British Journal of Pharmacology (1995) 114, 400–404

Mediation by 5-hydroxytryptamine$_{2B}$ receptors of endothelium-dependent relaxation in rat jugular vein

Elizabeth S. Ellis, Clare Byrne, Olive E. Murphy, Nicholas S. Tilford & [1]Gordon S. Baxter

SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AD

1 An 'atypical' 5-HT$_2$ receptor which is located on the endothelium of rat jugular vein has been described. In the present study we have further defined the nature of the 5-HT$_2$ receptor subtype present in this preparation.

2 In experiments conducted in the presence of ketanserin to preclude involvement of 5-HT$_2$ receptors, the mixed 5-HT$_{2B/2C}$ antagonist, SB 200646, acted as an antagonist of 5-HT at the endothelial 5-HT receptor (pA$_2$ = 7.2). Yohimbine, which exhibits negligible affinity for rat 5-HT$_{2C}$ receptors but has high 5-HT$_{2B}$ receptor affinity, acted as a potent but non-surmountable antagonist (pA$_2 \geqslant 7.3$) in rat jugular vein. Neither yohimbine nor SB 200646 affected endothelium-dependent relaxations induced by carbachol.

3 Mianserin also acted as a surmountable antagonist (pA$_2$ = 7.3) and the 5-HT$_{2B}$ agonist, BW 723C86, acted as a potent partial agonist (pEC$_{50}$ [95% C L], intrinsic activity ± s.e.mean = 7.9 [7.6–8.3], 0.84 ± 0.04). Responses to BW 723C86 were antagonized by SB 200646 (0.3 μM) yielding an 'apparent' pA$_2$ [95% CL] of 7.03 [6.76–7.32].

4 These data are consistent with the presence of 5-HT$_{2B}$ receptors mediating endothelium-dependent relaxation of rat jugular vein.

Keywords: 5-HT$_{2B}$ receptors vascular endothelium SB 200646 BW 723C86

Introduction

Atypical 5-hydroxytryptamine (5-HT) receptors which mediate endothelium-dependent relaxation have been identified in a number of blood vessels. Whilst some have been classified as 5-HT$_1$-like (Schoeffter & Hoyer, 1990; Gupta, 1992), others may be tentatively classified as subtypes of the 5-HT$_2$ receptor family. Thus, endothelial 5-HT receptors mediating relaxation of rabbit jugular vein (Leff *et al.*, 1987), rat jugular vein (Bodelsson *et al.*, 1992), piglet vena cava (Sumner, 1991) and pig pulmonary artery (Glusa, 1992) share some pharmacological identity with both 5-HT$_{2B}$ (Wainscott *et al.*, 1993) and 5-HT$_{2C}$ (Hoyer *et al.*, 1989) receptors. It has been proposed that the endothelial 5-HT receptor in rat jugular vein and pig pulmonary artery represent the first examples of peripheral 5-HT$_{2C}$ receptors (Bodelsson *et al.*, 1992; Glusa, 1992). However, as 5-HT$_{2C}$ and 5-HT$_{2B}$ receptors possess close pharmacological similarity (see Baxter *et al.*, 1994) it is plausible that 5-HT$_{2B}$ receptors may be involved. In this regard, a close relationship between the 5-HT receptor in rat jugular vein and 5-HT$_{2B}$ receptors in rat stomach fundus was discounted only on the basis of a difference in the antagonist potency of mianserin (Bodelsson *et al.*, 1992).

We have recently demonstrated that a novel ligand, SB 200646 (N-1-methyl-5-indolyl)-*N'*-(3-pyridyl) urea HCl) exhibits moderate affinity for cloned rat 5-HT$_{2C}$ receptors and putative 5-HT$_{2B}$ receptors in rat stomach fundus, but little or no affinity for rat 5-HT$_{2A}$ and many other 5-HT receptors (Forbes *et al.*, 1993; Baxter *et al.*, 1994). In the present study we have used SB 200646 and a number of other ligands which may permit discrimination of 5-HT$_2$ receptor subtypes in the rat to characterize further 5-HT$_{2C}$-like receptors which mediate relaxation of the jugular vein in this species. A preliminary account of this work has been reported previously (Ellis *et al.*, 1994).

