

5-Hydroxytryptamine-induced vasodilator responses in the hindquarters of the anaesthetized rat, involve β_2 -adrenoceptors

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Abstract

These studies were conducted to examine the role of the vasoactive mediators nitric oxide (NO) and adrenaline (epinephrine) in the serotonin (5-hydroxytryptamine; 5-HT)-induced vasodilator response in the hindquarter vascular bed of anaesthetized rats. Intra-arterial administration of doses of 5-HT in the range 0.12–25 ng kg⁻¹ produced a dose-independent vasodilator effect in the hindquarters. The selective 5-HT_{1D/1B} receptor agonist, L-694,247 at intra-arterial doses of 0.0012–1000 ng kg⁻¹, as well as adrenaline (at doses of 0.05–50 ng kg⁻¹ i.a.), mimicked the dose-independent vasodilator effect induced by intra-arterial administration of 5-HT. Intravenous pre-treatment with the selective β_2 -receptor antagonist ICI 118,551 (0.5 mg kg⁻¹) blocked the vasodilator effect of 5-HT, adrenaline and L-694,247. Additionally, the inhibitor of NO synthase N^G-nitro-L-arginine (L-NAME) (at a dose of 10 mg kg⁻¹ i.v.) blocked the vasodilator action of acetylcholine 300–3000 ng kg⁻¹) but did not modify 5-HT-induced vasodilatation. The vasodilator effect produced by intra-arterial administration of 5-HT in the hindquarters was significantly inhibited both 30 min after denervation of the lumbar sympathetic chains and 1 h after bilateral adrenalectomy. Our data suggest that in the in-situ autoperfused hindquarters of the rat 5-HT-induced vasodilatation is mediated by a local 5-HT_{1D} or 5-HT_{1D/1B} activation, which in turn mediates the adrenal release of adrenaline, which then produces β_2 -activation and vasodilatation.

Introduction

Since the discovery of serotonin (5-hydroxytryptamine; 5-HT) as an endogenous vasoactive compound (Rapport et al 1948), many studies have analysed the effect of this biogenic amine in the cardiovascular system and both vasoconstrictor and vasodilator effects have been described. For 5-HT, many authors have proposed several actions, depending on different factors such as the species of animal used, basal vascular tone, the type of vascular bed, the dose used, the experimental conditions, the nature of the 5-HT receptors involved and the presence of other vasoactive substances (Houston & Vanhoutte 1986; Vanhoutte 1987; Villalón et al 1993).

It has also been proposed that, due to the activation of different 5-HT receptor subtypes, some of the vascular effects of 5-HT would mainly be mediated through indirect mechanisms, such as the modification of sympathetic transmission in the vascular system (Morán et al 1994, 1998), the interaction between adrenergic and serotonergic systems (Watts et al 2000), the activation of angiotensin II (Morán et al 1997), interactions with the prostaglandin system (Blackshear et al 1986; Tuncer & Vanhoutte 1991), a central release of vasopressin (Pergola & Alper 1991; Bagdy et al 1992), increases in plasma renin activity (Blackshear et al 1986; Alper 1990; Zink et al 1990; Bagdy et al 1992) and different endothelial and endogenous factors (Sikorski et al 1991; Bruning et al 1993; Whiting & Cambridge 1995), such as nitric oxide (NO) or endothelins, which act as recognised mediators in the peripheral vascular resistance of different animal species (De Wit et al 1998; Krum et al 1998).

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Some studies carried out at our laboratory to investigate the haemodynamic effects of 5-HT in regional autoperfused vascular beds have revealed that, at the doses used by us, intra-arterial administration of 5-HT to the renal and mesenteric vascular beds decreases local blood flow. The serotonergic vasoconstrictor responses induced in the in-situ autoperfused rat kidney are mediated through angiotensin II activation through a local 5-HT₂ receptor mechanism (Morán et al 1997). In the mesenteric vascular bed, this vasoconstrictor action is mainly mediated by direct activation of 5-HT_{2B} or 5-HT_{2C} receptors (Fernández et al 2000).

By contrast, in the hindquarter vascular bed we have observed that intra-arterial administration of 5-HT modifies local blood flow in a dual way: it produces vasodilatation at the lowest doses used and vasoconstriction with the highest ones (Calama et al 2002). In the vasoconstrictor effect, the 5-HT receptor subtypes mainly involved are 5-HT₂ whereas the vasodilator response induced by low doses of 5-HT is mainly due to the activation of 5-HT_{1D/1B} receptors (Calama et al 2002).

To date, few other studies have addressed the actions of 5-HT in the hindquarter vascular bed. The results obtained so far suggest the existence of both vasodilator and vasoconstrictor effects. Anderson et al (1996) showed that intracerebrovascular administration of 5-HT to conscious Long-Evans and Brattleboro rats causes vasodilatation and increases hindquarter blood flow. However, in the hindquarter vascular bed of anaesthetized normal and diabetic rats (Sikorski et al 1991; Loke & Woodman 1996), mainly vasoconstrictor actions have been reported after intra-arterial administration of 5-HT. However, the mechanism by which 5-HT decreases vascular resistance in the hindquarter vascular bed of the rat remains to be elucidated.

Accordingly, based on previous findings, this study was undertaken to investigate the possible participation of the vasoactive mediators NO and adrenaline (epinephrine) in the 5-HT_{1D/1B}-mediated vasodilator responses observed by us in the hindquarter vascular bed of the rat. Also, the role of adrenergic innervation of the hindquarter vascular bed in mediating the vasodilator responses to 5-HT was investigated.

