British Journal of Pharmacology (1998) 123, 205–214

Pharmacological analysis of the haemodynamic effects of 5-$HT_{1B/D}$ receptor agonists in the normotensive rat

Fabrice Pagniez, Jean-Pierre Valentin, Sylvie Vieu, Francis C. Colpaert & [1]Gareth W. John

Centre de Recherche Pierre Fabre, Division of Cardiovascular Diseases, Castres, France

1 The receptors involved in mediating the haemodynamic effects of three 5-$HT_{1B/D}$ receptor agonists were investigated in pentobarbitone anaesthetized rats ($n=6-17$ per group).

2 Cumulative intravenous (i.v.) infusions of rizatriptan and sumatriptan (from 0.63 to 2500 μg kg^{-1}; each dose over 5 min) induced dose-dependent and marked hypotension (-42 ± 6 and -34 ± 4 mmHg at the highest dose, respectively; both $P<0.05$ vs vehicle: $+5\pm3$ mmHg) and bradycardia (-85 ± 16 and -44 ± 12 beats min^{-1} at the highest dose, respectively; both $P<0.05$ vs vehicle: $+16\pm6$ beats min^{-1}). Zolmitriptan evoked only moderate hypotension at the highest dose (-19 ± 9 mmHg; $P<0.05$ vs vehicle).

3 A high dose of the 5-$HT_{1B/D}$ receptor antagonist, GR 127935 (0.63 mg kg^{-1}, i.v.), failed to antagonize the hypotension and bradycardia evoked by sumatriptan (-35 ± 6 mmHg and -52 ± 19 beats min^{-1}, respectively; both not significant vs sumatriptan in untreated rats), but moderately reduced the hypotension and bradycardia evoked by rizatriptan (-20 ± 5 mmHg and -30 ± 17 beats min^{-1}, respectively; both $P<0.05$ vs vehicle and vs rizatriptan in untreated rats).

4 The selective 5-HT_{1A} receptor antagonist, WAY 100635 (0.16 and 0.63 mg kg^{-1}, i.v.), dose-dependently attenuated the haemodynamic responses evoked by rizatriptan and sumatriptan, which were almost abolished by the higher dose of WAY 100635 (-4 ± 3 mmHg and -15 ± 8 beats min^{-1}; both not significant vs vehicle and $P<0.05$ vs rizatriptan in untreated rats). A slight but statistically significant reduction in mean arterial pressure (MAP) persisted at the highest dose of sumatriptan (-13 ± 4 mmHg following the higher dose of WAY 100635; $P<0.05$ vs vehicle).

5 In pithed rats with MAP normalized by angiotensin II, rizatriptan failed to induce hypotension or bradycardia ($+5\pm4$ mmHg and -6 ± 16 beats min^{-1}, respectively; both NS vs vehicle and $P<0.05$ vs rizatriptan in untreated rats). Similarly, sumatriptan failed to induce bradycardia in pithed rats ($+5\pm6$ beats min^{-1}; not significant vs vehicle and $P<0.05$ vs sumatriptan in untreated rats), whereas a slight but statistically significant reduction in MAP, compared to controls, occurred at the highest dose (-9 ± 9 mmHg; $P<0.05$ vs both vehicle and sumatriptan in untreated rats).

6 In bilaterally vagotomized and atropine-treated (1 mg kg^{-1}, i.v.) rats, the reductions in MAP and heart rate evoked by rizatriptan (-31 ± 4 mmHg and -64 ± 9 beats min^{-1}, respectively; both $P<0.05$ vs vehicle and not significant vs rizatriptan in controls) and sumatriptan (-47 ± 8 mmHg and -56 ± 10 beats min^{-1}, respectively; both $P<0.05$ vs vehicle and not significant vs sumatriptan in controls) were not statistically significantly different from those observed in controls.

7 In conclusion, the 5-$HT_{1B/D}$ receptor agonists, rizatriptan and sumatriptan, elicit hypotension and bradycardia in the normotensive anaesthetized rat predominantly via activation of central 5-HT_{1A} receptors, and a consequent reduction in sympathetic outflow.

Keywords: 5-$HT_{1B/D}$ receptors; 5-HT_{1A} receptors; bradycardia; GR 127935; hypotension; sumatriptan; rizatriptan; WAY 100635; zolmitriptan

Introduction

Sumatriptan is a relatively selective 5-$HT_{1B/D}$ receptor agonist effective in the acute treatment of migraine (Ferrari & Saxena, 1993). Previous *in vivo* studies with sumatriptan have demonstrated vasopressor responses in the systemic circulation in several species. Arterial pressure (AP) was increased by sumatriptan in anaesthetized dogs (Cambridge *et al*., 1995), pigs (den Boer *et al*., 1992; Verscheure *et al*., 1995) and man (Mac Intyre *et al*., 1993; Hood *et al*., 1997; Sciberras *et al*., 1997b), whereas heart rate (HR) remained unaffected (den Boer *et al*., 1992; Mac Intyre *et al*., 1993; Cambridge *et al*., 1995; Verscheure *et al*., 1995; Hood *et al*., 1997; Sciberras *et al*., 1997b). These effects are mediated by activation of postjunctional vascular 5-$HT_{1B/D}$ receptors, as evidenced by blockade of sumatriptan-evoked vasoconstriction by 5-$HT_{1B/D}$ receptor antagonists (Cambridge *et al*., 1995; De Vries *et al*., 1996; 1997a; Valentin *et al*., 1996b; Villalon *et al*., 1996; 1997). In contrast, intravenous administration of sumatriptan to anaesthetized normotensive rats produced dose-dependent, marked hypotension and bradycardia (Valentin *et al*., 1995b). Furthermore recent studies showed that sumatriptan evoked only moderate or no hypotension in pithed or vagosympathectomized rats, respectively (De Vries *et al*., 1997b; Terron, 1997). Taken together, these observations suggest that the effects of sumatriptan upon AP and HR in rat involve different mechanisms which remain unidentified at present. The present study was therefore designed to elucidate the nature of the receptors involved in mediating the haemodynamic effects of three relatively selective 5-$HT_{1B/D}$ receptor agonists, sumatriptan (Humphrey *et al*., 1988), rizatriptan (Sciberras *et al*.,

[1]Author for correspondence at: Institut de Recherche Pierre Fabre, Division of Cardiovascular Diseases, 17, Avenue Jean Moulin, 81106 Castres cédex, France.

1997a,b) and zolmitriptan (Dixon *et al*., 1997; Martin *et al*., 1997) in anaesthetized normotensive rats.

Methods

Experiments were carried out in accordance with French law and the local ethical committee guidelines for animal research. Male Sprague-Dawley rats (280–400 g, OFA, Iffa-Credo, France) were housed in climate controlled conditions (21°C and 55% relative humidity with a 12 h light/dark cycle) and provided standard rat chow and water *ad libitum*. Animals were anaesthetized with an intraperitoneal injection of 60 mg kg^{-1} sodium pentobarbitone (Sanofi Laboratories, France), except when otherwise specified, and placed on a heated table to maintain rectal temperature at 37±0.5°C. Animals underwent tracheotomy and were mechanically ventilated (60 cycles min^{-1}; 2.5 ml/cycle; Harvard apparatus, South Natick, MA). They were prepared for acute experimentation as previously described (Valentin *et al*., 1996a). Experiments were started 15–30 min after completion of surgical procedures.

Effect of cumulative doses of rizatriptan, sumatriptan and zolmitriptan on mean arterial pressure (MAP) and heart rate (HR)

Fifty five rats received an intravenous (i.v.) bolus administration of sterile saline (i.e., vehicle of GR 127935 and WAY 100635; see below). Ten minutes later, they received 7 cumulative doses (0.63, 2.5, 10, 40, 160, 630 and 2500 μg kg^{-1}) of either rizatriptan ($n=9$), sumatriptan ($n=17$), zolmitriptan ($n=8$) or their respective vehicles (sterile saline, 0.9% for rizatriptan and sumatriptan and polyethyleneglycol (PEG) 300/saline 0.9%; 40/60; v/v for zolmitriptan; $n=16$ and 5, respectively). Each dose of drug or vehicle was administered as a solution at a rate of 40 μl min^{-1} over 5 min. After the infusion of the highest dose of drug or vehicle had ceased, AP was allowed to stabilize for several minutes, then flesinoxan, a selective 5-HT$_{1A}$ receptor agonist (Wouters *et al*., 1988), was administered as a bolus injection (0.63 mg kg^{-1}, i.v.).

