The bladder was also cannulated to prevent overfilling during the course of the experiments. Lead II E.C.G. was recorded for measurement of R-R intervals. Following placement in a stereotaxic head frame, a midline incision was made in the skin extending from the cervical vertebrae to the lamboid suture. The muscles were retracted by blunt dissection and the atlanto-occipital membrane exposed. A needle, constructed from 28 gauge hollow stainless steel tubing and connected to a 100 μl Hamilton syringe by polythene tubing (0.28 mm int. diam.) was used to pierce the membrane and gain access to the cisternal space. The phrenic nerve was isolated low in the neck by a lateral approach, cut, and the central end placed on bipolar silver wire recording electrodes. Activity was amplified (Neurolog NL104, Digitimer) and filtered (NL125 Digitimer, 0.2–2 kHz). The left renal nerve was isolated by a retro-peritoneal approach, placed on bipolar silver wire recording electrodes and isolated from the surrounding medium with President light body dental polyvinylsiloxane (Coltene). Renal nerve activity (RNA) was amplified (NL104), filtered (NL125, 100–500 Hz) and integrated (NL703, 20 ms time constant).

Protocol

A schematic diagram of the protocol used in these experiments is shown in Figure 1. Smoke, produced from commercially available nicotine-free herbal cigarettes (Honeyrose Products), was collected in a 50 ml glass syringe and blown retrogradely through the upper airways at constant rate (over approximately 5 s). Smoke challenges were performed at 10 min intervals using a volume of smoke (15–45 ml) which produced a submaximal bradycardia. In the intervening periods, warmed and humidified air was passed through the upper airways at 10 ml s^{-1}, to prevent smoke remaining in the airways, and to control for possible activation of airway receptors by changing flows, pressures or temperature (Sant'Ambrogio & Sant'Ambrogio, 1997). Smoke challenges were repeated until three consecutive heart rate responses of similar magnitude were produced. Five minutes following the third smoke challenge the selective β_1-adrenoceptor antagonist atenolol (1 mg kg^{-1}, i.v.) was administered. Five minutes later the smoke challenges were resumed until three consecutive responses of similar magnitude were produced. The test drug or vehicle was then administered either i.c. or i.v. in a volume of 20 μl given over 20 s. Five minutes later the smoke challenges were resumed. In some experiments a second test drug was given 20 min after the initial i.c. or i.v. pretreatment (Figure 1B). In all experiments, four smoke challenges were given following the final drug application. The doses of 5-HT receptor ligands used in the present experiments were determined from previously published data (Bogle *et al*., 1990, Futuro-Neto *et al*., 1993, Shepheard *et al*., 1990). If a novel ligand was used, then the dose was estimated by comparison of its published binding affinity, to the particular 5-HT receptor under investigation, with that of other 5-HT receptor ligands used previously.

Analysis of data

Arterial blood pressure, ECG, phrenic nerve activity, raw and integrated renal nerve activity were recorded onto a computer hard disk and optical disk (Panasonic LF7010) using a CED 1401+interface and SPIKE 2 (CED, Cambridge, U.K.) data collection software. Heart rate was derived electronically from the blood pressure signal using a purpose built discriminator. The R-wave of the ECG was discriminated using a spike processor (Digitimer D130) and R-R intervals calculated from the output using SPIKE2 data analysis software. R-R intervals were measured 2 s prior to a smoke challenge (baseline), 3 s following the challenge, and at the maximal R-R interval attained. The effects of drugs on R-R interval were assessed by comparing the R-R intervals 5 min prior to, and 5 and 15 min following the drug or vehicle application. Mean arterial pressure (MAP), calculated as diastolic pressure+(systolic pressure-diastolic pressure)/3, was derived 2 s prior to a smoke stimulus (baseline) and at the peak response. Changes in MAP (peak response MAP-baseline MAP) were calculated for each response. Renal nerve activity was rectified and integrated with a 20 ms time constant using an EMG integrator (Neurolog NL

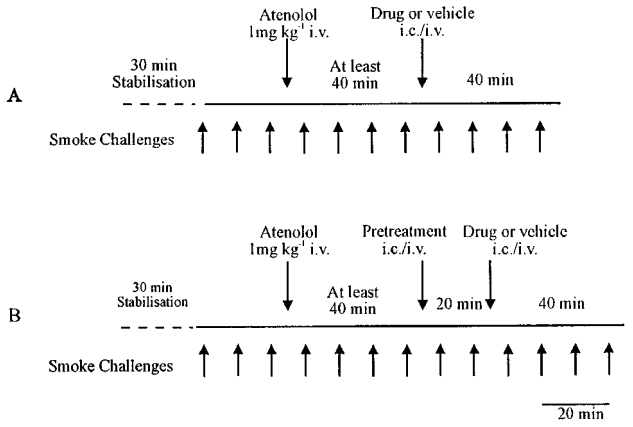

Figure 1 Schematic diagrams illustrating the experimental protocols used to investigate: (A) the effects of drug or vehicle applied i.c. or i.v. on the cardiorespiratory responses evoked by smoke challenges. (B) the effects of drug or vehicle applied i.c. or i.v. on the cardiorespiratory responses to smoke challenges following pretreatment with another drug i.c. or i.v.