Materials and Methods

Drugs

The following drugs were used: pentobarbital sodium (Sigma Chemical Company, St Louis, MO), atropine sulfate (Scharlau, Barcelona, Spain), heparin sodium (Roche, Madrid, Spain), acetylcholine chloride (Sigma Chemical Company, St Louis, MO), 5-hydroxytryptamine-creatinine sulphate (Sigma Chemical Company, St Louis, MO), 5 carboxamidotryptamine maleate (Research Biochemicals International, Natick, USA), L-694,247 (Tocris, Bristol, UK), L-arginine (Research Biochemicals International, Natick, USA), ICI 118,551 hydrochloride (Tocris, Bristol, UK), N^G-nitro-L-arginine methyl ester (L-NAME) (Sigma Chemical Company, St Louis, MO),

BRL-15572 hydrochloride (Tocris, Bristol, UK). All drugs used were dissolved in distilled water at the time of the experiments, with the exception of BRL 15572, which was dissolved in 20% propylene glycol.

Animal preparation

The experiments were carried out on anaesthetized (pentobarbital sodium; 60 mg kg⁻¹ i.p.) normotensive male Wistar rats, 300–450 g, from the Animal House of the University of Salamanca (P.A.E.-SA001). A midline incision was made in the cervical region, and the trachea and right jugular vein were cannulated, the latter for drug administration. The rats breathed room air spontaneously via a tracheal tube. An abdominal midline incision was made and a segment of the abdominal aorta between the left renal artery and the posterior aortic bifurcation was exposed and dissected free from the cava vein. Heparin (1000 IU kg⁻¹) was administered and the two carotid arteries were cannulated.

The penis vein was cannulated to connect a perfusion (2 mL h⁻¹) of saline solution (0.9% NaCl) using a Harvard 901 pump (MA). This perfusion was maintained throughout the experiments. A ligature was placed around the aorta and a cannula inserted distal to this ligature. Blood was withdrawn through the right carotid artery cannula and perfused at a constant flow rate (2–3 mL min⁻¹) to the hindquarters through the abdominal cannula using a Gilson peristaltic pump (Madrid, Spain). The distal portion of the external circuit was connected to a Spectramed model P23 XL pressure transducer (Madrid, Spain) and a Grass model 7 Physiograph recorder (USA). At the beginning of each experiment, the flow was adjusted to render the perfusion pressure equal to the systemic pressure. The flow was kept constant throughout the experiment. Changes in perfusion pressure reflected the changes in vascular resistance. For blood pressure measurements, the left carotid artery was connected to a Spectramed model P23 XL pressure transducer and a Grass model 7 Physiograph recorder. In all experiments (with the exception of those in which we tested the effect of acetylcholine), atropine (1 mg kg⁻¹) was administered intravenously to block the cholinergic effect. The rats were kept warm (at 37.5 ± 0.5 °C) with a heating lamp.

In one group of rats, the hindquarter vascular bed was denervated by ligating and cutting the lumbar sympathetic chain ganglia. In another group, a bilateral adrenalectomy was performed under anaesthesia, before the abdominal aorta was exposed and dissected.

All provisions regarding the protection of animals used for experimental purposes in Spanish law and European Community (EEC 1986) specifications were observed in this study.

Experimental protocols

After a 15-min period to allow blood pressure and perfusion pressure to stabilize, the corresponding experiments were performed using groups of 5 rats for each treatment. Each group was used to evaluate the vascular effect

induced by different doses of one agonist (5 min elapsing between each drug dose), either alone or after administration of the corresponding antagonist, in such way that each rat preparation was used to evaluate only one agonist, with or without antagonist ($n = 5$). In all experiments, two dose–response curves of the agonist used were obtained: the first one was the control curve and the second one was the same agonist dose–response curve obtained after the administration of vehicle (saline solution or propylene glycol 20%) or the corresponding antagonist (starting this curve 10 min after the administration of the vehicle or the antagonist).

5-HT (at doses of 0.12, 0.6, 3, 6, 12.5 and 25 ng kg⁻¹), (2-[5-[3-(4-methylsulphonylamino)benzyl]-1,2,4-oxadiazol-5-yl]-1*H*-indol-3-yl)ethanamine (L-694,247, at doses of 0.0012, 0.012, 0.12 and 1000 ng kg⁻¹), adrenaline (at doses of 0.05, 0.5, 5 and 50 ng kg⁻¹) and acetylcholine (at doses of 300, 1000 and 3000 ng kg⁻¹) were administered locally via the distal cannula intra-arterially by bolus injection of 10 μ L, using a microsyringe (Exmire microsyringe), 5 min elapsing between each drug dose. In the same way, a control group was administered intra-arterially with saline solution (10 μ L, 5 min elapsing between each saline administration).

To analyse the mechanisms involved in the action of 5-HT receptor agonists, adrenaline or acetylcholine, several compounds were administered intravenously 10 min before the corresponding dose–response curve of the agonist was obtained: the β_2 -receptor antagonist ICI 118,551 (at a dose of 0.5 mg kg⁻¹) was administered before adrenaline, 5-HT and L-694247 (a selective 5-HT_{1D/1B} agonist); N^G-nitro-L-arginine (L-NAME) at a dose of 10 mg kg⁻¹ and L-arginine (at a dose of 100 mg kg⁻¹) were administered before 5-HT and acetylcholine and BRL-15572 (a 5-HT_{1D/1B} antagonist) was administered at a dose of 1 mg kg⁻¹ before the 5-HT_{1D/1B} receptor agonist L-694247.

In the next set of experiments, we investigated the role of adrenergic innervation to the hindquarter vascular bed in mediating vasodilator responses to the intra-arterial administration of 5-HT. To this end, after a control curve of 5-HT had been obtained, the hindquarter vascular bed was denervated by ligating and cutting the lumbar sympathetic chains just below the level of the renal veins, after which the 5-HT-induced effects obtained at 30 min after the denervation were compared. We also tested the effect of a dose of 0.5 ng kg⁻¹ of adrenaline before and after the denervation.

To confirm a possible adrenal release of adrenaline, induced by 5-HT, a group of experiments were performed using acute adrenalectomized rats. One hour after the bilateral adrenalectomy, the effects obtained with 5-HT (at doses of 0.12, 0.6, 3, 6, 12.5 and 25 ng kg⁻¹) or adrenaline (0.05 ng kg⁻¹) were compared with the corresponding effects obtained in the 5-HT or adrenaline groups of rats without adrenalectomy.