Effect of GR 127935 and WAY 100635 on the responses to rizatriptan and sumatriptan

Nine groups of 6–10 anaesthetized rats received an i.v. injection of either the selective 5-HT$_{1B/D}$ receptor antagonist, GR 127935 (0.63 mg kg^{-1}; $n=25$ rats; Clitherow *et al*., 1994), or the selective 5-HT$_{1A}$ receptor antagonist, WAY 100635 (0.16 or 0.63 mg kg^{-1}; $n=23$ and 19 rats, respectively; Fletcher *et al*., 1994), followed 10 min later by 7 cumulative doses of either rizatriptan ($n=22$), sumatriptan ($n=24$) or vehicle ($n=21$). Flesinoxan (0.63 mg kg^{-1}, i.v.) was administered during the recovery period as previously described.

Influence of the autonomic nervous system on the responses to rizatriptan and sumatriptan

Any influence of the autonomic nervous system was removed by pithing the animals as previously described (Valentin *et al*., 1995a). In order to restore AP within the physiological range, angiotensin II (0.1–0.5 μg kg^{-1} min^{-1}; Fozard *et al*., 1987) was infused for the duration of the experiment at a rate of 20 μl min^{-1}. After stabilization of AP, rizatriptan ($n=8$), sumatriptan ($n=7$) or the vehicle ($n=10$) as well as flesinoxan were infused as described below.

Influence of the parasympathetic nervous system on the responses to rizatriptan and sumatriptan

Three groups of pentobarbitone-anaesthetized rats were subjected to bilateral vagotomy and received atropine (1 mg kg^{-1}, i.v.). After stabilization of AP, rizatriptan ($n=8$), sumatriptan ($n=9$) or vehicle ($n=8$), as well as flesinoxan, were administered as described below.

Drugs and solutions

Flesinoxan hydrochloride, rizatripan hemisulphate, sumatriptan hydrochloride, GR 127935 dihydrochloride (N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl] - 2(-methyl-1,2,4)-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide) and WAY 100635 (N-{2-[4-(2-methoxyphenyl) - 1-piperazinyl]ethyl}-N-(2-pyridinyl) cyclo-hexanecarboxamide) were dissolved in sterile saline 0.9%. Zolmitriptan was dissolved in PEG 300/saline 0.9% (40/60; v/v). Flesinoxan, rizatriptan, sumatriptan, zolmitriptan and WAY 100635 were synthesized by the Department of Analytical Chemistry and GR 127935 by the Division of Medicinal Chemistry IV (Centre de Recherche Pierre Fabre, Castres, France). Atropine sulphate was purchased from Sigma Chemical Co. (St Louis, MO) and dissolved in sterile saline. Drugs were administered in μg kg^{-1} base weight as 1 ml kg^{-1} bolus solutions or as 20–40 μl min^{-1} infusions as specified.

Calculations and statistical analysis

Data are expressed as mean absolute maximal changes ±s.e.mean of 6 to 17 rats per group. One way analysis of variance followed by Dunnett's test was used to assess significance between groups (StatView, Abacus Concepts Inc., Berkeley, CA). $P<0.05$ was considered the minimum level of significance.

Results

Effects of cumulative doses of rizatriptan, sumatriptan and zolmitriptan on AP and HR in anaesthetized rats

A typical recording of AP and HR following infusion of incremental doses of sumatriptan is presented in Figure 1. Sumatriptan induced rapid, dose-dependent and marked reductions in AP and HR which were reversible following cessation of the infusion. No significant changes in AP or HR were detected in vehicle-treated rats (Figures 1 to 3). The reductions in MAP elicited by rizatriptan and sumatriptan were statistically significantly different from vehicle from 40 and 2.5 μg kg^{-1}, i.v., respectively, with respective maxima of −42±6 and −34±4 mmHg being reached at the highest doses (Figures 2 and 3). HR was statistically significantly reduced compared to vehicle from 40 and 10 μg kg^{-1}, i.v., of rizatriptan and sumatriptan, respectively, with maxima of −85±16 and −44±12 beats min^{-1}, respectively, being reached at the highest doses. Zolmitriptan evoked moderate hypotension only at the highest dose studied (−19±9 mmHg; $P<0.05$ vs vehicle) and failed to induce bradycardia (data not shown).

Effect of a 5-HT$_{1B/D}$ receptor antagonist on the responses to rizatriptan and sumatriptan

We employed the relatively selective 5-HT$_{1B/D}$ receptor antagonist, GR 127935 (Clitherow *et al*., 1994), in order to determine whether 5-HT$_{1B/D}$ receptors were involved in

mediating the hypotension and bradycardia evoked by rizatriptan and sumatriptan. Baseline (post-GR127935 administration) values for MAP and HR are presented in Table 1. Neither MAP nor HR differed significantly between GR 127935- and vehicle-pretreated animals, suggesting that GR 127935 was devoid of significant intrinsic haemodynamic activity under these experimental conditions (Figure 1).

As shown in Figure 2, GR 127935, failed to attenuate significantly the hypotension and bradycardia evoked by sumatriptan (-35 ± 6 mmHg and -52 ± 19 beats min^{-1}, respectively at the highest dose; both not significant vs sumatriptan in untreated rats), with the exception of the hypotension evoked by 40 μg kg^{-1}, i.v., of sumatriptan (-2 ± 2 mmHg; not significant vs vehicle ($+3\pm2$ mmHg) and $P<0.05$ vs sumatriptan in untreated rats -11 ± 4 mmHg). Moreover, GR 127935 moderately antagonized the hypotension and bradycardia evoked by rizatriptan (-20 ± 5 mmHg and -30 ± 17 beats min^{-1}, respectively at the highest dose; both $P<0.05$ vs vehicle and vs rizatriptan in untreated rats; Figure 3). Moreover, GR 127935 failed to affect flesinoxan-evoked hypotension and bradycardia (-41 ± 11 vs -41 ± 4 mmHg and -76 ± 7 vs -92 ± 16 beats min^{-1} in GR 127935 and vehicle-pretreated rats, respectively; $n=5$ and 8, respectively; no significant difference between groups; Figure 4).

Effect of a 5-HT$_{1A}$ receptor antagonist on the responses to rizatriptan and sumatriptan

As presented in Table 1 and Figure 1, baseline MAP was slightly lower in rats following the high dose of WAY 100635 (i.e. 0.63 mg kg^{-1}, i.v.) whereas HR was slightly greater in rats