703; Digitimer Ltd.). The mean level of integrated RNA (intRNA) 30 s prior to (baseline) and 30 s following (response) a smoke stimulus was then derived using a SPIKE 2 data analysis script. Since the absolute values of RNA vary substantially between animals, the values of baseline and response intRNA measured 5 min prior to a drug treatment were taken as 100%. Changes evoked by the drug were then compared to these pre-drug values. Raw phrenic nerve activity (PNA) was displayed using the SPIKE 2 data analysis package. The mean rate of phrenic bursts over the 30 s period prior to each stimulus and the duration of the evoked apnoea were calculated. For all measured variables statistical analyses were performed using a 2-way ANOVA and least significant difference test to make time-matched comparisons of drug-induced changes with vehicle alone changes. Changes in baseline or response size were calculated by subtracting the absolute value at any given time after treatment from the absolute value 5 min prior to treatment. The changes in these values were compared with the relevant values obtained at the same time points in the vehicle treatment group. All values are expressed as mean ± s.e.mean. Differences between means were taken as significant when $P<0.05$.

Drugs and solutions

The following drugs were freshly dissolved in 0.9% saline: 8-(4-(4-(2-pyrimidinyl)-1-piperazinyl)butyl)-8-azaspirol(4,5)decane-7,9-dione hydrochloride (buspirone); 8-hydroxy-2-(di-n-propylamino)tetralin HBr ((+)8-OH-DPAT) all from Research Biochemicals, Semat Technical Ltd., St Albans, U.K.; N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide trichloride (WAY-100635), a gift from Wyeth Research, Maidenhead, U.K.; 3-(2-(dimethylamino)ethyl)-N-methyl-1H-indole-5-methanesulphonamide succinate (sumatriptan), a gift from Wellcome Laboratories, Beckenham, U.K. (−)pindolol HCl from Research Biochemicals, Semat Technical Ltd., St Albans was dissolved in 0.01 N hydrochloric acid and then diluted with 0.9% saline. N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide (GR127935), a gift from Glaxo Research Group, Ware, U.K. was dissolved in distilled water.

Results

Effect of 5-HT$_{1A}$ receptor ligands on baseline variables

The resting baseline values of the cardio-respiratory variables measured in each of the experimental groups of animals are given in Table 1. For each experimental drug regime, the change in these baseline variables at 5 and 15 min following drug or vehicle administration are given in Table 2. Administration of the 5-HT$_{1A}$ receptor antagonist WAY-100635 (100 μg kg^{-1}) i.v. had no significant effects on the resting values of any of the variables measured. The same dose given i.c. also had no significant effects, though R-R interval tended to rise at 5 min. When another 5-HT$_{1A}$ receptor antagonist (−)pindolol (100 μg kg^{-1}) was given i.c. it evoked a small but significant increase in R-R interval (12 ± 4 ms) at 5 min and later falls in phrenic rate of −13 ± 9 bursts min^{-1}.

Table 1 Resting mean arterial blood pressure (MAP; mmHg), R-R interval (ms) and phrenic rate (bursts min^{-1}) before vehicle or drug challenge in atenolol (1 mg kg^{-1}; i.v.) pretreated spontaneously breathing anaesthetized rabbits

Drug and route	n	*MAP*	*R-R interval*	*Phrenic rate*
Saline, i.c.	5	55 ± 3	243 ± 8	50 ± 5
Acidified saline, i.c.	5	48 ± 5	273 ± 5	38 ± 3
WAY-100635, i.c.	5	55 ± 5	265 ± 7	53 ± 4
WAY-100635, i.v.	5	61 ± 5	262 ± 4	56 ± 3
(−)Pindolol, i.c.	5	41 ± 2	265 ± 3	54 ± 8
Buspirone, i.c.	5	64 ± 6	259 ± 13	56 ± 5
(+)8-OH-DPAT, i.c.	5	52 ± 7	262 ± 11	55 ± 3
Buspirone, i.c. (after WAY-100635, i.v.)	5	62 ± 6	256 ± 6	55 ± 2
(+)8-OH-DPAT, i.c (after WAY-100635, i.v)	4	69 ± 4	269 ± 11	59 ± 3
Sumatriptan, i.c. (after WAY-100635, i.v.)	4	53 ± 4	250 ± 11	53 ± 4
Distilled water, i.c.	4	65 ± 3	264 ± 6	67 ± 3
GR127935, i.c.	5	65 ± 5	252 ± 7	71 ± 7
GR127935, i.v	4	55 ± 6	273 ± 11	66 ± 14
(+)8-OH-DPAT, i.c. (after GR127935, i.v.)	4	46 ± 2	265 ± 6	66 ± 13

Table 2 Changes (Δ) in baseline mean arterial blood pressure (MAP; mmHg), R-R interval (ms), Phrenic rate (bursts min^{-1}) and renal nerve activity (RNA%) 5 and 15 min after vehicle or drug challenge in atenolol (1 mg kg^{-1}; i.v.) pretreated spontaneously breathing anaesthetized rabbits. *$P<0.05$; **$P<0.01$ compared to vehicle. †$P<0.05$; ††$P<0.01$ compared to buspirone alone. #$P<0.05$; ##$P<0.01$ compared to (+)8-OH-DPAT alone