Statistical analysis

The results of the experiments are given as means $\pm$ s.e.m of the perfusion pressure and systemic blood pressure

calculated using the following expression: mean blood pressure = diastolic pressure + 1/3(diastolic pressure – systolic pressure). Changes in hindquarter vascular resistance are given in mmHg as increases or decreases in perfusion pressure in comparison with the baseline values of the control group. Statistical significance was calculated by one-way analysis of variance, followed by the Newman–Keuls multiple comparison test. Differences were considered significant when $P < 0.05$.

Results

The mean basal blood pressure, mean basal perfusion pressure and heart rate in the experiments with no adrenalectomized rats were 90.5 $\pm$ 2.5 mmHg ($n = 70$), 91.3 $\pm$ 3.1 mmHg ($n = 70$) and 340 $\pm$ 10 beats/min ($n = 70$), respectively. These values only changed significantly in the rats treated with L-NAME. In these experiments, the increases in perfusion pressure and mean blood pressure at the end of the experiment were 55 $\pm$ 5.2 mmHg and 36 $\pm$ 1.7 mmHg, respectively, whereas for the rest of the experiments the variations were -1.31 ± 1.4 and -1.52 ± 1.5 mmHg, respectively and the change in heart rate was -1.38 ± 12 beats/min.

Effect of local administration of 5-HT and L-694,247 in autoperfused hindquarters of anaesthetized rats

As previously reported (Calama et al 2002), intra-arterial administration of increasing doses of 5-HT (0.12–25 ng kg⁻¹) in autoperfused hindquarters of anaesthetized rats had no effect on systemic blood pressure but produced a dose-independent vasodilator effect that subsequently decreased.

At doses of 0.0012–1000 ng kg⁻¹, the selective 5-HT_{1D} receptor agonist L-694,247 (Beer et al 1993), which has recently been proposed to show high affinity for both the 5-HT_{1D} and 5-HT_{1B} receptor subtypes in pig (Bhalla et al 2001), mimicked the dose-independent vasodilator effect induced by intra-arterial administration of 5-HT – as previously described (Calama et al 2002) – without modifying systemic blood pressure.

The vasodilator effect induced by both 5-HT and L-694,247 was statistically significant when compared with a control group which received saline intra-arterially, 10 μ L at 5-min intervals.

Hindquarter vascular effect induced by local adrenaline or acetylcholine administration

Intra-arterial administration of adrenaline (at doses of 0.05, 0.5, 5 and 50 ng kg⁻¹) produced a dose-independent vasodilator effect (Figure 1), whereas acetylcholine (at doses of 300, 1000 and 3000 ng kg⁻¹) produced a dose-dependent vasodilatation in the hindquarter vascular bed (Figure 2). Neither adrenaline nor acetylcholine modified systemic blood pressure.

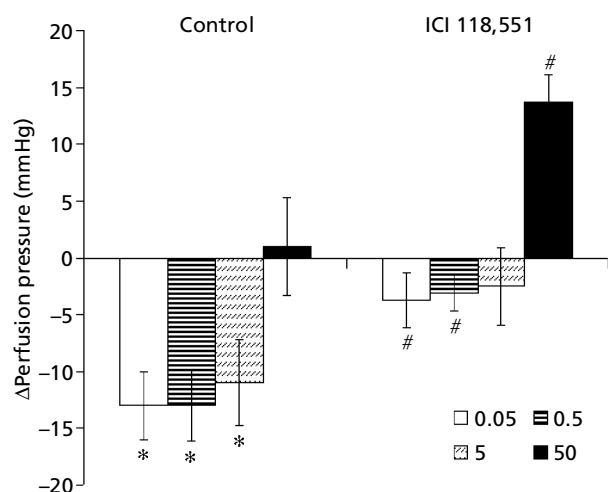


Figure 1 Effect of intra-arterial administration of different doses of adrenaline ($0.05\text{--}50\text{ ng kg}^{-1}$) (control) on the perfusion pressure of the in-situ autoperfused hindquarters of the rat and effect of intravenous pre-treatment with ICI 118,551 (0.5 mg kg^{-1}) on the adrenaline-induced responses. $*P < 0.05$ with respect to the basal perfusion pressure of the hindquarters; $\#P < 0.05$ with respect to the corresponding dose in the adrenaline curve, $n = 5$ for each group.

Pre-treatment with 0.5 mg kg^{-1} of the β_2 -receptor antagonist ICI 118,551 (Bilski et al 1983) inhibited the vasodilator effect of adrenaline (Figure 1), whereas pre-treatment with L-NAME (at a dose of 10 mg kg^{-1}) blocked the acetylcholine-induced vasodilatation (Figure 2). The vasodilator inhibitory effect of L-NAME was reversed after treatment with L-arginine (at a dose of 100 mg kg^{-1}), which re-established the acetylcholine-induced vasodilatation (Figure 2).

Effect of intravenous administration of antagonists on the vasodilator action caused by 5-HT or L-694,247

Pre-treatment with 0.5 mg kg^{-1} of the selective β_2 -receptor antagonist ICI 118,551 inhibited the vasodilator effect of 5-HT (doses of 0.12 , 0.6 , 3 and 6 ng kg^{-1}) and L-694,247 (Figure 3A and B, respectively). The effect of the latter was also inhibited by intravenous administration of 1 mg kg^{-1} of BRL-15572, a preferential 5-HT_{1D} receptor antagonist (Price et al 1997; Villalón et al 2000) (Figure 3B). The administration of either ICI 118,551 or BRL-15572 failed to modify either the perfusion or the systemic blood pressure (Table 1).