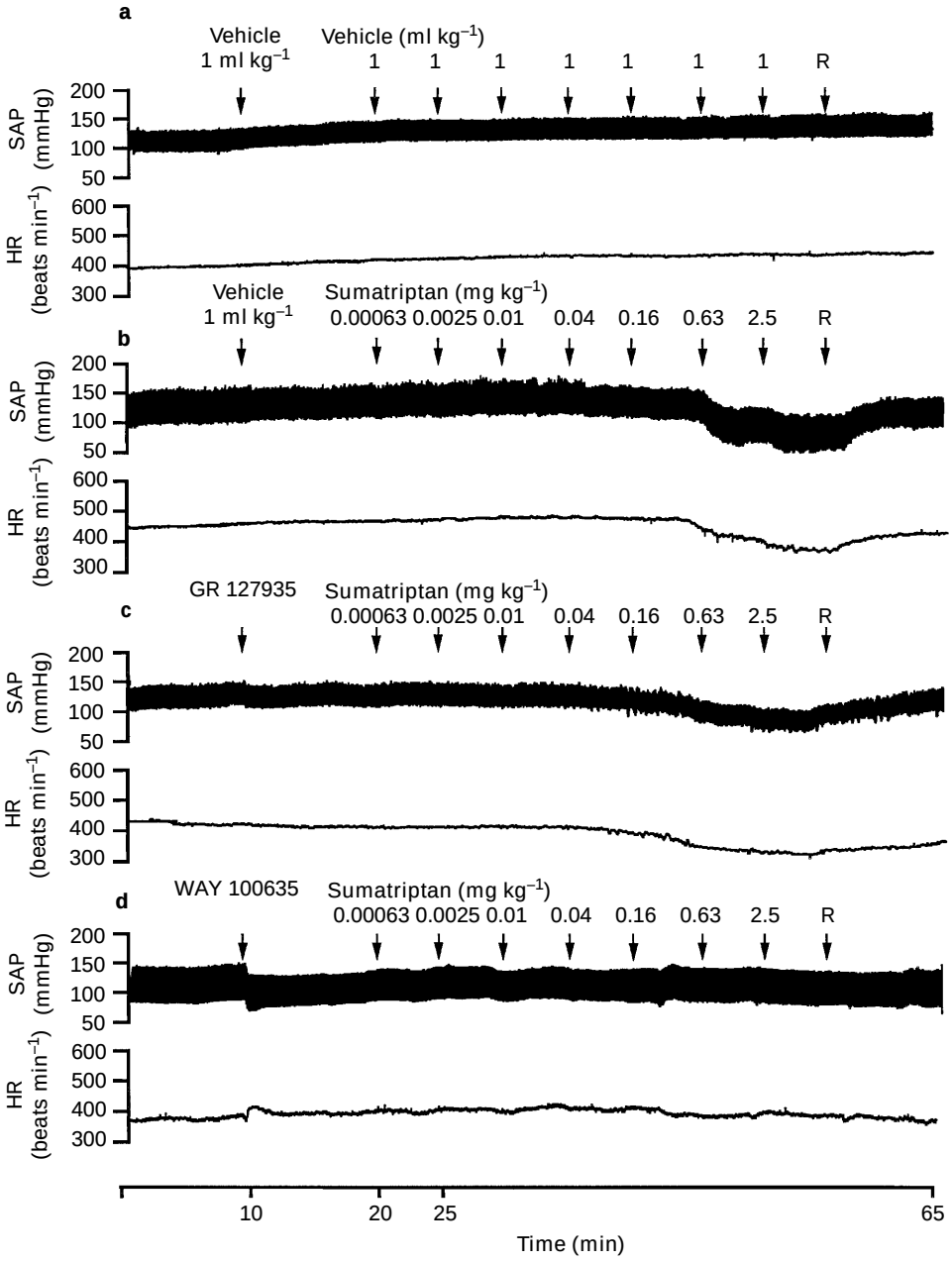

Figure 1 Typical recordings of systemic arterial pressure (SAP) and heart rate (HR) following cumulative doses of sumatriptan (0.63, 2.5, 10, 40, 160, 630 and 2500 μg kg^{-1}, i.v. as indicated by the arrows; b,c,d) or vehicle (sterile saline, 0.9%, 40 μl min^{-1}; a) following pretreatment with GR 127935 (0.63 mg kg^{-1}, i.v.; c), WAY 100635 (0.63 mg kg^{-1}, i.v.; d) or vehicle (saline, 0.9%; a,b). Sumatriptan induced dose-dependent and reversible hypotension and bradycardia which were only slightly attenuated by GR 127935 and fully prevented by WAY 100635. R = recovery.

pretreated with the low dose of WAY 100635 (i.e. 0.16 mg kg^{-1}, i.v.).

As shown in Figure 2, the haemodynamic responses evoked by rizatriptan were dose-dependently antagonized by WAY 100635 (0.16 mg kg^{-1}, i.v.) and almost abolished by the higher dose of WAY 100635 (-4 ± 3 mmHg and -15 ± 8 beats min^{-1} for MAP and HR, respectively, at the highest dose; both not significant vs vehicle and $P<0.05$ vs rizatriptan in untreated rats). As shown in Figure 3, WAY 100635, dose-dependently reduced the haemodynamic responses elicited by sumatriptan, although a slight but statistically significant reduction in MAP still persisted at the highest dose of sumatriptan (-13 ± 4 mmHg following the higher dose of WAY 100635; $P<0.05$ vs vehicle).

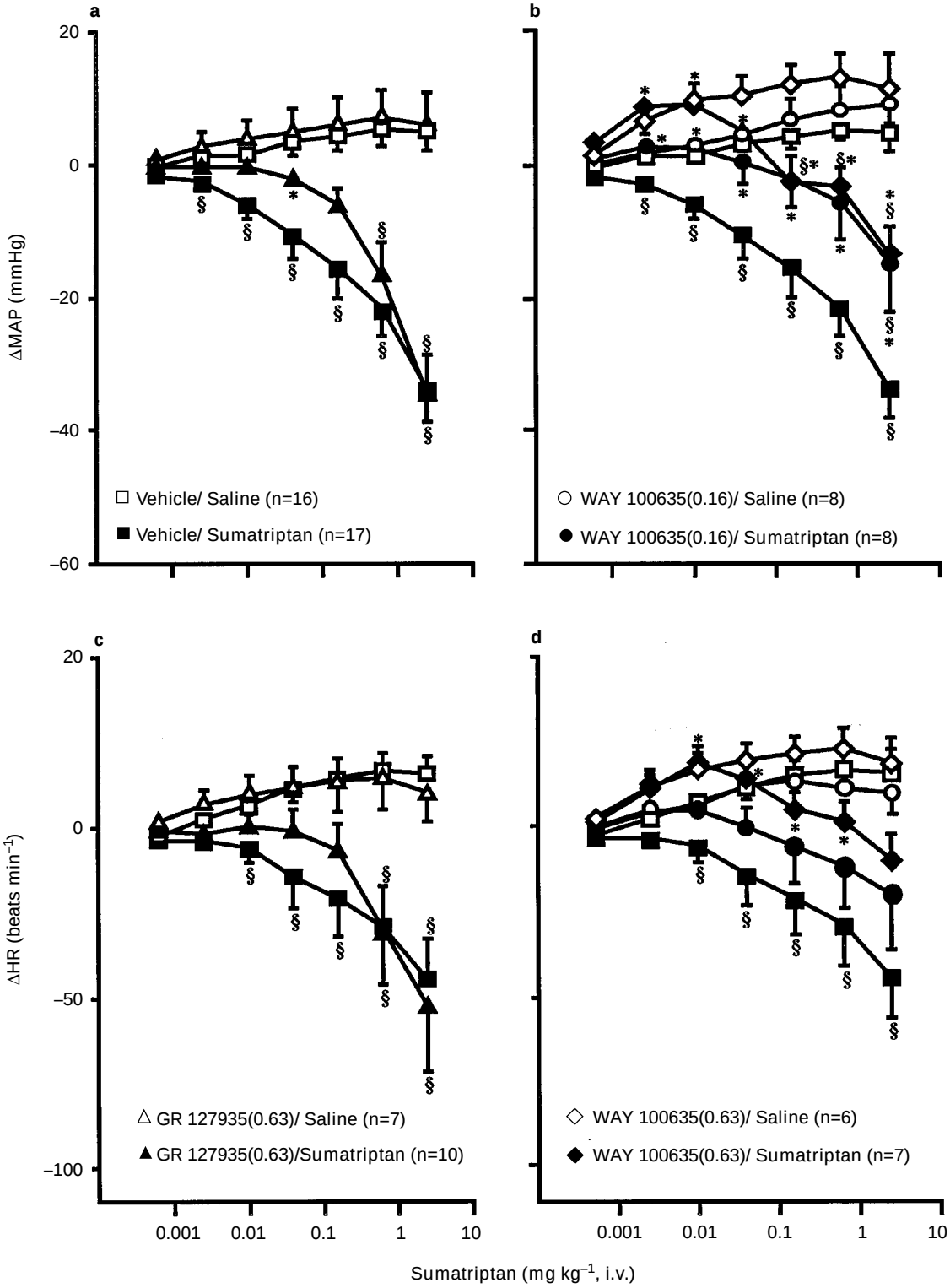

Figure 2 Effect of pretreatment with GR 127935 (0.63 mg kg^{-1}, i.v.; a,c) and WAY 100635 (0.16 and 0.63 mg kg^{-1}, i.v.; b,d) on the hypotension and bradycardia evoked by sumatriptan. Values are absolute changes in mean arterial pressure (ΔMAP; a,b) and heart rate (ΔHR; c,d) observed in rats pretreated with GR 127935, WAY 100635 or vehicle receiving cumulative doses of sumatriptan or vehicle. §$P<0.05$ vs vehicle of sumatriptan; *$P<0.05$ vs sumatripan in vehicle-pretreated rats.