Drug and route	*Dose*	n	Δ*MAP*		Δ*R-R interval*		Δ*Phrenic rate*		Δ*RNA(%)*	
Time after drug			*5 min*	*15 min*	*5 min*	*15 min*	*5 min*	*15 min*	*5 min*	*15 min*
Saline, i.c.	20 µl	5	−2 ± 2	−4 ± 2	1 ± 1	2 ± 3	1 ± 1	2 ± 2	−9 ± 5	−12 ± 4
Acidified saline, i.c.	20 µl	5	−1 ± 1	−4 ± 2	−3 ± 1	−3 ± 3	6 ± 4	9 ± 4	0 ± 6	1 ± 6
WAY-100635, i.c.	100 µg kg^{-1}	5	−1 ± 4	−3 ± 2	13 ± 7	8 ± 6	−2 ± 9	−2 ± 6	7 ± 2	−13 ± 15
WAY-100635, i.v.	100 µg kg^{-1}	5	7 ± 1	−1 ± 3	1 ± 1	−2 ± 3	−2 ± 2	2 ± 2	3 ± 1	−2 ± 1
(−)Pindolol, i.c.	100 µg kg^{-1}	5	−6 ± 4	−12 ± 3	12 ± 4*	8 ± 5	−8 ± 5	−13 ± 9*	−16 ± 18	−33 ± 17
Buspirone, i.c.	200 µg kg^{-1}	5	−12 ± 4**	−11 ± 1*	23 ± 8**	22 ± 3**	16 ± 7*	27 ± 5**	−37 ± 21	−35 ± 25
(+)8-OH-DPAT, i.c.	25 µg kg^{-1}	5	−5 ± 3	2 ± 6	3 ± 3	17 ± 5*	55 ± 10*	91 ± 24**	−41 ± 12	−37 ± 12*
Buspirone, i.c. (after WAY-100635; 100 µg kg^{-1}, i.v.)	200 µg kg^{-1}	5	−7 ± 4	−18 ± 4	6 ± 3††	7 ± 3†	−3 ± 3†	1 ± 2††	13 ± 4†	−14 ± 7
(+)8-OH-DPAT, i.c. (after WAY-100635; 100 µg kg^{-1}, i.v.)	25 µg kg^{-1}	4	−10 ± 4	−10 ± 3	0 ± 1	0 ± 1##	14 ± 7	38 ± 11#	−2 ± 6##	−8 ± 7#
Sumatriptan, i.c. (after WAY-100635; 100 µg kg^{-1}, i.v.)	50 µg kg^{-1}	4	3 ± 3	5 ± 2*	−3 ± 1	−4 ± 3	3 ± 4	17 ± 4*	14 ± 4	32 ± 10**
Distilled water, i.c.	20 µl	4	−1 ± 2	−2 ± 3	−5 ± 2	−8 ± 3	2 ± 5	2 ± 6	−1 ± 6	3 ± 9
GR127935, i.c.	20 µg kg^{-1}	5	−4 ± 1	−6 ± 2	1 ± 1	1 ± 1*	−3 ± 5	−3 ± 5	−2 ± 4	−2 ± 7
GR127935, i.v.	100 µg kg^{-1}	4	−2 ± 1	−5 ± 3	0 ± 4	−1 ± 5	1 ± 1	2 ± 3	−4 ± 2	−10 ± 2
(+)8-OH-DPAT, i.c. (after GR127935; 100 µg kg^{-1}, i.v.)	25 µg kg^{-1}	4	−3 ± 1	6 ± 3	15 ± 5	21 ± 7	97 ± 15	119 ± 15	−23 ± 15	74 ± 46#

Within 5 min of administration of buspirone (200 μg kg^{-1}, i.c.) R-R interval (259±13 ms) increased significantly by 23±8 ms and this was maintained (22±3 ms) at 15 min following the drug. Phrenic rate also increased from 56±5 bursts min^{-1} by 16±7 bursts min^{-1} and 27±5 bursts min^{-1} at 5 and 15 min respectively, while MAP fell by −12±4 mmHg and −11±4 mmHg at 5 and 15 min. Following administration of (+)8-OH-DPAT (25 μg kg^{-1}, i.c.) baseline R-R interval (262±11 ms) slowly increased, reaching significance at 15 min. Associated with this change in R-R interval there were significant increases in phrenic rate (55±10 bursts min^{-1} and 91±24 bursts min^{-1} from a resting value of 55±3 bursts min^{-1}) and falls in RNA (−41±12% and −37±12%) at 5 and 15 min respectively but no significant changes in MAP. Pretreatment of animals with WAY-100635 (100 μg kg^{-1}) i.v., which itself had no significant effects, attenuated the effects of i.c. administered buspirone and (+)8-OH-DPAT on baseline R-R interval, phrenic rate and RNA (Table 2).

Effect of 5-HT$_{1A}$ receptor ligands on reflex-evoked changes

Passing a bolus of nicotine-free herbal cigarette smoke (15–45 ml) through the upper airways of spontaneously breathing anaesthetized rabbits evokes a pattern of response often termed the 'diving response' (Figure 2). Taking the whole group of animals (n=65), an apnoea was evoked which lasted for 20±4 s, accompanied by increases in R-R interval of 116±33 ms from the resting value of 260±10 ms and MAP of 26±3 mmHg from the baseline value of 57±5 mmHg. The change in MAP is likely to be due to peripheral vasoconstriction, since in these experiments renal nerve activity increased by 211±27%. The sizes of the reflex responses evoked in the individual experimental groups of animals are shown in Table 3. For each drug or vehicle administration the effect on the size of the reflex response at 5 and 15 min following the treatment is shown in Table 4. The 5-HT$_{1A}$ receptor antagonist WAY-100635 (100 μg kg^{-1}, i.c.) significantly attenuated the evoked increase in R-R interval at both 5 and 15 min post-drug (−68±27 ms and −57±24 ms; Figure 2) and reduced the pressor response (−9±5 mmHg) at 5 min but was without effect on both the apnoeic response (3±6 s and −3±3 s) and the sympathoexcitation in the renal nerve (Figure 3). These changes are likely to be due to a central action of the drug since the same dose given i.v. had no significant effects on any of the reflex responses. Similarly, (−)pindolol (100 μg kg^{-1} i.c.) markedly attenuated the reflex increase in R-R interval at 5 and 15 min post-drug (−109±27 ms and −207±60 ms) and the pressor response at 15 min (−16±5 mmHg). In addition, it also attenuated the renal sympathoexcitation, reaching significance at 15 min (−39±10%) (Figure 3).