In another series of experiments, the effects of 5-HT (doses of 0.06 , 6 and 25 ng kg^{-1}) were tested after intravenous administration of L-NAME, an inhibitor of NO synthase (Furchgott & Vanhoutte 1989; Moncada et al 1991), at a dose of 10 mg kg^{-1} (a dose that blocked the effect of acetylcholine), either alone or in the presence of L-arginine, a precursor of NO synthesis (Schmidt et al 1993) (at a dose of 100 mg kg^{-1}). Intravenous administration of L-NAME produced a maintained increase of $36 \pm 1.7\text{ mmHg}$ in mean blood pressure and also in perfusion pressure

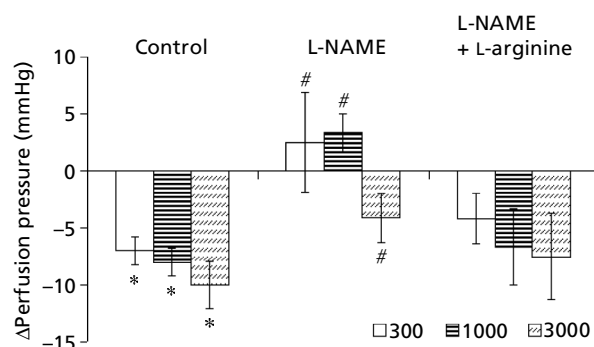


Figure 2 Effect of intra-arterial administration of different doses of acetylcholine ($300\text{--}3000\text{ ng kg}^{-1}$) (control) on the perfusion pressure of the in-situ autoperfused hindquarters of the rat and effect of intravenous pre-treatment with L-NAME (10 mg kg^{-1}) or L-NAME (10 mg kg^{-1}) and L-arginine (100 mg kg^{-1}) on the acetylcholine-induced responses. $*P < 0.05$ with respect to the basal perfusion pressure of the hindquarters; $\#P < 0.05$ with respect to the corresponding dose in the acetylcholine curve, $n = 5$ for each group.

($55 \pm 5.2\text{ mmHg}$), and both pressures returned to basal levels only after intravenous administration of L-arginine. Neither pre-treatment with L-NAME nor pre-treatment with L-arginine modified the 5-HT-induced vasodilatation (Figure 4).

Influence of sympathetic denervation or bilateral adrenalectomy on the 5-HT-induced vasodilator action

In our experiments, sympathectomy produced a non-significant decrease in the perfusion pressure and mean blood pressure (Table 1).

The vasodilator effect produced by intra-arterial administration of 5-HT in the rat hindquarter vascular bed was significantly inhibited 30 min after this denervation (Figure 5). However, the response to the intra-arterial administration of 0.5 ng kg^{-1} of adrenaline (which decreased the perfusion pressure by $13 \pm 4.1\text{ mmHg}$) did not change significantly with sympathectomy ($13.32 \pm 1.7\text{ mmHg}$). These results showed that, at this level, the vasodilator effect of this biogenic amine was dependent on the adrenergic nervous system.

One hour after the bilateral adrenalectomy, the rats presented values of mean blood pressure and perfusion pressure of $70.4 \pm 5.7\text{ mmHg}$ and $67.3 \pm 5.3\text{ mmHg}$, respectively. These values were lower than the control. In these rats, the doses of 5-HT tested ($0.12\text{--}25\text{ ng kg}^{-1}$) did not produced a vasodilator effect (Figure 5) but 0.05 ng kg^{-1} of adrenaline induced a vasodilator response similar to the control group ($-13.0 \pm 3.0\text{ mmHg}$).

Discussion

According to Brody et al (1963) and other authors, the technique used in our experiments allows continuous measurement of responses in an isolated portion of the

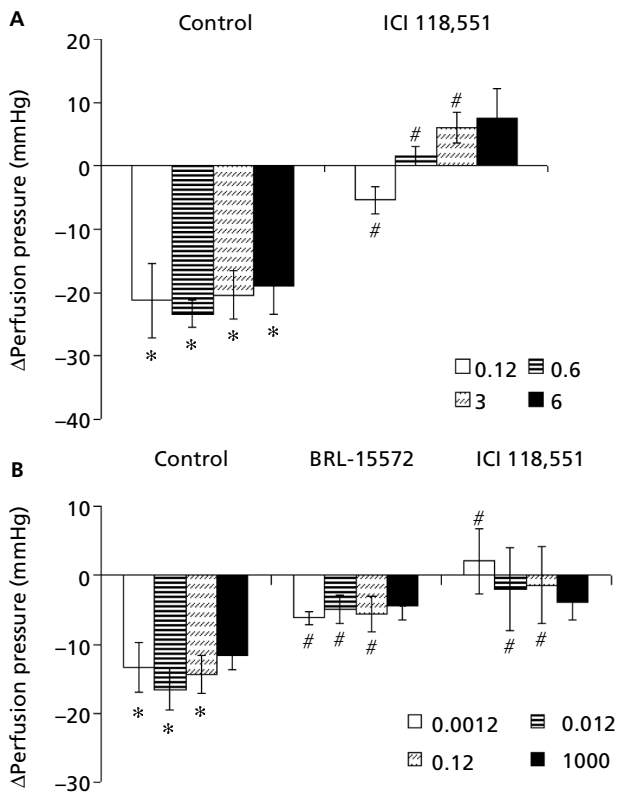


Figure 3 Effect of intra-arterial administration of different doses of 5-HT (0.12–6 ng kg⁻¹) (control) on the perfusion pressure of the in-situ autoperfused hindquarters of the rat and effect of intravenous pre-treatment with ICI 118,551 (0.5 mg kg⁻¹) on the 5-HT-induced responses (A) and effect of intra-arterial administration of different doses of L-694,247 (0.0012–1000 ng kg⁻¹) on the perfusion pressure of the in-situ autoperfused hindquarters of the rat and effect of intravenous pre-treatment with BRL-15572 (1 mg kg⁻¹) or ICI 118,551 (0.5 mg kg⁻¹) on the L-694,247-induced responses of the hindquarters (B). * $P < 0.05$ with respect to the basal perfusion pressure of the hindquarters; # $P < 0.05$ with respect to the corresponding dose in the agonist curve, $n = 5$ for each group.