[1] Author for correspondence.

Methods

Rat jugular vein was set up as described by Bodelsson *et al.* (1992). Briefly, segments of the external sub-maxillary branch of the jugular vein were carefully removed from male Sprague Dawley rats (250–300 g) and placed in Krebs solution of the following composition (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 11.1, $NaHCO_3$ 25, $CaCl_2$ 2.5 and indomethacin 0.003. Vessels were cleared of excess connective tissue and ring segments (5 mm long) were suspended, under a tension equivalent to 5 mN, between stainless steel wires (150 μm diameter) in oxygenated (95% O_2/5% CO_2) Krebs solution at 37°C. To expose relaxant responses to agonists, tone was induced in each preparation with the stable thromboxane-mimetic, U-46619 (0.1 μM). This concentration of U-46619 produced a contraction which was approximately 70% of the maximum response attainable with this agonist. In many preparations the responses to U-46619 were not well maintained and this interfered with the measurement of 5-HT-induced relaxation. For this reason, each preparation was challenged with U-46619 (0.1 μM) prior to construction of concentration-effect curves to determine their suitability. Any preparations which did not maintain a reasonably stable contractile response to U-46619, or upon which relaxant responses to 5-HT were ill defined, were not used. All experiments were conducted in the presence of ketanserin (0.1 μM) to prevent interactions of agonists at 5-HT$_{2A}$ receptors (Bodelsson *et al.*, 1992) and after exposure of tissues to pargyline (100 μM for 15 min followed by washout) to inhibit monoamine oxidase.

Agonist concentration-effect curves were fitted to the following equation using Kaleidagraph (Synergy Software) on an Apple Macintosh II Ci computer.

$$E = \frac{\alpha}{1 + (EC_{50}/[A])^n} \quad (1)$$

α, [A] and n represent the maximum response, agonist concentration and curve mid-point slope factor, respectively. The EC$_{50}$ is the concentration of agonist that produces 50% of the maximal response. On repetition, curves to 5-HT were

reproducible and EC_{50} location parameters differed by less than 2 fold. For this reason, correction factors were not routinely applied to account for changes in sensitivity between first and second concentration-effect curves.

Antagonist affinity

pA_2 values were calculated according to the method of Arunlakshana & Schild (1959). A single pA_2 value was obtained for each antagonist by plotting $-\log_{10}$ molar antagonist concentration against the $-\log_{10}$ of all of the concentration-ratios determined in individual experiments. Concentration-ratios were obtained by dividing the EC_{50} location parameter for the 2nd agonist concentration-effect curve by that for the 1st concentration-effect curve constructed in the presence and absence of antagonist respectively. Tissues were equilibrated with antagonists for 1 h before the construction of second concentration-effect curves.

Responses were recorded with Dynamometer UH1 isometric transducers coupled to a MACLAB/8 (AD instruments) recording system and Apple Macintosh II Ci computer.

Compounds used

5-Hydroxytryptamine (5-HT), carbachol HCl and yohimbine HCl were obtained from Sigma Chemical Company (Dorset). SB 200646 (N-1-methyl-5-indolyl-)*N'*-(3-pyridyl) urea HCl) and mianserin (racaemic) were synthesized at SmithKline Beecham Pharmaceuticals, Harlow, Essex. Sincere thanks to Dr Graeme Martin for supplying a sample of BW 72386 ([±]-1-[5-(2-thenyloxy)-1H-indol-3-yl]-propan-2-amine HCl).

Results

Mechanical removal of the endothelium, by rotating/sliding the vessel along a 200 μm o.d. stainless steel wire, abolished relaxant responses to both carbachol and 5-HT confirming the previously established endothelium-dependent nature of the relaxant response to both agonists in this preparation (Bodelsson *et al.*, 1992). In experiments where care was taken not to damage the endothelium, cumulative addition of 5-HT (0.1 nM–0.3 μM) caused concentration-dependent relaxations (Figure 1).