Following administration of buspirone (200 μg kg^{-1}, i.c.) the smoke-induced increase in R-R interval was significantly potentiated by 189±65 and 148±65 ms at 5 and 15 min, respectively and there was a small attenuation of the pressor response (−5±1 mmHg) at 5 min. There were no significant changes in the duration of the evoked apnoea or the renal sympathoexcitation (Figure 3). In contrast, following administration of (+)8-OH-DPAT (25 μg kg^{-1}, i.c.) the evoked increase in R-R interval was significantly inhibited (−98±23 ms and −103±21 ms) at 5 and 15 min respectively and at these same times, the duration of the evoked apnoea (−11±3 s and −13±2 s) and sympathoexcitation

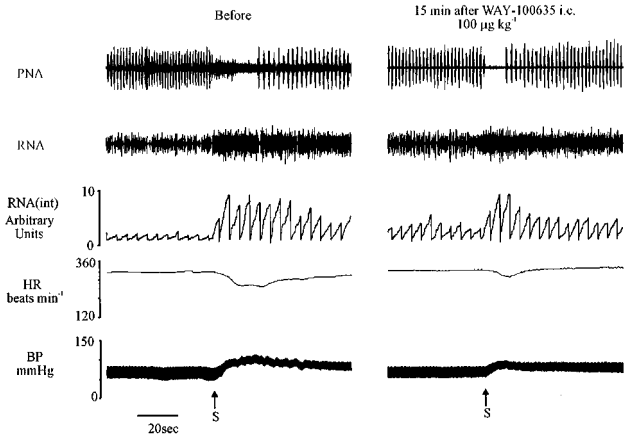

Figure 2 Traces from an anaesthetized spontaneously breathing rabbit pretreated with atenolol (1 mg kg^{-1}, i.v.) showing the effects on phrenic nerve activity (PNA), renal sympathetic nerve activity (RNA), integrated renal sympathetic nerve activity (RNAint), heart rate (HR) and arterial blood pressure (BP) produced by passing a bolus of smoke (S) retrogradely through the upper airways before and 15 min after administration of WAY-100635 (100 μg kg^{-1}, i.c.).

Table 3 Size of the reflex-evoked increase in mean arterial blood pressure (MAP; mmHg), increases in R-R interval (ms) and apnoea duration (s) induced by passing smoke through the upper airways before vehicle or drug challenge in atenolol (1 mg kg^{-1}, i.v.) pretreated spontaneously breathing anaesthetized rabbits

Drug and route	n	*Increase in MAP*	*Increase in R-R interval*	*Apnoea duration*
Saline, i.c.	5	22±4	128±41	25±5
Acidified saline, i.c.	5	25±1	167±51	29±5
WAY-100635, i.c.	5	32±2	158±43	22±2
WAY-100635, i.v.	5	29±3	77±19	22±3
(−)Pindolol, i.c.	5	27±3	218±60	29±6
Buspirone, i.c.	5	29±1	135±26	22±5
(+)8-OH-DPAT, i.c.	5	23±3	141±22	21±3
Busipirone, i.c. (after WAY-100635, i.v.)	5	24±3	62±12	19±3
(+)8-OH-DPAT, i.c. (after WAY-100635, i.v.)	4	19±5	114±48	19±2
Sumatriptan, i.c (after WAY-100635, i.v.)	4	28±4	153±31	22±3
Distilled water, i.c.	4	32±3	90±53	18±6
GR127935, i.c.	5	23±2	56±15	10±2
GR127935, i.v.	4	29±3	86±32	20±1
(+)8-OH-DPAT, i.c. (after GR127935, i.v)	4	26±3	88±17	15±4

Table 4 Changes (Δ) in the size of the reflex changes in mean arterial blood pressure (MAP; mmHg), R-R interval (ms), apnoea duration (s) and renal nerve activity (RNA, %) evoked by passing smoke through the upper airways in atenolol (1 mg kg^{-1}) pretreated spontaneously breathing anaesthetized rabbits 5 and 15 min after vehicle or drug challenge. *$P<0.05$; **$P<0.01$ compared to vehicle. †$P<0.05$; ††$P<0.01$ compared to buspirone alone. #$P<0.05$; ##$P<0.01$ compared to (+)8-OH-DPAT alone