Table 1 Changes in perfusion pressure (mmHg) and mean blood pressure (mmHg) in the rat hindquarter vascular bed after different treatments.

Treatment	Δ Perfusion pressure	Δ Mean blood pressure
Saline	0.36 ± 1.9	0.20 ± 2.1
ICI 118,551	1.03 ± 2.1	1.32 ± 3.2
BRL-15572	-1.43 ± 2.0	-1.91 ± 1.9
L-NAME	55.0 ± 5.2	36.0 ± 1.7
L-arginine	-52.0 ± 5.1	-34.0 ± 3.1
Sympathectomy	-5.53 ± 3.9	-5.68 ± 4.1
Adrenalectomy	-20.6 ± 5.7	-24.3 ± 5.3

Values are given as means $\pm$ s.e.m. For saline treatment, the mean values correspond to five consecutive 10- μ L intra-arterial administrations.

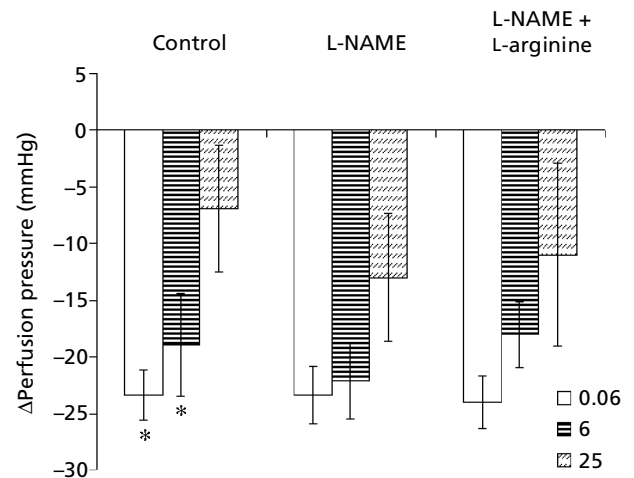


Figure 4 Effect of intra-arterial administration of different doses of 5-HT (0.06–25 ng kg⁻¹) on the perfusion pressure of the in-situ autoperfused hindquarters of the rat and effect of intravenous pre-treatment with L-NAME (10 mg kg⁻¹) or L-NAME (10 mg kg⁻¹) and L-arginine (100 mg kg⁻¹) on the 5-HT-induced responses. * $P < 0.05$ with respect to the basal perfusion pressure of the hindquarters, $n = 5$ for each group.

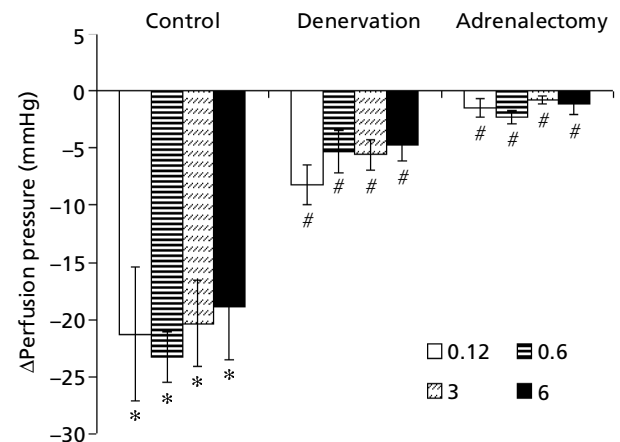


Figure 5 Effect of intra-arterial administration of different doses of 5-HT (0.12–6 ng kg⁻¹) (control) on the perfusion pressure of the in-situ autoperfused hindquarters of the rat before, and 30 min after, sectioning the lumbar sympathetic chains or 1 h after bilateral adrenalectomy. * $P < 0.05$ with respect to the basal perfusion pressure of the hindquarters; # $P < 0.05$ with respect to the control group, $n = 5$ for each group.

peripheral vasculature and hence quantification of the vascular reactivity of hindquarter vascular beds. It is therefore possible to measure (in an indirect way) local blood flow and evaluate rapid changes in hindquarter blood flow induced by direct intra-arterial 5-HT administration to the hindquarters. In turn, this makes it possible to evaluate, at least in anaesthetised rats, both direct and indirect actions (induced by the release of vasoconstrictor or vasodilator humoral agents), which

have been suggested to be related to the mechanism of action of 5-HT in other vascular beds and in other animal species (Vanhoutte 1987, 1991).

As previously noted, in the hindquarter vascular bed of the anaesthetized rat, at the lowest doses 5-HT induces vasodilator responses (Calama et al 2002). These are inhibited by methiothepin, but not by mesulergine, and are mimicked by the administration of 5-CT, a selective 5-HT₁ receptor agonist (Hoyer et al 1994) that also activates 5-HT₇ receptors, and L-694,247, a selective 5-HT_{1D} agonist (Beer et al 1993) that has recently been proposed to show high affinity for both the 5-HT_{1D} and 5-HT_{1B} receptor subtypes (Bhalla et al 2001). Therefore, we initially excluded the participation of 5-HT₇ receptors and considered that 5-HT_{1D/1B} receptors would be involved in the 5-HT-induced vasodilator response (Calama et al 2002). Furthermore, in the above work, the administration of 8-OH-DPAT, a selective 5-HT_{1A} agonist (see Hoyer et al 1994), or CGS-12066B, a selective 5-HT_{1B} agonist (Neale et al 1987), failed to mimic the vasodilator effect 5-HT. We therefore suggested that mainly 5-HT_{1D} receptors would be responsible for the vasodilator activity of 5-HT in the hindquarter vascular bed of the rat.

In this work we also investigated whether the vasodilator response found for 5-HT in the hindquarters of the rat might be due to a direct activation of 5-HT_{1D/1B} receptors or, as has been proposed in different vascular beds by other authors, whether other vasodilator agents could be involved in this response (Blackshear et al 1986).