Whilst some inter-tissue variability was apparent, location parameters of 5-HT concentration-effect curves constructed in the same tissues were reproducible (pEC_{50} [95% CL] estimates of 8.6 [8.5–8.7], and 8.6 [8.4–8.7], $n = 22$ for control and repeat curves, respectively). SB 200646 (0.3–3.0 μM) and mianserin (0.1 and 0.3 μM) caused concentration-dependent rightward displacements of concentration-effect curves for 5-HT with no significant effect on maximum response (Figures 2 and 3). Schild regression analysis yielded pA_2 estimates of 7.2 for SB 200646 and 7.3 for mianserin (Figures 2 and 3). The slopes of the regression were 1.05 and 1.1, respectively, and were not significantly different from unity. In contrast, yohimbine acted as a non-surmountable antagonist, reducing the maximum response and causing a dextral displacement ± s.e.mean of concentration-effect curves for 5-HT by 1.6 ± 0.5, 14.9 ± 5 and 41.3 ± 13 fold at concentrations of 0.03, 0.1 and 0.3 μM respectively (Figure 4). The mean ± s.e.mean reduction is maximum response to 5-HT at these same concentrations were 11.8 ± 4.0, 22.6 ± 4.8 and 28.3 ± 8, respectively. These data are consistent with an apparent pA_2 for yohimbine of ≥ 7.3. Neither yohimbine (0.3 μM) nor SB 200646 (3 μM) antagonized responses to carbachol (Figure 5).

BW 723C86, which acts as a potent partial agonist at 5-HT$_{2B}$ receptors in rat stomach fundus and a weak agonist at 5-HT$_{2A}$ receptors in rat caudal artery (*see* Ellis *et al.*, 1994), also caused potent relaxation of rat jugular vein (Figure 6) and was a partial agonist relative to 5-HT (pEC_{50} [95% CL] = 7.9, [7.6–8.2], mean intrinsic activity ± s.e. mean = 0.84 ± 0.04, $n = 4$). Relaxations induced by BW 723C86 were antagonized by 1.0 μM SB 200646 yielding an *apparent* mean pA_2 [95% CL] of 7.03 [6.76–7.32], $n = 3$, indicating a common site of action.

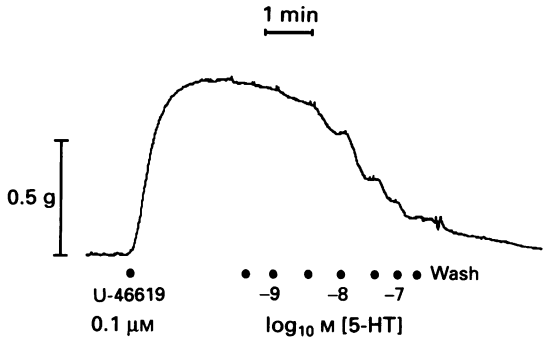

Figure 1 Typical trace of a cumulative (0.5 $\log_{10}$ increments) concentration-effect curve to 5-HT in rat jugular vein.

Discussion

In the present study we have confirmed the 5-HT$_2$-like status of the endothelial 5-HT receptor mediating relaxation of rat jugular vein (Bodelsson *et al.*, 1992) using selective pharmacological probes which allow discrimination of rat 5-HT$_2$ receptor subtypes *in vitro*. In the presence of the 5-HT$_{2A}$ antagonist, ketanserin (Van Neuten *et al.*, 1981), the mixed