Drug and route *Time after drug*	*Dose*	n	*ΔMAP* 5 min	*ΔMAP* 15 min	*ΔR-R interval* 5 min	*ΔR-R interval* 15 min	*ΔApnoea* 5 min	*ΔApnoea* 15 min	*ΔRNA* 5 min	*ΔRNA* 15 min
Saline, i.c.	20 µl	5	−1±1	0±1	−4±9	−7±11	1±2	−1±2	−3±8	2±4
Acidified saline, i.c.	20 µl	5	−2±1	−2±1	4±27	2±15	3±4	1±3	1±4	8±4
WAY-100635, i.c.	100 µg kg^{-1}	5	−9±5**	−5±3	−68±27*	−57±24*	3±6	−3±3	0±16	−8±9
WAY-100635, i.v.	100 µg kg^{-1}	5	−5±1	−1±1	−5±7	−16±11	−1±2	−2±2	−3±1	−4±3
(−)Pindolol, i.c.	100 µg kg^{-1}	5	−6±4	−16±5**	−109±27*	−207±60**	4±7	5±9	−15±14	39±10**
Buspirone, i.c.	200 µg kg^{-1}	5	−5±1	0±3	189±65**	148±65*	−7±5	−4±5	21±15	−2±8
(+)8-OH-DPAT, i.c.	25 µg kg^{-1}	5	−6±3	−10±2**	−98±23**	−103±21**	−11±3**	−13±2**	−48±11**	−41±9**
Buspirone, i.c. (after WAY-100635; 100 µg kg^{-1}, i.v.)	200 µg kg^{-1}	5	4±2††	1±2	21±19†	15±20†	−1±3	−4±2	9±3	3±7
(+)8-OH-DPAT, i.c. (after WAY-100635; 100 µg kg^{-1}, i.v.)	25 µg kg^{-1}	4	−5±4	−6±3	−68±30	−87±40	−4±1	−5±1#	−2±14##	0±3##
Sumatriptan, i.c. (after WAY-100635; 100 µg kg^{-1}, i.v.)	50 µg kg^{-1}	4	−5±2	−12±4**	−54±19	−68±13*	−7±3	−5±3	−4±5	2±7
Distilled water, i.c.	20 µl	4	−7±4	−4±2	−11±11	−16±15	−6±1	−5±3	−4±6	−2±8
GR127935, i.c.	20 µg kg^{-1}	5	4±3*	3±2	31±19	22±26	0±2	−1±3	−1±6	1±12
GR127935, i.v.	100 µg kg^{-1}	4	−3±2	−3±1	−4±14	2±37	−2±2	−5±3	−7±3	−13±3
(+)8-OH-DPAT, i.c. (after GR127935; 100 µg kg^{-1}, i.v.)	25 µg kg^{-1}	4	−13±4	−12±5	3±21##	−6±23##	−7±4	−9±4	−25±9#	−11±4#

(−48±11% and −41±9%) were also significantly reduced. The pressor response was significantly reduced (−10±2 mmHg) only after 15 min (Figure 4).

When animals were pretreated with WAY-100635 (100 µg kg^{-1}, i.v.), which itself had no effect on the evoked reflex, buspirone (200 µg kg^{-1}, i.c.) still potentiated the evoked prolongation of R-R interval but this was significantly less than without the pretreatment (21±19 ms and 15±20 ms at 5 and 15 min). In addition, the attenuation of the pressor response was reversed (Figure 3). In contrast, the ability of (+)8-OH-DPAT (25 µg kg^{-1}, i.c.) to attenuate the reflex bradycardia was unaffected by pretreatment with WAY-100635 (100 µg kg^{-1} i.v.), though the attenuation of the apnoeic response and the sympathoexcitation were reversed (Figure 4).

Effect of 5-HT$_{1B/1D}$ receptor ligands on baseline variables and reflex effects

In animals pretreated with WAY-100635 (100 µg kg^{-1}, i.v.), the 5-$HT_{IB/ID}$ receptor agonist sumatriptan (50 µg kg^{-1}, i.c.) had no effect on resting R-R interval but caused small increases at 15 min in MAP (5±2 mmHg), phrenic rate (17±4 bursts min^{-1}) and RNA (32±10%) (Table 2). The 5-$HT_{IB/ID}$ receptor antagonist GR127935, given either centrally (20 µg kg^{-1}, i.c.) or peripherally (100 µg kg^{-1} i.v.), was without effect on baseline variables (Table 2). Although sumatriptan had no effect on baseline R-R interval, it markedly reduced the evoked increase in R-R interval (153±31 ms) by −54±19 ms and by 15 min this had become significant reaching −68±13 ms. It also attenuated the pressor response at 15 min (Table 4 and Figure 4). However, central administration of GR127935 (20 µg kg^{-1}, i.c.) had no significant effect on either the evoked apnoea or RNA but tended to potentiate the pressor response, this being significant at 5 min. It also tended to augment the evoked bradycardia but this did not reach significance. When given peripherally (100 µg kg^{-1}, i.v.) GR127935 had no significant effect on any of the reflexively-evoked responses (Table 4). However, in animals pretreated with GR127935 (100 µg kg^{-1}, i.v.) the attenuation of the reflex increases in