NO has been reported to be involved in the vasodilatation of vascular beds, such as the mesenteric arterial bed of the rat (Warner et al 1989), and this compound has an attenuating effect on constriction as a possible mechanism in controlling the changes occurring in total peripheral vascular resistance elicited by vasoconstrictors in cremaster arterioles (De Wit et al 1998). In the canine renovasculature, Whiting & Cambridge (1995) studied the modulation through endothelial 5-HT₁-like receptors by endogenous NO. NO also suppresses sympathetic vasoconstriction in the mesentery and kidney at the spinal level, whereas hindquarter tone is mediated at supraspinal and synaptic levels (Iida 1999).

In view of all the foregoing, we analysed the possible participation of NO release in the vasodilator effects of 5-HT in the hindquarter vascular bed of the rat using L-NAME, an NO-synthase inhibitor, at a dose able to block the vasodilator action of acetylcholine, and the substrate for NO formation, L-arginine, at a dose able to re-establish the vasodilator effect of acetylcholine previously inhibited by the administration of L-NAME. In contrast to the acetylcholine-induced vasodilator effect, neither the administration of L-NAME nor the administration of L-arginine before L-NAME modified the vasodilator effect of 5-HT. These results allow us to propose that the 5-HT_{1D} receptor-induced vasodilatation observed in the hindquarter vascular bed of the rat is not dependent on NO release.

On the other hand, different studies have shown the relationship between 5-HT and vascular sympathetic transmission (Morán et al 1994, 1998). Such evidence concerning the 5-HT-induced inhibition and stimulation of

sympathetic nervous activity, together with the fact that in most vascular smooth muscles adrenaline and other adrenergic agonists induce vasodilatation, mainly mediated through β_2 -adrenoceptor activation (Gardiner et al 1991; Guimaraes & Moura 2001), prompted us to consider a possible 5-HT-induced release of adrenaline because ICI 118,551 (a selective β_2 adrenoceptor antagonist (Bilski et al 1983)), administered at a dose that inhibits the vasodilator action of adrenaline, inhibited the vasodilatation induced by both 5-HT and L-694,247.

In agreement with our results, two important questions must be considered. It is well known that noradrenaline (norepinephrine) is the main neurotransmitter of sympathetic post-ganglionic fibres and that adrenaline is mainly released by the adrenal medulla. On the other hand, 5-HT_{1D/1B} receptors are negatively coupled to adenylyl cyclase, a transduction mechanism associated with the inhibition of the release of neurotransmitters, and it is known that pre-junctional 5-HT_{1D/1B} receptor activation inhibits noradrenaline release from vascular sympathetic nerve terminals (see Hoyer et al 1994). Recently, however, different studies have suggested that other signalling pathways different from adenylyl cyclase inhibition, such as mitogen-activated protein kinase activation, would be linked to this receptor subtype (Hinton et al 2000; Albert & Tiberi 2001; Watts et al 2001). In particular, the studies of Lin et al (2002) provide evidence that although all 5-HT₁ receptors negatively regulate adenylyl cyclase to some extent, they differentially couple to G proteins and consequently exhibit differences in coupling to cellular signals.

Thus, in the light of our results we propose that 5-HT_{1D/1B} activation, perhaps through a pathway not related to adenylyl cyclase, would have stimulated the adrenal medulla and that the release of adrenaline from adrenal glands, and not from sympathetic terminal nerves, would have produced the vasodilator effect. The initial studies with adrenalectomized animals confirm this theory because no vasodilator activity was obtained with 5-HT in these animals.

There is increasing evidence that endothelial cells may contribute to β -adrenergic vasorelaxation, although the relative contribution of endothelial and smooth muscle β -adrenoceptors to vasodilatation may vary between the different vascular beds and also between different species studied (Rebich et al 1995; Dawes et al 1997). In the rabbit femoral artery in-vivo, β -adrenoceptor activation stimulates NO production, giving rise to vasodilatation (Xu et al 2000). Also in in-vitro studies, a relationship between β -adrenoceptors and NO has been shown. Priest et al (1997) proposed that β -mediated vasorelaxation in the large pulmonary arteries of the rat would be largely NO-dependent, whereas in small arteries a significant proportion would be NO-independent. In our experiments, carried out in the hindquarters of rats, we failed to observe this relationship since β -adrenoceptors, but not NO, seemed to be involved in the serotonergic vasodilator response.

To confirm adrenergic involvement in 5-HT agonist-induced vasodilatation, we performed experiments with

denervated hindquarter vascular beds after ligating and cutting the lumbar sympathetic chains. This experimental procedure has been used by other authors to confirm the dependence or independence of vasodilator responses on the adrenergic nervous system (Champion et al 1996; Czapla et al 1997). In concordance with the results reported above, the vasodilatation produced by intra-arterial administration of 5-HT was significantly blocked 30 min after denervation due to a decrease in the adrenaline reserve and hence, at least at this level, the vasodilator effect of 5-HT must be dependent on the adrenergic nervous system.

Conclusions

Our data suggest that in the in-situ autoperfused hindquarter vascular bed of the rat the serotonergic vasodilator effect would be mediated by a local 5-HT_{1D} or 5-HT_{1D/1B} activation. This would in turn mediate the release of adrenaline from the adrenal medulla more than from the lumbar sympathetic chains, thereby producing β_2 -activation, which finally ends in vasodilatation.