Moreover, WAY 100635 dose-dependently attenuated the hypotension and bradycardia evoked by flesinoxan (Figure 4). The decrease in MAP and HR evoked by flesinoxan in vehicle-pretreated rats (-41 ± 4 mmHg and -76 ± 7 beats min^{-1}) was statistically significantly reduced in rats pretreated with the low (-17 ± 4 mmHg and -38 ± 12 beats min^{-1}; $n=8$; both $P<0.05$ vs vehicle-controls) and high (-9 ± 2 mmHg and -17 ± 7 beats min^{-1}; $n=5$; both $P<0.05$ vs vehicle-controls) dose of WAY 100635.

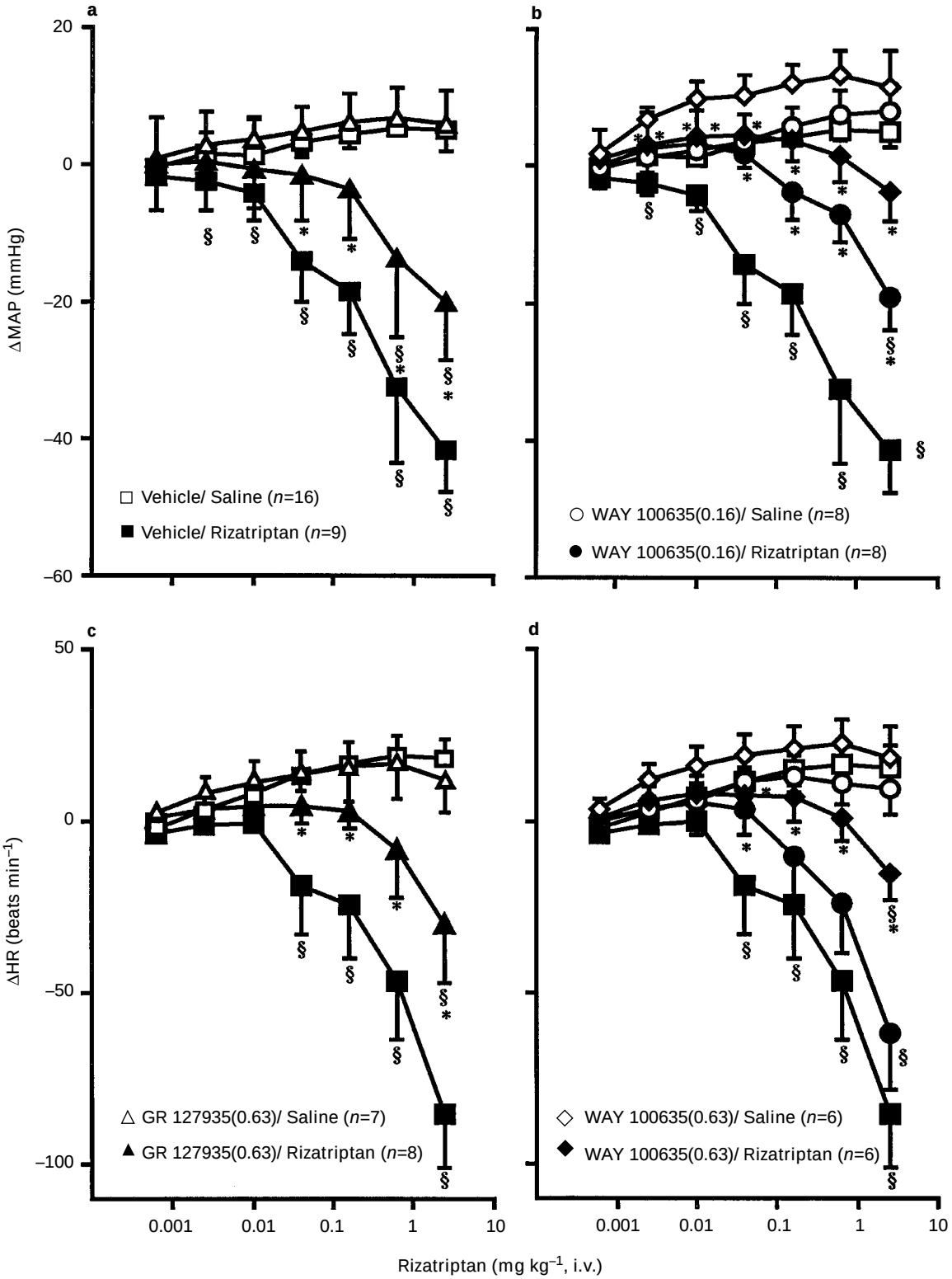

Figure 3 Effect of pretreatment with GR 127935 (0.63 mg kg^{-1}, i.v.; a,c) and WAY 100635 (0.16 and 0.63 mg kg^{-1}, i.v.; b,d) on the hypotension and bradycardia evoked by rizatriptan. Values are absolute changes in mean arterial pressure (ΔMAP; a,b) and heart rate (ΔHR; c,d) observed in rats pretreated with GR 127935, WAY 100635 or vehicle and receiving a perfusion of cumulative doses of rizatriptan (0.63 to 2500 μg kg^{-1}, i.v.) or vehicle (sterile saline, 0.9%). §$P<0.05$ vs vehicle of rizatriptan; *$P<0.05$ vs rizatriptan in vehicle-pretreated rats.

Table 1 Effect of pretreatments on mean arterial pressure (MAP) and heart rate (HR)

Pretreatment	*Dose* (mg kg^{-1}, i.v.)	n	*MAP* (mmHg)	*HR* (beats min^{-1})
Vehicle (saline)	1 ml kg^{-1}	55	113±2	402±5
GR 127935	0.63	25	118±3	420±7
WAY 100635	0.16	23	113±3	433±8*
WAY 100635	0.63	19	100±3*	405±7
Pithing[1]		25	115±4	403±15
Bilateral vagotomy + atropine	1	25	129±4**	385±11

Values are mean±s.e.mean. *n*, number of rats. *$P<0.05$ and **$P<0.01$ versus vehicle pretreated group. [1]With angiotensin II normalized MAP.

Influence of the autonomic nervous system on the responses to rizatriptan and sumatriptan

Angiotensin II administered as an intravenous infusion to pithed rats normalized MAP to values not statistically significantly different from those of intact anaesthetized rats (Table 1). HR did not significantly differ between pithed and intact anaesthetized animals.

In pithed rats, rizatriptan failed to evoke hypotension or bradycardia (+5±4 mmHg and −6±16 beats min^{-1}, respectively; both not significant vs vehicle and $P<0.05$ vs rizatriptan in untreated rats; Figure 5). Similarly, sumatriptan failed to induce bradycardia in pithed rats (+5±6 beats min^{-1}; not significant vs vehicle and $P<0.05$ vs sumatriptan in untreated rats) whereas a slight but statistically significant reduction in MAP, compared to controls, subsisted at the highest dose (−9±9 mmHg; $P<0.05$ vs both vehicle and sumatriptan in untreated rats; Figure 5).

Moreover, the hypotension and bradycardia evoked by flesinoxan in control rats were abolished in pithed rats (−5±3 mmHg and −4±6 beats min^{-1}, respectively; $n=8$; both $P<0.05$ vs flesinoxan in control rats; Figure 4).

Influence of the parasympathetic nervous system on the responses to rizatriptan and sumatriptan

As shown in Table 1, MAP was slightly higher in bilaterally vagotomized and atropine-treated rats compared to vehicle-pretreated intact anaesthetized rats.

As depicted in Figure 5, in bilaterally vagotomized and atropine-treated rats, the reductions in MAP and HR evoked by rizatriptan (−31±4 mmHg and −64±9 beats min^{-1}, respectively; both $P<0.05$ vs vehicle and not significant vs rizatriptan in untreated rats) and sumatriptan (−47±8 mmHg and −56±10 beats min^{-1}, respectively; both $P<0.05$ vs vehicle and not significant vs sumatriptan in untreated rats) were not statistically significantly different from those observed in untreated controls.