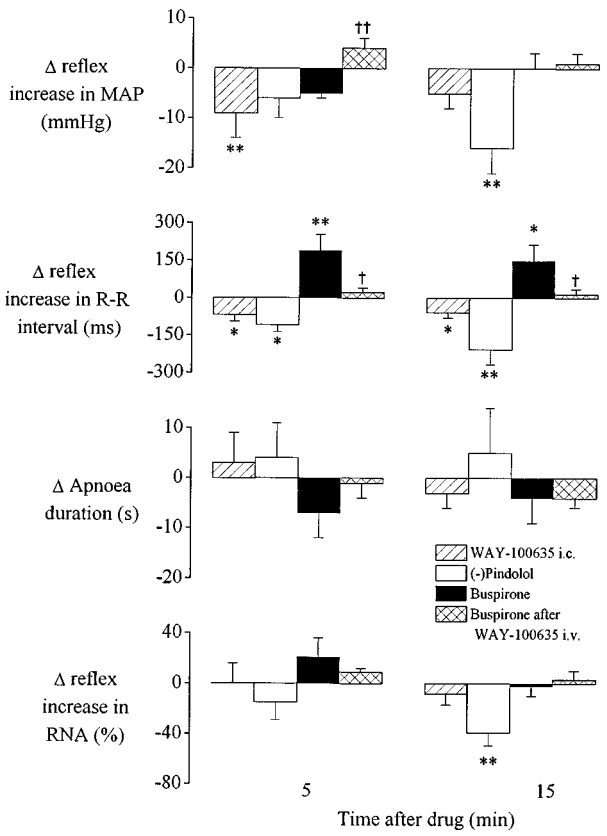

Figure 3 Atenolol (1 mg kg^{-1}; i.v.) pretreated spontaneously breathing anaesthetized rabbits: histograms showing the changes (Δ) in the size of the smoke-induced increase in mean arterial pressure (MAP), increase in R-R interval, apnoea duration and increase in renal sympathetic nerve activity (RNA) at 5 (left) and 15 min (right) following administration of WAY-100635 (100 µg kg^{-1}, i.c.), (−)pindolol (100 µg kg^{-1}, i.c.), buspirone (200 µg kg^{-1}, i.c.), and buspirone (200 µg kg^{-1}, i.c.) following pretreatment with WAY-100635 (100 µg kg^{-1}, i.v.). Each column shows the mean change in the size of the reflex and bars show s.e.mean. Changes caused by the treatment have been compared with time-matched controls. *$P<0.05$, **$P<0.01$ compared to vehicle, †$P<0.05$, †† $P<0.01$ compared to buspirone alone.

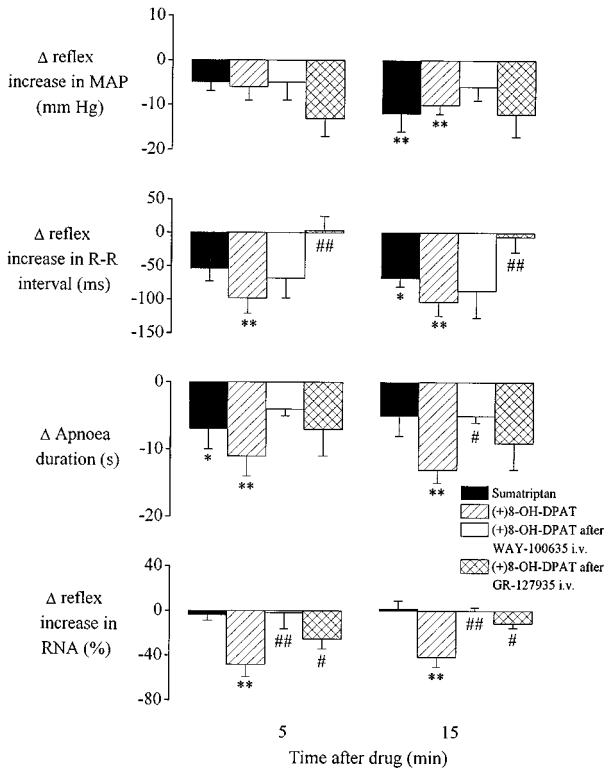

Figure 4 Atenolol (1 mg kg^{-1}; i.v.) pretreated spontaneously breathing anaesthetized rabbits: histograms showing the changes (Δ) in the size of the smoke-induced increases in mean arterial pressure (MAP), in R-R interval, apnoea duration and in renal sympathetic nerve activity (RNA) at 5 (left) and 15 min (right) following administration of sumatriptan (50 μg kg^{-1}, i.c.), (+)8-OH-DPAT (25 μg kg^{-1}, i.c.), (+)8-OH-DPAT (25 μg kg^{-1}, i.c.) following pretreatment with WAY-100635 (100 μg kg^{-1}, i.v.) and (+)8-OH-DPAT (25 μg kg^{-1}, i.c.) following pretreatment with GR127935 (100 μg kg^{-1}, i.v.). Each column shows the mean change in the size of the reflex and bars show s.e.mean. Changes caused by the treatment have been compared with time-matched controls. $^*P<0.05$, $^{**}P<0.01$ compared to vehicle; $\#P<0.05$, $\#\#P<0.01$ compared to (+)8-OH-DPAT alone.

R-R interval and RNA normally produced by (+)8-OH-DPAT (25 μg kg^{-1}, i.c.) were reduced (Table 4 and Figure 4).