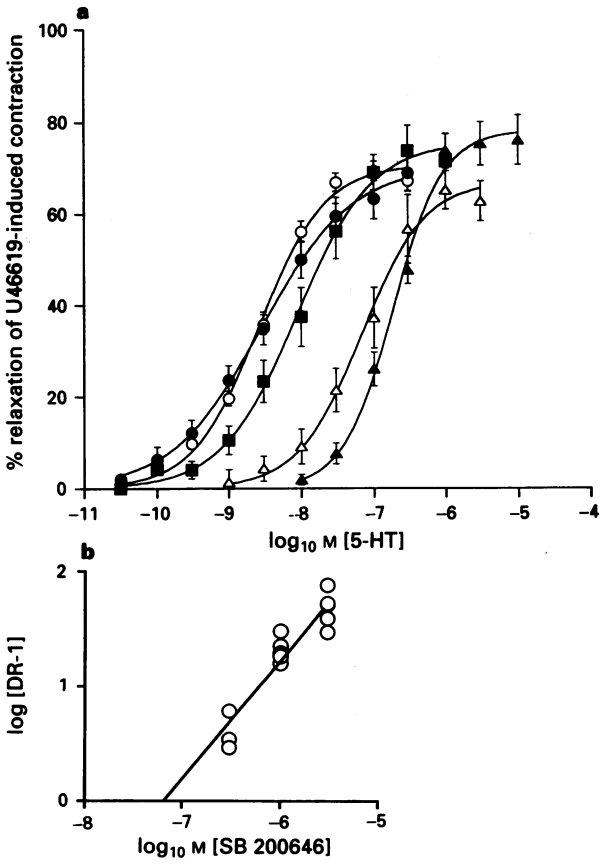

Figure 2 (a) Cumulative concentration-effect curve to 5-HT in the absence (○, first curve, ●, second curve) and presence of 0.3 (■), 1 (△) and 3 (▲) μM SB 200646 in rat jugular vein. Each point represents the arithmetic mean ± s.e.mean of $n \geq 4$ experimental determinations. (b) Schild regression analysis derived from agonist concentration-ratios determined at 0.3, 1 and 3 μM SB 200646.

5-HT$_{2C/2B}$ receptor ligand, SB 200646, acted as a potent and surmountable antagonist of 5-HT in rat jugular vein. The affinity for SB 200646 (pA$_2$ = 7.2) is of the same order as those determined at both rat stomach fundus 5-HT$_{2B}$ receptors (pA$_2$ = 7.5; Baxter *et al.*, 1994) and cloned rat 5-HT$_{2C}$ receptors (pK_i = 6.86; Forbes *et al.*, 1993). The finding that yohimbine also possessed antagonist activity in rat jugular vein at concentrations consistent with its pA$_2$ at 5-HT$_{2B}$ receptors permits further definition of the subtype involved. Yohimbine exhibits low affinity for rat 5-HT$_{2A}$ receptors (pA$_2$ = $<$6.3) and cloned rat 5-HT$_{2C}$ receptors (pK_i = $<$5.3, Martyn Wood personal communication) but high affinity for cloned rat 5-HT$_{2B}$ receptors and 5-HT$_{2B}$ receptors in rat stomach fundus (Clineschmidt *et al.*, 1985; Wainscott *et al.*, 1993). Unfortunately it was not possible to obtain a more robust estimate of the affinity for yohimbine versus 5-HT in the rat jugular vein as it was a non-surmountable antagonist in this preparation. Whilst yohimbine does act as a surmountable antagonist at 5-HT$_{2B}$ receptors in the smooth muscle of rat stomach fundus (Clineschmidt *et al.*, 1985; Baxter *et al.*, 1994), this preparation shows relatively stable, well maintained contractile responses to 5-HT. In contrast, activation of endothelial 5-HT$_{2B}$ receptors in rat jugular vein leads to production of a metabolically labile messenger, nitric oxide, which causes relaxant responses which are not