Furthermore, in vehicle-treated, bivagotomized and atropine-treated rats flesinoxan reduced MAP and HR by −37±6 mmHg and −64±19 beats min^{-1}, respectively ($n=8$); effects not statistically significantly different from those observed in vehicle-pretreated intact anaesthetized rats (−41±4 and −76±7 beats min^{-1}; both NS between groups; Figure 4).

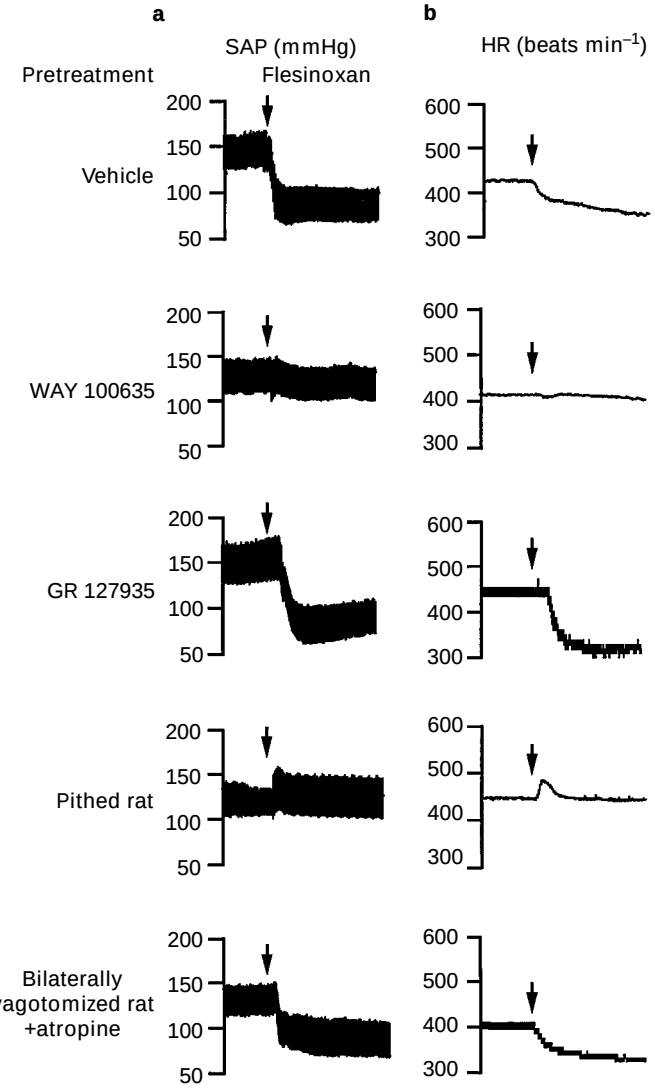

Figure 4 Typical recordings of systemic arterial pressure (SAP; a) and heart rate (HR; b) following flesinoxan administration (0.63 mg kg^{-1}, i.v. as indicated by the arrows) in intact anaesthetized rats pretreated with WAY 100635 (0.63 mg kg^{-1}, i.v.), GR 127935 (0.63 mg kg^{-1}, i.v.) or vehicle (sterile saline, 0.9%), in pithed rats and in bilaterally vagotomized and atropine-treated rats. Flesinoxan induced marked, sustained hypotension and bradycardia which was antagonized by WAY 100635 but not by GR 127935. The responses to flesinoxan were not altered in bilaterally vagotomized and atropinized rats but were abolished in pithed rats.

Discussion

5-HT$_{1B/D}$ receptor agonists, including sumatriptan and rizatriptan, have been shown to raise AP without affecting HR in several species including man (Perrin *et al*., 1989; Hood *et al*., 1997; Sciberras *et al*., 1997b). However, in anaesthetized rats rizatriptan and sumatriptan, but not zolmitriptan, elicited hypotension and bradycardia, confirming our preliminary

observations (Valentin *et al*., 1995b). These contrasting effects of sumatriptan and rizatriptan upon AP and HR in the rat prompted us to undertake experiments to elucidate the nature of the receptors involved in mediating the hypotension and bradycardia evoked by rizatriptan and sumatriptan.

The 5-HT$_{1B/D}$ receptor antagonist, GR 127935 (Clitherow *et al*., 1994), at a dose (0.63 mg kg^{-1}, i.v.) that is higher than that required to block fully 5-HT$_{1B/D}$ receptor-mediated carotid vasoconstriction in anaesthetized dogs (Villalon *et al*., 1996), pigs (De Vries *et al*., 1996) and rabbits (De Vries *et al*., 1997a) failed to attenuate significantly the hypotension and bradycardia evoked by sumatriptan, although it partially antagonized the responses elicited by rizatriptan. Furthermore, the estimated ED_{50} values for sumatriptan and rizatriptan in eliciting hypotension (i.e., around 160 μg kg^{-1}, i.v.) are at least one order of magnitude higher than those required to evoke carotid vasoconstriction (Choppin & O'Connor, 1996; De Vries *et al*., 1996; Villalon *et al*., 1996). Taken together, these results strongly suggest that 5-HT$_{1B/D}$ receptors have little or no involvement in mediating rizatriptan and sumatriptan-induced hypotension and bradycardia in anaesthetized rats. However, it may be speculated that the slight attenuation by GR 127935 of the haemodynamic effects evoked by sumatriptan and rizatriptan, under these experimental conditions, resulted from a reduction of sympathetic vascular tone secondary to activation of sympathetic prejunctional 5-HT$_{1B/D}$ receptors, as previously demonstrated in rats (Shepheard *et al*., 1997) as well as in man (Sciberras *et al*., 1997a).

In addition to possessing nanomolar affinity for 5-HT$_{1B/D}$ receptors, rizatriptan and sumatriptan also have micromolar affinity for 5-HT$_{1A}$ receptors (Pauwels *et al*., 1997; Newman-Tancredi *et al*., 1997; Terron, 1997). Furthermore, the fact that