References

- Albert, P. R., Tiberi, M. (2001) Receptors signalling and structure: insights from serotonin-1 receptors. *Trends Endocrinol. Metab.* **12**: 453–460
- Alper, R. (1990) Hemodynamic and renin responses to ($\pm$)-DOI, a selective 5-HT₂ receptor agonist, in conscious rats. *Eur. J. Pharmacol.* **175**: 323–332
- Anderson, I. K., Ramage, A. G., Gardiner, S. M. (1996) Cardiovascular effects of serotonin and DP-5-CT in conscious Long-Evans and Brattleboro rats. *Am. J. Physiol.* **271**: R455–R463
- Bilski, A. J., Halliday, S. E., Fitzgerald, J. D., Wale, J. L. (1983) The pharmacology of a β_2 -selective adrenoceptor antagonist (ICI 118,551). *J. Cardiovasc. Pharmacol.* **5**: 430–437
- Bagdy, G., Sved, A. F., Murphy, D. L., Szemerédi, K. (1992) Pharmacological characterization of serotonin receptor subtypes involved in vasopressin and plasma renin activity responses to serotonin agonists. *Eur. J. Pharmacol.* **210**: 285–289
- Beer, M. S., Stanton, J. A., Bevan, Y., Heald, A., Reeve, A. J., Street, L. J., Matassa, V. G., Hargreaves, R. J., Middlemiss, M. D. (1993) L 694247: a potent 5-HT_{1D} receptor agonist. *Br. J. Pharmacol.* **110**: 1196–1200
- Bhalla, P., Sharma, H. S., Ma, X., Wurch, T., Pauwels, P. J., Saxena, P. (2001) Molecular cloning, pharmacological properties and tissue distribution of the porcine 5-HT_{1B} receptor. *Br. J. Pharmacol.* **133**: 891–901
- Blackshear, J. L., Orlandi, C., Hollenberg, N. K. (1986) Serotonin and renal blood supply: role of prostaglandins and the 5-HT₂ receptor. *Kidney Int.* **30**: 304–310
- Brody, M. J., Shaffer, R. A., Dixon, R. L. (1963) A method for the study of peripheral vascular response in the rat. *J. Appl. Physiol.* **18**: 645–647
- Bruning, T. A., Chang, P. C., Blauw, G. J., Vermeij, P., Van Zwieten, P. A. (1993) Serotonin-induced vasodilatation in the human forearm is mediated by the “nitric oxide-pathway”: no evidence for involvement of the 5-HT₃-receptor. *J. Cardiovasc. Pharmacol.* **22**: 44–51
- Calama, E., Fernández, M. M., Morán, A., Martín, M. L., San Román, L. (2002) Vasodilator and vasoconstrictor responses induced by 5-hydroxytryptamine in the *in situ* blood autoperfused hindquarters of the anaesthetized rat. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **366**: 110–116
- Champion, H. C., Czapla, M. A., Friedman, D. E., Lambert, D. G., Murphy, W. A., Coy, D. H., Kadowitz, P. J. (1996) Tone-dependent vasodilator responses to proadrenomedullin NH₂-terminal 20 peptide in the hindquarters vascular bed of the rat. *Peptides* **18**: 513–519
- Czapla, M. A., Champion, H. C., Kadowitz, P. J. (1997) Decreases in systemic arterial and hindquarters perfusion pressure in response to nociceptin are not inhibited by naloxone in the rat. *Peptides* **18**: 1197–1200
- Dawes, M., Chowiecnyk, P. J., Ritter, J. M. (1997) Effects of inhibition of the L-arginine/nitric oxide pathway on vasodilatation caused by β -adrenergic agonists in human forearm. *Circulation* **95**: 2293–2297
- De Wit, C., Jahrbeck, B., Schafer, C., Bolz, S. S., Pohl, U. (1998) Nitric oxide opposes myogenic pressure responses predominantly in large arterioles in vivo. *Hypertension* **31**: 787–794
- Fernández, M. M., Morán, A., Martín, M. L., San Román, L. (2000) Mesenteric vasoconstrictor response to 5-hydroxytryptamine in the *in situ* blood autoperfused rat mesentery: involvement of 5-HT_{2B} and/or 5-HT_{2C} receptor activation. *Eur. J. Pharmacol.* **401**: 221–227
- Furchgott, R. F., Vanhoutte, P. M. (1989) Endothelium-derived relaxing and contracting factors. *FASEB J.* **3**: 2007–2118
- Gardiner, S. M., Kemp, P. A., Bennet, T. (1991) Effects of N^G-nitro-L-arginine methyl ester on vasodilator responses to adrenaline or BRL 38227 in conscious rats. *Br. J. Pharmacol.* **104**: 731–737
- Guimaraes, S., Moura, D. (2001) Vascular adrenoceptors: an update. *Pharmacol. Rev.* **53**: 319–356
- Hinton, J. M., Hill, P., Jeremy, J., Garland, C. (2000) Signalling pathways activated by 5-HT(1B)/5-HT(1D) receptors in native smooth muscle and primary cultures of rabbit renal artery smooth muscle cells. *J. Vasc. Res.* **37**: 457–468
- Houston, D. S., Vanhoutte, P. M. (1986) Serotonin and the vascular system. Role in health and disease, and implications for therapy. *Drugs* **31**: 149–163
- Hoyer, D., Clarke, D. E., Fozard, J. R., Hartig, P. R., Martin, G. R., Myle-Charane, E. J., Saxena, P. R., Humphrey, P. P. A. (1994) International Union of Pharmacology classification of receptors of 5-hydroxy-tryptamine (serotonin). *Pharmacol. Rev.* **46**: 157–203
- Iida, N. (1999) Nitric oxide mediates sympathetic vasoconstriction at supraspinal, spinal, and synaptic levels. *Am. J. Physiol.* **276** (3 Pt 2): H918–H925
- Krum, H., Viskoper, R. J., Lacourciere, Y., Budde, M., Charlon, V. (1998) The effect of an endothelin-receptor antagonist, bosentan, on blood pressure in patients with essential hypertension. *N. Engl. J. Med.* **338**: 784–790
- Lin, S. L., Setya, S., Johnson-Farley, N. N., Cowen, D. S. (2002) Differential coupling of 5-HT₁ receptors to G proteins of the Gi family. *Br. J. Pharmacol.* **136**: 1072–1078
- Loke, K. E., Woodman, O. L. (1996) Effect of ischaemic preconditioning on vascular dysfunction induced by ischaemia and reperfusion in rat hindquarters. *Eur. J. Pharmacol.* **32**: 1081–1087
- Moncada, S., Palmer, R. M. J., Higgs, E. A. (1991) Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol. Rev.* **43**: 109–142
- Morán, A., Velasco, C., Salvador, T., Martín, M. L., San Román, L. (1994) Inhibitory 5-hydroxytryptamine receptors involved in pressor effects obtained by stimulation of sympathetic outflow from spinal cord in pithed rats. *Br. J. Pharmacol.* **113**: 1358–1362
- Morán, A., Velasco, C., Martín, M. L., San Román, L. (1997) Renal vasoconstrictor response to 5-hydroxytryptamine in the *in situ*