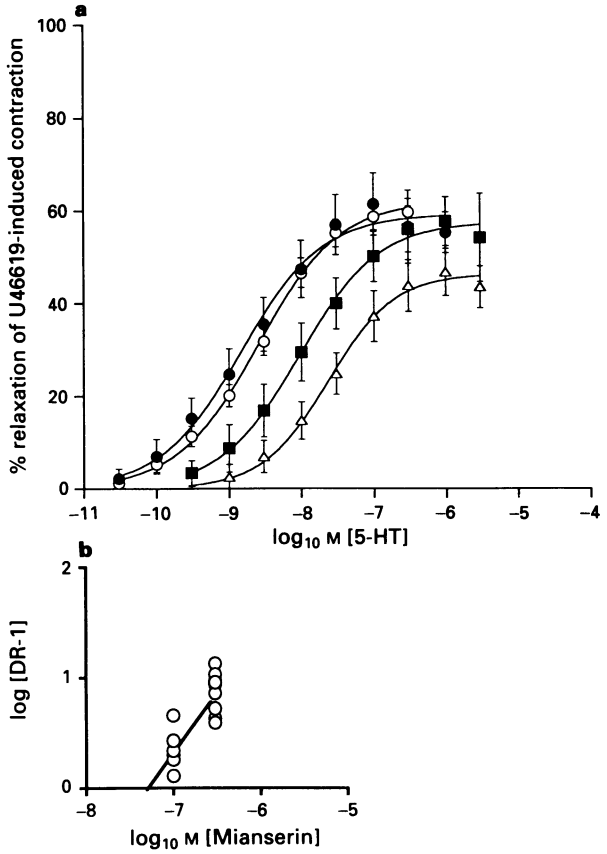

Figure 3 (a) Cumulative concentration-effect curve to 5-HT in the absence (○, first curve, ●, second curve) and presence of 0.1 (■) and 0.3 (△) μM mianserin in rat jugular vein. Each point represents the arithmetic mean ± s.e.mean of $n \geq 4$ experimental determinations. (b) Schild regression analysis derived from agonist concentration-ratios determined at 0.1 and 0.3 μM mianserin.

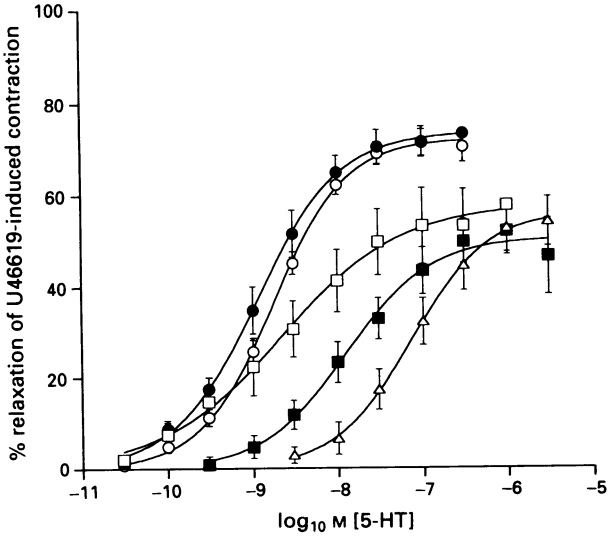

Figure 4 Cumulative concentration-effect curve to 5-HT in the absence (○, first curve, ●, second curve) and presence of 0.03 (□), 0.1 (■) and 0.3 (△) μM yohimbine in rat jugular vein. Each point represents the arithmetic mean ± s.e.mean of $n \geq 4$ experimental determinations.

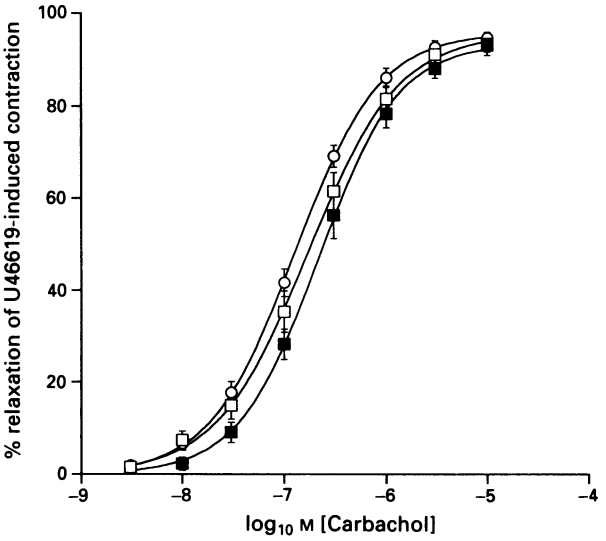

Figure 5 Cumulative concentration-effect curve to carbachol in the absence and presence of 3 μM SB 200646 (□) and 0.3 μM (■) yohimbine in rat jugular vein. Each point represents the arithmetic mean ± s.e.mean of $n \geq 4$ experimental determinations.