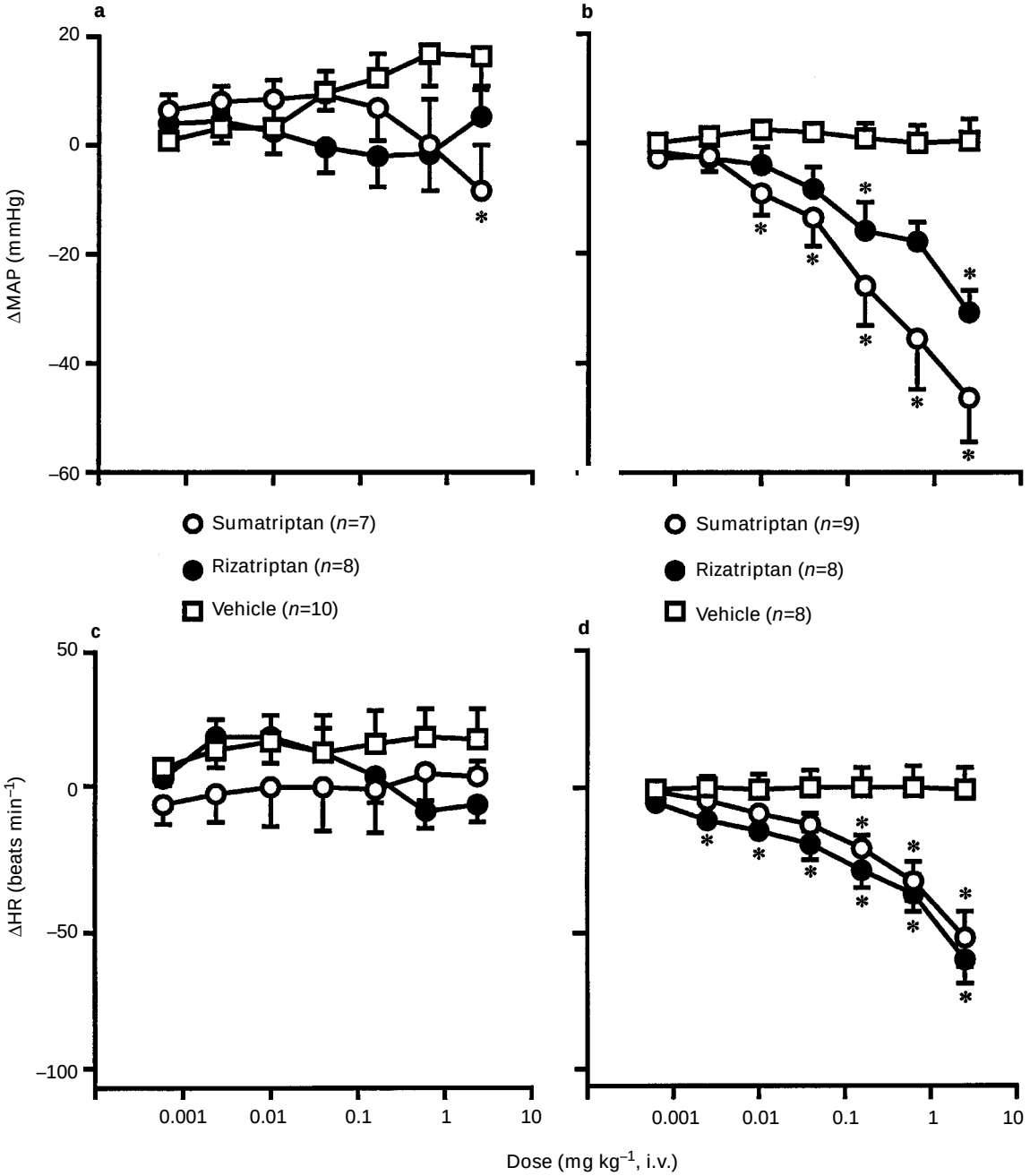

Figure 5 Influence of the autonomic (a,c) and parasympathetic (b,d) nervous system on the responses to rizatriptan and sumatriptan or vehicle (sterile saline, 0.9%). Values are absolute changes in mean arterial pressure (ΔMAP; a,b) and heart rate (ΔHR; c,d) observed in pithed rats (a,c) or bilaterally vagotomized and atropine-treated (b,d). *$P<0.05$ vs vehicle.

the hypotension and bradycardia evoked by sumatriptan and rizatriptan occurred at relatively high doses is compatible with their affinity at 5-HT$_{1A}$ receptors. Therefore, we addressed the possibility of whether the hypotension and bradycardia elicited by rizatriptan and sumatriptan were mediated by 5-HT$_{1A}$ receptors. The recently described and relatively selective 5-HT$_{1A}$ receptor antagonist, WAY 100635 (Fletcher *et al*., 1994), at doses (0.16 and 0.63 mg kg^{-1}, i.v.) which attenuated 5-HT$_{1A}$ receptor-mediated responses in the rat (Critchley *et al*., 1994; present study) dose-dependently reduced and almost abolished the haemodynamic responses evoked by rizatriptan and sumatriptan. Thus, these results suggest that rizatriptan and sumatriptan-induced hypotension and bradycardia are mediated predominantly by 5-HT$_{1A}$ receptor activation. Interestingly, a recent study demonstrated agonist activity of antimigraine drugs, including sumatriptan, at transfected, overexpressed h 5-HT$_{1A}$ receptors (Newman-Tancredi *et al*., 1997); our findings are consistent with activation of native 5-HT$_{1A}$ receptors by sumatriptan and rizatriptan *in vivo* in the rat.

As previously mentioned, GR 127935 partly antagonized the haemodynamic responses evoked by rizatriptan; an effect which could result from antagonist properties of GR127935 at 5-HT$_{1A}$ receptors. However, this is unlikely, since GR 127935 did not affect the hypotension and bradycardia induced by the 5-HT$_{1A}$ receptor agonist, flesinoxan.

We next explored the possibility of whether the hypotension and bradycardia evoked by rizatriptan and sumatriptan could be mediated via activation of 5-HT$_{1A}$ receptors located within the central nervous system, as established for other drugs (Fozard *et al*., 1987; Saxena & Villalon, 1990). In the pithed rat rizatriptan as well as the selective 5-HT$_{1A}$ receptor agonist, flesinoxan (Wouters *et al*., 1988), failed to induce hypotension or bradycardia. Similarly, sumatriptan failed to induce bradycardia or hypotension, although a slight reduction in AP was noted at the highest dose. These results suggest that 5-HT$_{1A}$ receptors localized within the central nervous system mediated rizatriptan, sumatriptan and flesinoxan-induced hypotension and bradycardia. Our observations are in agreement with those of Fozard *et al*. (1987), showing that the 5-HT$_{1A/7}$ receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), failed to reduce AP or HR in pithed rats. In the rat, a high density of 5-HT$_{1A}$ receptors are located within the nucleus tractus solitarius and the dorsal root of the spinal cord (Thor *et al*., 1992). Furthermore, microinjection studies revealed that the responsive sites of 5-HT$_{1A}$ receptor-mediated cardiovascular actions in the rat included the raphe pallidus, the parapyramidal region and the rostral ventrolateral medulla (Helke *et al*., 1993). These areas are poorly protected by the blood brain barrier, which is compatible with the observed activity of sumatriptan in the present study which is considered to penetrate the blood brain barrier poorly (Ferrari & Saxena, 1993). Gallacher and Ramage (1995) showed that activation of forebrain 5-HT$_{1A}$ receptors mediated sumatriptan evoked hypertension and tachycardia following i.c.v. administration. It may be speculated that these forebrain receptors were not readily accessed when sumatriptan was administered via the i.v. route.

Interestingly, a slight but significant hypotension persisted at the highest dose of sumatriptan in both WAY 100635-pretreated rats and in pithed rats, which may suggest the involvement of peripheral non-5-HT$_{1A}$ receptors, as also observed by others (Terron, 1997). Based on the rank order of agonist potencies, antagonism of 5-hydroxytryptamine, 5-carboxamidotryptamine, 5-methoxytryptamine and sumatriptan-induced responses by a series of drugs showing high affinity for the cloned 5-HT$_7$ receptor and a lack of blockade by GR 127935, Terron (1997) and De Vries *et al*. (1997b) demonstrated that the depressor responses evoked by these agonists in sympathectomized and vagosympathectomized rats, respectively, were mediated via activation of 5-HT$_7$ receptors. Thus, in agreement with these findings, it is proposed that the residual hypotension evoked by sumatriptan in pithed rats may be due to activation of 5-HT$_7$ receptors. Interestingly, rizatriptan failed to evoke hypotension in pithed rats, possibly due to lower affinity and/or efficacy at these receptors.

There is no consensus regarding the precise autonomic pathway involved in the cardiovascular effects mediated by 5-HT$_{1A}$ receptors; activation of the parasympathetic nervous system (Gradin *et al*., 1985; Ramage & Fozard, 1987; McCall *et al*., 1994) and/or inhibition of the sympathetic nervous system (McCall *et al*., 1987; Clement & McCall, 1990; 1992) have been demonstrated. In bivagotomized rats pretreated with atropine the hypotension and bradycardia evoked by sumatriptan, rizatriptan as well as flesinoxan was not significantly modified, thus excluding any major contribution of the parasympathetic nervous system in mediating the haemodynamic effects resulting from central 5-HT$_{1A}$ receptor activation. In contrast, the bradycardia elicited by 8-OH-DPAT was found to be prevented in vagotomized cats (Ramage & Fozard, 1987) or rats (Gradin *et al*., 1985). However, Clement and McCall (1990) and King and McCall (1991) showed that 8-OH-DPAT elicited decreases in HR and AP via reduced sympathetic outflow, resulting from inhibition of neuronal firing in the rostral ventrolateral medulla (Clement & McCall, 1990). Our results further indicate that sumatriptan, rizatriptan and flesinoxan activated central 5-HT$_{1A}$ receptors, which in turn probably reduced sympathetic outflow. Moreover, zolmitriptan, a compound shown to cross the blood brain barrier readily (Goadsby & Edvinsson, 1994; Goadsby & Hoskin, 1996; Martin *et al*., 1997), failed to induce major haemodynamic effects, possibly because (i) zolmitriptan activated both 5-HT$_{1A}$ receptors located within the forebrain which induce sympathoexcitation (Gallacher & Ramage, 1995), and those located within the nucleus tractus solitarius and the dorsal root of the spinal cord (Thor *et al*., 1992) which result in sympathoinhibition, and/or (ii) low intrinsic activity and partial agonist behaviour of zolmitriptan at 5-HT$_{1A}$ receptors.