- autoperfused rat kidney: involvement of angiotensin II and the 5-HT₂ receptor activation. *Eur. J. Pharmacol.* **330**: 205–211
- Morán, A., Fernández, M. M., Velasco, C., Martín, M. L., San Román, L. (1998) Characterization of prejunctional 5-HT₁ receptors that mediated the inhibition of pressor effects elicited by sympathetic stimulation in the pithed rats. *Br. J. Pharmacol.* **123**: 1205–1213
- Neale, R. F., Fallon, S. L., Boyar, W. C., Waslev, J. W., Martin, L. L., Stone, G. A., Glaeser, B. S., Sinton, C. M., Williams, M. (1987) Biochemical and pharmacological characterization of CGS 12066B, a selective serotonin-1B agonist. *Eur. J. Pharmacol.* **136**: 1–9
- Pergola, P. E., Alper, R. H. (1991) Vasopressin and autonomic mechanisms mediate cardiovascular actions of central serotonin. *Am. J. Physiol.* **260**: R1188–R1193
- Price, G. W., Burton, M. J., Collin, L. J., Duckworth, M., Gaster, L., Göther, M., Jones, B. J., Roberts, C., Watson, J. M., Middlemiss, D. N. (1997) SB-216641 and BRL-15572 compounds to pharmacologically discriminate h5-HT_{1B} and h5-HT_{1D} receptors. *Naunyn Schmiedeberg's Arch. Pharmacol.* **356**: 312–320
- Priest, R. M., Hucks, D., Ward, J. P. T. (1997) Noradrenaline, β -adrenoceptor mediated vasorelaxation and nitric oxide in large and small pulmonary arteries of the rat. *Br. J. Pharmacol.* **122**: 1375–1384
- Rapport, M. M., Green, A. A., Page, I. H. (1948) Serum vasoconstrictor (serotonin) IV. Isolation and characterization. *J. Biol. Chem.* **176**: 1243–1251
- Rebich, S., Devine, J. O., Armstead, W. M. (1995) Role of nitric oxide and cAMP in β -adrenoceptor-induced pial artery vasodilation. *Am. J. Physiol.* **268**: H1071–H1076
- Schmidt, K., Klatt, P., Mayer, B. (1993) Characterization of endothelial cell amino acid transport systems involved in the actions of nitric oxide synthase inhibitors. *Mol. Pharmacol.* **44**: 615–621
- Sikorski, B. W., Hodgson, W. C., King, R. G. (1991) Thromboxane A₂ receptor stimulation similarly potentiates pressor responses to 5-hydroxytryptamine in perfused hindquarters of non-diabetic and alloxan diabetic rats. *Clin. Exp. Pharmacol. Physiol.* **18**: 237–244
- Tuncer, M., Vanhoutte, P. M. (1991) Role of prostanoids in the increased vascular responsiveness and delayed tachyphylaxis to serotonin in the kidney of spontaneously hypertensive rats. *J. Hypertens.* **9**: 623–629
- Vanhoutte, P. M. (1987) Cardiovascular effects of serotonin. *J. Cardiovasc. Pharmacol.* **10**: S8–S11
- Vanhoutte, P. M. (1991) Platelet-derived serotonin, the endothelium and cardiovascular disease. *J. Cardiovasc. Pharmacol.* **17** (Suppl. 5): S6–S12
- Villalón, C. M., Terrón, J., Hong, E., Saxena, P. R. (1993) Efectos cardiovasculares de agonistas y antagonistas de los receptores a la serotonina (Parte 1). *Arch. Inst. Cardiol. Mex.* **63**: 441–447
- Villalón, C. M., Sánchez-López, A., Centurión, D., Saxena, P. R. (2000) Unravelling the pharmacological profile of the canine external carotid vasodilator 5-HT₁-like receptors: coexistence of sympatho-inhibitory 5-HT_{1b} and postjunctional 5-HT₇ receptors. *Naunyn Schmiedeberg's Arch. Pharmacol.* **363**: 73–80
- Warner, T. D., De Nucci, G., Vane, J. R. (1989) Comparison of the survival of endothelium-derived relaxing factor and nitric oxide within the isolated perfused mesenteric arterial bed of the rat. *Br. J. Pharmacol.* **97**: 777–782
- Watts, S. W., Fink, D. G., Silver, P. J., Cushing, D. J. (2000) Interaction of the β adrenergic receptor antagonist bucindolol with serotonergic receptors. *J. Cardiovasc. Pharmacol.* **35**: 29–36
- Watts, S. W., Yang, P., Banes, A. K., Baez, M. (2001) Activation of Erk mitogen-activated protein kinase proteins by vascular serotonin receptors. *J. Cardiovasc. Pharmacol.* **38**: 539–551
- Whiting, M. V., Cambridge, D. (1995) Canine renovascular responses to sumatriptan and carboxamidotryptamine: modulation through endothelial 5-HT₁-like receptors by endogenous nitric oxide. *Br. J. Pharmacol.* **114**: 969–974
- Xu, B., Li, J., Gao, L., Ferro, A. (2000) Nitric oxide-dependent vasodilatation of rabbit femoral artery by β_2 -adrenergic stimulation or cyclic AMP elevation *in vivo*. *Br. J. Pharmacol.* **129**: 969–974
- Zink, M. H., Pergola, P. E., Doane, J. F., Sved, A. F., Alper, R. H. (1990) Quipazine increases renin release by a peripheral hemodynamic mechanism. *J. Cardiovasc. Pharmacol.* **15**: 1–9