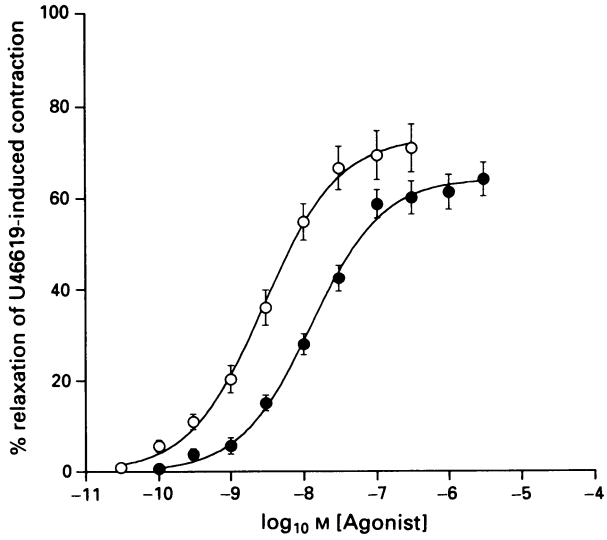

Figure 6 Cumulative concentration-effect curve to 5-HT (○) and BW 723C86 (●) in rat jugular vein. Each point represents the arithmetic mean ± s.e.mean of $n \geq 4$ experimental determinations.

generally as well maintained (*see* Bodelsson *et al.*, 1992). It is also likely that the jugular vein, like the rat stomach fundus, possesses a low 'effective receptor reserve' for 5-HT (Clineschmidt *et al.*, 1985; Baxter *et al.*, 1994). A combination of these factors may contribute to the exposure of non-surmountable antagonism with yohimbine in the present study. It is also worth pointing out that the depression of maximum response to 5-HT after exposure to yohimbine appeared to be concentration-independent. We can offer no satisfactory explanation for these observations at this time.

Other investigators have discounted a possible affiliation of the rat jugular vein 5-HT receptor with the rat stomach fundus receptor (5-HT$_{2B}$) on the basis of differences in sensitivity to antagonism by mianserin (Bodelsson *et al.*, 1992). In the present study the pA_2 estimated for mianserin agreed well with that reported for cloned 5-HT$_{2B}$ receptors ($pK_i = 7.3$; Wainscott *et al.*, 1992) and naturally expressed 5-HT$_{2B}$ receptors in rat stomach fundus longitudinal muscle ($pA_2 = 7.6$; Baxter *et al.*, 1994). The reason for the discrepancy between the present study and that conducted by Bodelsson *et al.* (1992) is not known; however, it is worth pointing out that mianserin possesses a complex profile of action in rat stomach fundus. In the studies of Clineschmidt *et al.* (1985) mianserin antagonized the effects of 5-HT in a concentration-independent manner and evoked methysergide-insensitive contractions in its own right. This contractile response to mianserin in the rat fundus preparation has also been observed by other investigators (Frankhuyzen & Bonta, 1974), however, in contrast to the findings of Clineschmidt *et al.* (1985), these investigators reported that mianserin failed to antagonize responses to 5-HT at concentrations up to 1 μM whilst still conferring protection versus the non-surmountable properties of phenoxybenzamine, methysergide and cyproheptadine. Such observations suggest that the use of mianserin for the characterization of 5-HT$_2$ receptors must be regarded with some caution, especially in view of its high affinity for other receptor subtypes.