These observations raise the question regarding the role, if any, of 5-HT$_{1B/D}$ receptors in the rat cardiovascular system. Several hypotheses can be entertained to explain the lack of major involvement of these receptors and the predominant role of 5-HT$_{1A}$ receptors in mediating hypotension and bradycardia in the rat: (i) the expression, distribution and/or coupling of 5-HT$_{1B/D}$ receptors within the cardiovascular system of the rat is low or insufficient for them to be detected by functional pressor responses, following activation by an agonist even at relatively high doses. Interestingly, evidence has been obtained for 5-HT$_{1B}$ receptor-mediated contractile responses in the rat tail artery (Craig & Martin, 1993), which may suggest high expression and/or coupling of 5-HT$_{1B}$ receptors in this vascular preparation, although it appears unlikely that this is the case throughout the systemic vasculature. (ii) 5-HT$_{1A}$ receptors are expressed abundantly within the cardiovascular system of the rat, so that their activation predominates largely over 5-HT$_{1B/D}$ receptors in mediating functional

responses elicited by mixed 5-HT$_{1B/D}$ and 5-HT$_{1A}$ receptor agonists. However, if this were the case, then 5-HT$_{1B/D}$ receptor-mediated responses should have been observed in the presence of WAY 100635. This was not the case, strongly suggesting that 5-HT$_{1B/D}$ receptors play little or no role in effecting systemic haemodynamic responses in the rat.

In conclusion, the results of the present study indicate that rizatriptan and sumatriptan evoke hypotension and bradycardia in the rat predominantly via activation of central 5-HT$_{1A}$ receptors, which in turn reduce sympathetic outflow; and suggest that 5-HT$_{1B/D}$ receptors play no major role in mediating cardiovascular responses in the normotensive anaesthetized rat.

The authors are grateful to Drs S. Halazy, C. Jorand, J.P. Ribet and J.L. Maurel, Centre de Recherche Pierre Fabre, Castres, France, for the synthesis of sumatriptan, rizatriptan, zolmitriptan, GR 127935 and WAY 100635.

References

CAMBRIDGE, D., WHITING, M.V., BUTTERFIELD, L.J. & MARSTON, C. (1995). Vascular 5-HT$_1$-like receptors mediating vasoconstriction and vasodilatation: their characterization and distribution in the intact canine cardiovascular system. *Br. J. Pharmacol.*, **114,** 961 – 968.

CHOPPIN, A. & O'CONNOR, S.E. (1996). Influence of vascular tone on vasoconstrictor responses to the 5-HT$_1$-like receptor agonist sumatriptan in anesthetized rabbits. *Eur. J. Pharmacol.*, **304,** 87 – 92.

CLEMENT, M.E. & MCCALL, R.B. (1990). Studies on the site and mechanism of the sympatholytic action of 8-OH-DPAT. *Brain Res.*, **525,** 232 – 241.

CLEMENT, M.E. & MCCALL, R.B. (1992). Evidence that the lateral tegmental field plays an important role in the central sympathoinhibitory action of 8-OH-DPAT. *Brain Res.*, **587,** 115 – 122.

CLITHEROW, J.W., SCOPES, D.I.C., SKINGLE, M., JORDAN, C.C., FENIUK, W., CAMPBELL, I.B., CART, M.C., COLLINGTON, E.W., CONNOR, H.E., HIGGINS, G.A., BEATTIE, D., KELLY, H.A., MITCHELL, W.L., OXFORD, A.W., WADSWORTH, A.H. & TYERS, M.B. (1994). Evolution of a novel series of [(N,N-dimethylamino)propyl]- and piperazinylbenzanilides as the first selective 5-HT$_{1D}$ antagonists. *J. Med. Chem.*, **37,** 2253 – 2257.

CRAIG, D.A. & MARTIN, G.R. (1993). 5-HT$_{1B}$ receptors mediate potent contractile responses to 5-HT in rat caudal artery. *Br. J. Pharmacol.*, **109,** 609 – 611.

CRITCHLEY, D.J.P., WILSON, K.M.R., MIDDLEFELL, V.C. & DOURISH, C.T. (1994). Plasma glucose and cardiovascular studies in the rat with WAY 100635, a selective, potent 5-HT$_{1A}$ receptor antagonist. *Br. J. Pharmacol.*, **112,** 109P.

DE VRIES, P., VILLALON, C.M., HEILIGERS, J.P.C. & SAXENA, P.R. (1996). Blockade of porcine carotid vascular responses to sumatriptan by GR 127935, a selective 5-HT$_{1D}$ receptor antagonist. *Br. J. Pharmacol.*, **118,** 85 – 92.

DE VRIES, P., APAYDIN, S., VILLALON, C.M., HEILIGERS, J.P.C. & SAXENA, P.R. (1997a). Interactions of GR127935, a 5-HT$_{1B/D}$ receptor ligand, with functional 5-HT receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **355,** 423 – 430.

DE VRIES, P., VILLALON, C.M., HEILIGERS, J.P.C. & SAXENA, P.R. (1997b). Nature of 5-HT$_1$-like receptors mediating depressor responses in vagosympathectomized rats; close resemblance to the cloned 5-ht$_7$ receptor. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **356,** 90 – 99.

DEN BOER, M.O., SOMERS, J.A.E. & SAXENA, P.R. (1992). Lack of effect of the antimigraine drugs, sumatriptan, ergotamine and dihydroergotamine on arteriovenous anastomotic shunting in the dura mater of the pig. *Br. J. Pharmacol.*, **107,** 577 – 583.

DIXON, R., GILLOTIN, C., GIBBENS, M., POSNER, J. & PECK, R.W. (1997). The pharmacokinetics and effects on blood pressure of multiple doses of the novel anti-migraine drug zolmitriptan (311C90) in healthy volunteers. *Br. J. Clin. Pharmacol.*, **43,** 273 – 281.

FERRARI, M.D. & SAXENA, P.R. (1993). Clinical and experimental effects of sumatriptan in humans. *Trends Pharmacol. Sci.*, **14,** 129 – 133.

FLETCHER, A., BILL, D.J., CLIFFE, I.A., FORSTER, E.A., JONES, D. & REILLY, Y. (1994). A pharmacological profile of WAY 100635, a potent and selective 5-HT$_{1A}$ receptor antagonist. *Br. J. Pharmacol.*, **112,** 91P.

FOZARD, J.R., MIR, A.K. & MIDDLEMISS, D.N. (1987). Cardiovascular response to 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) in the rat: site of action and pharmalogical analysis. *J. Cardiovasc. Pharmacol.*, **9,** 328 – 347.

GALLACHER, M. & RAMAGE, A.G. (1995). Antagonism by WAY-100635 of the haemodynamic effects of activation of forebrain 5-HT$_{1A}$ receptors with DP-5-CT and sumatriptan in anaesthetized rats. *Br. J. Pharmacol.*, **114,** P448.

GOADSBY, P.J. & EDVINSSON, L. (1994). Peripheral and central trigeminovascular activation in cat is blocked by the serotonin (5HT)-$_{1D}$ receptor agonist 311C90. *Headache*, **34,** 394 – 399.

GOADSBY, P.J. & HOSKIN, K.L. (1996). Inhibition of trigeminal neurons by intravenous administration of the serotonin (5HT)$_{1B/D}$ receptor agonist zolmitriptan (311C90): are brain stem sites therapeutic target in migraine? *Pain*, **67,** 355 – 359.

GRADIN, K., PETTERSON, A., HEDNER, T. & PERSSON, B. (1985). Acute administration of 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), a selective 5-HT receptor agonist in Sprague-Dawley rat and the spontaneously hypertensive rat. *J. Neural Transm.*, **62,** 305 – 319.

HELKE, C.J., MCDONALD, C.H. & PHILLIPS, E.T. (1993). Hypotensive effects of 5-HT$_{1A}$ receptor activation: ventral medullary sites and mechanisms of action in the rat. *J. Auton. Nerv. Syst.*, **42,** 177 – 188.