Discussion

Reflex bradycardia

The present experiments have confirmed the observations of Futuro-Neto *et al*., (1993) that buspirone and 8-OH-DPAT have opposing effects on the reflex activation of cardiac preganglionic vagal motoneurones, as measured by changes in R-R interval, evoked by stimulation of the upper airways with smoke in atenolol pretreated, spontaneously breathing anaesthetized rabbits. It should be noted that changes in R-R interval, in the presence of such sympathetic blockade (atenolol pretreatment) are linearly related to changes in cardiac vagal activity (Parker *et al*., 1984). The present data also confirm the view that central 5-HT$_{1A}$ receptors play a facilitatory role in the reflex activation of cardiac preganglionic vagal motoneurones, as the reflex-evoked increase in R-R interval was attenuated by pretreatment with the 5-HT$_{1A}$ receptor antagonists (−)pindolol (Schoeffter & Hoyer, 1988) and WAY-100635 (Forster *et al*., 1995) and potentiated by buspirone, a 5-HT$_{1A}$ receptor partial agonist (Schoeffter & Hoyer, 1988). The fact that i.v. WAY-100635 blocked the potentiating action of buspirone on this reflex increase in R-R interval, indicates that buspirone is acting as an agonist not as an antagonist as suggested by Futuro-Neto *et al*., (1993). Furthermore, the present data demonstrate that this facilitating action of 5-HT$_{1A}$ receptors is due to a central rather than a peripheral location of these receptors, as the same dose of WAY-100635 given i.v. had no effect on the reflex responses. The mechanism by which i.v. WAY-100635 is capable of blocking the potentiating effects of buspirone, but not the reflex itself, may be simply one of concentration, i.e. when given i.v. the brainstem concentration of WAY-100635 is high enough to block the effects of an exogenously applied agonist but not synaptic transmission involved in this reflex pathway, the brainstem being the site most accessible to i.c. administration. A similar difference between i.v. and i.c. application of WAY-100635 has also been reported on reflex-evoked bronchoconstriction in anaesthetized cats and guinea-pigs (Bootle *et al*., 1996, 1998). Alternatively, it is possible that the different routes of injection could access different central sites. Further, the present experiments demonstrate that the inhibitory effect of (+)8-OH-DPAT on the reflex increase in R-R interval, i.e. vagal bradycardia, is not mediated by 5-HT$_{1A}$ receptors as suggested by Futuro-Neto *et al*., (1993), but by 5-HT$_{1B/1D}$ receptors. This is because the inhibitory effect of (+)8-OH-DPAT on the reflex still occurred in animals pretreated with WAY-100635 but was prevented by pretreatment with the 5-HT$_{1B/1D}$ receptor antagonist GR127935 (Skingle *et al*., 1996), indicating that activation of central 5-HT$_{1B/1D}$ receptors inhibits the reflex increase in the R-R interval. This conclusion is further supported by the observation that, in the presence of WAY-100635, the 5-HT$_{1B/1D}$ receptor agonist sumatriptan (see Hoyer *et al*., 1994) also inhibited the reflex increase in R-R interval. Interestingly, GR127935 given i.c. tended to augment the evoked increase in R-R interval but this did not reach statistical significance. However, as can be seen from the standard error of the mean, there was a large variability in this facilitatory action. In some animals GR127935 markedly potentiated the reflex bradycardia, whilst in others it was totally without effect. However, a significant potentiation could not be observed even at a higher dose (100 μg kg^{-1}, i.c., unpublished observations). Taken together, these observations suggest that this upper airway reflex activation of cardiac vagal preganglionic motoneurones involves the release of 5-HT which activates 5-HT$_{1A}$ but not 5-HT$_{1B/1D}$ receptors. In addition, these receptors have opposing actions in that 5-HT$_{1A}$ receptors facilitate while 5-HT$_{1B/1D}$ receptors attenuate the reflex activation of cardiac vagal preganglionic motoneurones by stimulation of upper airway receptors. This conclusion is similar to data reported recently for the reflex activation of bronchoconstrictor preganglionic vagal neurones in the guinea-pig (Bootle *et al*., 1998).

Non-5-HT$_{1A}$ receptor actions of 8-OH-DPAT

It is surprising that the archetypal 5-HT$_{1A}$ receptor agonist (+)8-OH-DPAT (see Tricklebank *et al*., 1984) inhibited the reflex increase in R-R interval by an action on 5-HT$_{1B/1D}$ receptors. However, 8-OH-DPAT does have affinity for this receptor subtype (pKi of approximately 7 at the human 5-HT$_{1D}$ receptor; Hartig *et al*., 1992). The effects of (+)8-OH-DPAT on baseline values are due to activation of 5-HT$_{1A}$ receptors since they were attenuated by pretreatment with WAY-100635. Therefore (+)8-OH-DPAT does activate 5-HT$_{1A}$ receptors in this animal model. Subsequent to blocking

5-$HT_{1B/1D}$ receptors it may be expected that 8-OH-DPAT would potentiate the reflex-evoked increase in the R-R interval. Further, as 8-OH-DPAT has approximately 100 times higher affinity for 5-HT_{1A} than 5-$HT_{1B/1D}$ receptors (see Hoyer *et al*., 1994) it would be expected that the 5-HT_{1A} receptor-mediated effects would predominate over the 5-$HT_{1B/1D}$ receptor-mediated effects. One possible explanation for the failure to observe any 5-HT_{1A} receptor-mediated effects by (+)8-OH-DPAT on the reflex increase in R-R interval is that 5-HT_{1A} receptors are antecedent to 5-$HT_{1B/1D}$ receptors in this reflex pathway to cardiac vagal preganglionic neurones. Therefore activation of 5-$HT_{1B/1D}$ receptors would block the facilitating effect of activation of 5-HT_{1A} receptors. It is also possible that (+)8-OH-DPAT may be activating other brainstem receptors to block the expected effects of 5-HT_{1A} receptor activation. In this respect, 8-OH-DPAT has been shown to have a partial agonist action at 5-HT_7 receptors (Lovenberg *et al*., 1994) and these receptors have been implicated in the ability of 8-OH-DPAT to modulate the short-latency reflex evoked in gastrocnemius medialis motoneurones (Wigglesworth *et al*., 1997) in decerebrate rabbits. Interestingly, in the cat and guinea-pig, 8-OH-DPAT potentiated the reflex activation of bronchoconstrictor preganglionic motoneurones (Bootle *et al*., 1996, 1998), an effect which is consistent with activation of 5-HT_{1A} receptors. Therefore these data suggest that there may be some species variation in the affinity of 8-OH-DPAT for 5-$HT_{1B/1D}$ receptors and/or the pharmacological selectivity of this agonist.