The antagonist data obtained in this study present compelling evidence for the existence of 5-HT$_{2B}$ receptors mediating the endothelium-dependent relaxation of the jugular vein of the rat. We have extended this characterization by our finding that BW 723C86, which acts as a potent agonist at 5-HT$_{2B}$ receptors in rat stomach fundus, was also a potent agonist in rat jugular vein. This ligand shows some selectivity for a vascular endothelial receptor in rabbit jugular vein over a number of other 5-HT receptors and displays low potency at 5-HT$_{2A}$ receptors in rabbit aorta and low affinity for 5-HT$_{2C}$ binding sites in pig choroid plexus (Martin *et al.*, 1993 and personal communication). We have confirmed the low potency of BW 723C86 at 5-HT$_{2A}$ receptors in the caudal artery of the rat ($pEC_{50} \leqslant 5.3$, intrinsic activity $\geqslant 0.4$, Ellis *et al.*, 1994). The data suggest that BW723C86 may act as a reasonably selective 5-HT$_{2B}$ agonist, however, no data has been reported regarding 'functional' activity of BW 723C86 at rat 5-HT$_{2C}$ receptors. In this regard, whilst functional data at the rat homologue are not available, we have observed some agonist activity with BW 723C86 at human cloned 5-HT$_{2C}$ receptors expressed in SH-SY5Y cells ($pEC_{50} = 6.5$, intrinsic activity relative to 5-HT = 1, Martyn Elliot personal communication).

It is plausible that 5-HT$_{2B}$ receptors may mediate endothelium-dependent relaxation induced by 5-HT in other species. However, although the endothelial receptors which are present in blood vessels of pig and rabbit have a similar pharmacological profile to that observed in rat jugular vein (Leff *et al.*, 1987; Sumner, 1991; Glusa, 1992), the occurrence of robust operational differences confound explicit classification as 5-HT$_{2B}$. For example, mesulergine (1.0 μM) which is ineffective as an antagonist in piglet vena cava (Sumner, 1991), is a moderately potent antagonist in rabbit ($pA_2 \geqslant 7.3$, Martin *et al.*, 1993) and rat jugular vein ($pA_2 = 7.84$, Bodelsson *et al.*, 1992). Yohimbine also appears to discriminate between endothelial 5-HT receptors in rat and rabbit jugular vein preparations in that it possesses an approximately 50 fold higher affinity for the receptor in the former preparation (Bodelsson *et al.*, 1992; Martin *et al.*, 1993). It is notable that the mouse and rat cloned 5-HT$_{2B}$ receptor homologues show quite profound differences in pharmacology (Compare Loric *et al.*, 1992; Waiscott *et al.*, 1993). For example, the rat receptor possessed high affinity for 5-HT ($pK_i = 8.0$), whilst that displayed by the mouse homologue is much lower ($pK_i = 5.9$). Differences also exist with respect to antagonist binding. It is therefore possible that the pharmacological differences between members of the 'atypical' endothelial receptor family reflect only species variation in pharmacology of 5-HT$_{2B}$ receptors.

In conclusion, by using pharmacological probes that possess selectivity for rat 5-HT$_2$ receptor subtypes, we present compelling evidence that an 'atypical' receptor mediating relaxation of rat jugular vein is a 5-HT$_{2B}$ receptor. The use of ligands such as SB 200646 and BW 723C86 should aid definition of 5-HT$_2$ receptor subtypes in other species and permit a greater understanding of the physiological role of 5-HT receptors on vascular endothelium.

References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonism. *Br. J. Pharmacol. Chemother.*, **14**, 48–58.

BAXTER, G.S., MURPHY, O.E. & BLACKBURN, T.P. (1994). Further characterisation of 5-hydroxytryptamine receptors (putative 5-HT$_{2B}$) in rat stomach fundus longitudinal muscle. *Br. J. Pharmacol.*, **112**, 323–331.

BODELSSON, M., TÖRNEBRANDT, K. & ARNEKLO-NOBIN, B. (1992). Endothelial relaxing 5-hydroxytryptamine receptors in the rat jugular vein: similarity with the 5-hydroxytryptamine$_{1C}$ receptor. *J. Pharmacol. Exp. Ther.*, **264**, 709.

CLINESCHMIDT, B.V., REISS, D.R., PETTIBONE, D.J. & ROBINSON, J.L. (1985). Characterization of 5-hydroxytryptamine receptors in rat stomach fundus. *J. Pharmacol. Exp. Ther.*, **235**, 696–708.