HOOD, S., BIRNIE, D. & HILLIS, S. (1997). The haemodynamic effects of subcutaneous sumatriptan, a 5-HT$_1$-receptor agonist. *Br. J. Clin. Pharmacol.*, **43,** 327 – 328.

HUMPHREY, P.P.A., FENIUK, W., PERREN, M.J., CONNOR, H.E., OXFORD, A.W., COATES, I.H. & BUTINA, D. (1988). GR43175, a selective agonist for the 5-HT$_1$-like receptor in dog isolated saphenous vein. *Br. J. Pharmacol.*, **94,** 1123 – 1132.

KING, K.A. & MCCALL, R.B. (1991). The effects of 8-OH-DPAT on medullary 5-HT neurones and sympathetic activity in baroreceptor-denervated animals. *Eur. J. Pharmacol.*, **200,** 357 – 360.

MACINTYRE, P.D., BHARGAVA, B., HOGG, K.J., GEMMILL, J.D. & HILLIS, W.S. (1993). Effect of subcutaneous sumatriptan, a selective 5HT$_1$ agonist, on the systemic, pulmonary and coronary circulation. *Circulation*, **87,** 401 – 405.

MARTIN, G.R., ROBERTSON, A.D., MACLENNAN, S.J., PRENTICE, D.J., BARRETT, V.J., BUCKINGHAM, J., HONEY, A.C., GILES, H. & MONCADA, S. (1997). Receptor specificity and trigeminovascular inhibitory actions of a novel 5-HT$_{1B/1D}$ receptor partial agonist, 311C90 (zolmitriptan). *Br. J. Pharmacol.*, **121,** 157 – 164.

MCCALL, R.B., PATEL, N.P. & HARRIS, L.T. (1987). Effects of serotonin$_1$ and serotonin$_2$ receptor agonists and antagonists on blood pressure, heart rate and sympathetic nerve activity. *J. Pharmacol. Exp. Ther.*, **242,** 1152 – 1159.

MCCALL, R., ESCANDON, N.A., HARRIS, L.T. & CLEMENT, M.E. (1994). Tolerance development to the vagal-mediated bradycardia produced by 5-HT$_{1A}$ receptor agonists. *J. Pharmacol. Exp. Ther.*, **271,** 776 – 781.

NEWMAN-TANCREDI, A., CONTE, C., CHAPUT, C., VERRIELE, L., AUDINOT-BOUCHEZ, V., LOCHON, S., LAVIELLE, G. & MILLAN, M.J. (1997). Agonist activity of antimigraine drugs at recombinant human 5-HT$_{1A}$ receptors: potential implications for prophylactic and acute therapy. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **355,** 682 – 688.

PAUWELS, P.J., TARDIF, S., PALMIER, C., WURCH, T. & COLPAERT, F.C. (1997). How efficacious are 5-HT$_{1B/D}$ receptor ligands: an answer from GTPγS binding studies with stably transfected C6-glial cell lines. *Neuropharmacology*, **36,** 499 – 512.

PERRIN, V.L., FARKKILA, M., GOASGUEN, J., DOENICKE, A., BRAND, J. & TFELT-HANSEN, P. (1989). Overview of initial clinical studies with intravenous and oral GR43175 in acute migraine. *Cephalalgia*, **9** (Suppl 9), 63 – 72.

RAMAGE, A.G. & FOZARD, J.R. (1987). Evidence that the putative 5-HT_{1A} receptor agonist 8OH-DPAT and ipsapirone have a central hypotensive action that differs from that of clonidine in anesthetized cats. *Eur. J. Pharmacol.*, **138,** 179–191.

SAXENA, P.R. & VILLALON, C.M. (1990). Cardiovascular effects of serotonin agonists and antagonists. *J. Cardiovasc. Pharmacol.*, **15,** (Suppl. 7) S17–S34.

SCIBERRAS, D.G., MAJMUDAR, N., BOWMAN, A.J., MASSEY, A., BRADSTREET, T., CLAYTON, R., REED, J., SHADLE, C., GOLDBERG, M.R. & FORD, G.A. (1997a). A study of the effects of MK-462 (rizatriptan), clonidine and sumatriptan on autonomic function. *Br. J. Clin. Pharmacol.*, **43,** 535P.

SCIBERRAS, D.G., POLVINO, W.J., GERTZ, B.J., CHENG, H., STEPANAVAGE, M., WITTREICH, J., OLAH, T., EDWARDS, M. & MANT, T. (1997b). Initial human experience with MK-462 (rizatripan): a novel 5-HT_{1D} agonist. *Br. J. Clin. Pharmacol.*, **43,** 49–54.

SHEPHEARD, S.L., WILLIAMSON, D.J., COOK, D.A., HILL, R.G. & HARGREAVES, R.J. (1997). Preclinical comparative studies on the sympatholytic effects of 5-$HT_{1B/D}$ receptor agonists *in vivo*. In *Frontiers in Headache Research*. ed. Olesen, J. & Tfelt-Hansen, P. Vol. 6, pp. 293–300. Philadelphia, New York: Lippicott Raven Publishers.

TERRON, J.A. (1997). Role of 5-ht_7 receptors in the long-lasting hypotensive response induced by 5-hydroxytryptamine in the rat. *Br. J. Pharmacol.*, **121,** 563–571.

THOR, K.B., BLITZ-SIEBERT, A. & HELKE, C.J. (1992). Autoradiographic localisation of 5-HT_1 binding sites in autonomic areas of the rat dorsomedial medulla oblongata. *Synapse*, **10,** 217–227.

VALENTIN, J.P., BESSAC, A.M., COLPAERT, F.C. & JOHN, G.W. (1995a). Use of the pithed rat model to determine the relative potencies of 5-$HT_{2A/2C}$ receptor antagonists following acute intravenous and oral administration. *Meth. Find. Exp. Clin. Pharmacol.*, **17,** 267–271.

VALENTIN, J.P., VIEU, S., JORAND, C., PEREZ, M., HALAZY, S., COLPAERT, F.C. & JOHN, G.W. (1995b). Hypotension and bradycardia evoked by novel 5-$HT_{1B/D}$ receptor agonists in the anesthetized rat. *Cephalalgia*, **15** (Suppl 14), 208.

VALENTIN, J.P., BESSAC, A.M., MAFFRE, M. & JOHN, G.W. (1996a). Nitric oxide regulation of TP receptor-mediated pulmonary vasoconstriction in the anesthetized, open chest rat. *Eur. J. Pharmacol.*, **317,** 335–342.

VALENTIN, J.P., BONNAFOUS, R. & JOHN, G.W. (1996b). Influence of the endothelium and nitric oxide on the contractile responses evoked by 5-HT_{1D} receptor agonists in the rabbit isolated saphenous vein. *Br. J. Pharmacol.*, **119,** 35–42.

VERSCHEURE, Y., DE COURTOIS, F. & JOHN, G.W. (1995). Hemodynamic effects of the 5-$HT_{1B/D}$ receptor agonists, sumatriptan and naratriptan in anesthetized, open-chest pigs. *Cephalalgia*, **15** (Suppl 14), 210.

VILLALON, C.M., SANCHEZ-LOPEZ, A. & CENTURION, D. (1996). Operational characteristics of the 5-HT_1-like receptors mediating external carotid vasoconstriction in vagosympathectomized dogs. Close resemblance to the 5-HT_{1D} receptor subtype. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **354,** 550–556.

VILLALON, C.M., CENTURION, D., LUJAN-ESTRADA, M., TERRON, J.A. & SANCHEZ-LOPEZ, A. (1997). Mediation of 5-HT-induced external carotid vasodilatation in GR127935-pretreated vagosympatectomized dogs by the putative 5-HT_7 receptor. *Br. J. Pharmacol.*, **120,** 1319–1327.

WOUTERS, W., TULP, M.T.M. & BEVAN, P. (1988). Flesinoxan lowers blood pressure and heart rate in cats via 5-HT_{1A} receptors. *Eur. J. Pharmacol.*, **149,** 213–223.

(Received July 8, 1997
Revised October 6, 1997
Accepted October 8, 1997)