Respiratory modulation

It is possible that these 5-HT receptor ligands are modulating the reflex activation of cardiac vagal preganglionic neurones by interfering with central respiratory drive as these neurones are subject to respiratory modulation (see Daly, 1995). Thus, if the drug treatments consistently changed respiratory drive and/or the duration of the apnoeas, then these secondary effects may result in altered cardiac responses. In the present experiments phrenic nerve activity was monitored as an index of central respiratory drive. Since WAY-100635 had no effect on either baseline respiratory rate or reflex apnoea duration, the inhibition of the reflex bradycardia produced by WAY-100635 cannot be attributed to secondary respiratory related effects. Although buspirone increased and (−)pindolol decreased baseline respiratory rates, neither ligand altered the duration of the evoked apnoea. Again, these changes could not account for the observed effects on the reflex bradycardia. Indeed, the increased respiratory rate would oppose the potentiation of the reflex bradycardia produced by buspirone, whereas the decreased respiratory rate would tend to oppose the inhibition of the reflex bradycardia produced by (−)pindolol. Increases in baseline respiratory rate associated with decreased apnoea duration were observed following the administration of (+)8-OH-DPAT. These respiratory changes could account for the observed attenuation of the bradycardia. However, in animals pretreated with WAY-100635, (+)8-OH-DPAT still produced an attenuation of the reflex bradycardia though changes in respiratory variables were abolished, indicating that modulation of the cardiac response is not secondary to changes in central respiratory drive. Further, in animals made hyperoxic, to remove any possible hypoxic stimulation of the arterial chemoreceptors which could cause potentiation of the evoked bradycardia, buspirone still potentiated the reflex increase in R-R interval (Dando *et al*., 1994a).

Sympathoexcitation

Another action of the upper airway reflex is to evoke sympathoexcitation, as indicated by increases in renal nerve activity and blood pressure. WAY-100635 had no effect on either the reflex-evoked sympathoexcitation or on baseline renal nerve activity, while (−)pindolol and (+)8-OH-DPAT caused delayed decreases in baseline and reflexively-evoked renal nerve activity. The effects of (+)8-OH-DPAT on baseline and evoked renal nerve activity were blocked by WAY-100635, indicating that they are mediated by 5-HT_{1A} receptor activation. In this respect, buspirone tended to decrease renal nerve activity, but not significantly, although this tendency was prevented by WAY-100635. Buspirone had no effect on the reflex-evoked increase in renal nerve activity. Overall these data suggest that activation of central 5-HT_{1A} receptors may inhibit the reflex increase in nerve activity, however this could be related to changes in background central sympathetic tone. The effects of buspirone and (−)pindolol may be related to their reported partial agonist actions at 5-HT_{1A} receptors (see Hoyer *et al*., 1994). The ability of GR127935 to partially block the inhibitory action of (+)8-OH-DPAT suggests involvement of 5-$HT_{1B/1D}$ receptors in the sympathoinhibitory actions of (+)8-OH-DPAT. However, activation of 5-$HT_{1B/1D}$ receptors by sumatriptan increased baseline renal nerve activity. Nevertheless, this is consistent with the report that central activation of these receptors can have either a pressor or a depressor action (Gallacher & Ramage, 1996).

Site of action

The question arises as to the possible central site(s) at which these 5-HT receptor ligands act to modify the pattern of reflex responses evoked by stimulation of the upper airway afferents, which has been termed the 'diving response' (see Daly, 1986). Afferent activity evoked during this response is transmitted to the nucleus tractus solitarius in the dorsal medulla. From there, activity will access the brainstem respiratory groups, vagal preganglionic neurones in the dorsal vagal nucleus and nucleus ambiguus and sympathetic premotor neurones in the rostral ventrolateral medulla (see Jordan, 1995; Daly, 1995). All these areas receive serotonergic innervation (Steinbusch, 1981) and application of 5-HT and its selective receptor subtype ligands have been demonstrated to modify neuronal activity in the nucleus tractus solitarius (Wang *et al*., 1997), the ventral respiratory group (Lalley *et al*., 1994), the dorsal vagal nucleus (Wang *et al*., 1995, 1996) and the rostral ventrolateral medulla (Wang & Lovick, 1992). Further, as with 5-HT_{1A} receptors (see Introduction), 5-HT_{1B} receptor binding sites have been reported in the NTS, DVN and nucleus ambiguus (Thor *et al*., 1992; Castro *et al*., 1997), while low levels of 5-HT_{1D} receptor binding has been reported in the NTS (Castro *et al*., 1997). Since in the present experiments ligands were administered in small volumes (20 μl) into the fourth ventricle it is not possible to delineate with any certainty the location of the receptors affected. However, ligands are likely to access all these brainstem sites, but since the modulatory effects reported here differentiated between different components of the 'diving response', they are more likely to be due to actions at the level of the motor outflow than at the afferent input. This view is supported by other studies which have demonstrated that buspirone similarly potentiates reflex vagal bradycardias evoked by stimulating pulmonary C-fibre afferents (Dando *et al*., 1994b; Skinner *et al*., 1997a, b), aortic baroreceptor afferents (Skinner *et al*., 1997a) and arterial chemoreceptors

(Skinner *et al*., 1997b) and these potentiations are also prevented in animals pretreated with WAY-100635 (Skinner *et al*., 1997c).

In conclusion, these data support the view that central 5-HT_{1A} and 5-$HT_{1B/1D}$ receptors have opposing roles, facilitation and inhibition respectively, on the reflex activation of cardiac vagal preganglionic neurones by upper airway stimulation with smoke. For the other motor components of the reflex, i.e. apnoea, renal sympathoexcitation and hypertension, it is difficult to assess the role of these receptors. Furthermore, these data also suggest that the selectivity of (+)8-OH-DPAT for 5-HT_{1A} receptors over other 5-HT receptor subtypes may vary between species and more care should be taken when attributing the effect of agonists to a receptor type without supporting antagonist data.

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