ELLIS, E.S., BYRNE, C., MURPHY, O.E. & BAXTER, G.S. (1994). 5-HT$_{2B}$-like receptors mediate endothelium-dependent relaxation of rat jugular vein. *Br. J. Pharmacol.*, **112**, 477P.

FORBES, I.T., KENNETT, G.A., GADRE, A., HAM, P., HAYWARD, C.J., MARTIN, R.T., THOMPSON, M., WOOD, M.D., BAXTER, G.S., GLEN, A., MURPHY, O.E., STEWART, B.A. & BLACKBURN, T.P. (1993). N-(1-methyl-5-indolyl)-N'-(-3-pyridyl) urea hydrochloride: the first selective 5-HT$_{1C}$ receptor antagonist. *J. Med. Chem.*, **36**, 1104–1107.

FRANKHUYZEN, A.L. & BONTA, I.L. (1974). Effect of mianserin, a potent anti-serotonin agent on the isolated rat stomach fundus preparation. *Eur. J. Pharmacol.*, **25**, 40–50.

GLUSA, E. (1992). Evidence for 5-HT$_{1C}$ receptor-mediated, endothelium-dependent relaxation of porcine pulmonary arteries in vitro. In: *5-Hydroxytryptamine Mechanisms in Primary Headaches*. ed. Olesen, J. & Saxena, P.R. pp. 168–172. New York: Raven Press.

GUPTA, P. (1992). An endothelial 5-HT receptor that mediates relaxation in guinea-pig isolated jugular vein resembles the 5-HT$_{1D}$ subtype. *Br. J. Pharmacol.*, **106**, 703–709.

HOYER, D., WAEBER, C., SCHOEFFTER, P., PALACIOS, J.M. & DRAVID, A. (1989). 5-HT$_{1C}$ receptor-mediated stimulation of inositol phosphate production in pig choroid plexus. *Naunyn-Schmeid Arch. Pharmacol.*, **339**, 252–258.

LEFF, P., MARTIN, G.R. & MORSE, J.M. (1987). Differential classification of vascular smooth muscle and endothelial cell 5-HT receptors by use of tryptamine analogues. *Br. J. Pharmacol.*, **91**, 321–331.

LORIC, S., LAUNAY, J.M., COLAS, J.F. & MAROTEAUX, L. (1992). New mouse 5-HT$_2$-like receptor, expression in brain, heart and intestine. *Fed. Eur. Biochem. Soc.*, **312**, 203–207.

MARTIN, G.R., BROWNING, C. & GILES, H. (1993). Further characterisation of an atypical 5-HT receptor mediating endothelium-dependent vasorelaxation. *Br. J. Pharmacol.*, **110**, 137P.

SCHOEFFTER, P. & HOYER, D. (1990). 5-Hydroxytryptamine (5-HT)-induced endothelium-dependent relaxation of pig coronary arteries is mediated by 5-HT receptors similar to the 5-HT$_{1D}$ receptor subtype. *J. Pharmacol. Exp. Ther.*, **252**, 387–395.

SUMNER, M.J. (1991). Characterization of the 5-HT receptor mediating endothelium-dependent relaxation in porcine vena cava. *Br. J. Pharmacol.*, **102**, 938–942.

VAN NEUTEN, J.M., JANSSEN, P.A.J., VAN BEEK, J., XHONNEUX, R., VERBEUREN, T.J. & VANHOUTTE, P.M. (1981). Vascular effects of ketanserin (R 41 468), a novel antagonist of 5-HT$_2$ serotonergic receptors. *J. Pharmacol. Exp. Ther.*, **218**, 217–230.

WAINSCOTT, D.B., COHEN, M.L., SCHENCK, K.W., ADIA, J.E., NISSEN, J.S., BAEZ, M., KURSAR, J.D., LUCAITES, V.L. & NELSON, D.L. (1993). Pharmacological characteristics of the newly cloned 5-Hydroxytryptamine$_{2F}$ receptor. *Mol. Pharmacol.*, **43**, 419–426.

(Received June 8, 1994
Revised September 15, 1994
Accepted September 19, 1994)