Changes in systemic and regional haemodynamics during 5-HT7 receptor-mediated depressor responses in rats

Abstract The 5-hydroxytryptamine (5-HT)-induced late depressor response in rats is mainly mediated by vascular 5-HT7 receptors. The present study was devoted to determining the systemic and regional haemodynamic changes during this response, with particular emphasis on localising vascular beds that may contribute to the increase in total systemic vascular conductance. In vagosympathectomised, pentobarbital-anaesthetised rats pretreated with the 5-HT2 receptor antagonist ritanserin (50 µg kg \(^{-1}\), i.v.), 5-HT (1, 3 and 10 µg kg \(^{-1}\) min \(^{-1}\) during 10 min; i.v.) produced a dose-dependent decrease in mean arterial blood pressure by up to 46 ± 3%. This decrease was accompanied by increases in systemic vascular conductance by up to 83 ± 15%; cardiac output was unaffected. 5-HT increased regional vascular conductance in skeletal muscle, carcass, mesentery/pancreas and adrenals by up to 740 ± 141%, 117 ± 18%, 135 ± 26% and 88 ± 22%, respectively, but decreased ‘lung’ (mainly arteriovenous anastomotic) conductance by up to 81 ± 2%. Pretreatment with \(R(+)\)lisuride (100 µg kg \(^{-1}\), i.v.) abolished all 5-HT-induced systemic and regional haemodynamic effects. In contrast, i.v. pretreatment with \(S(-)\)lisuride (100 µg kg \(^{-1}\)) or GR127935 (300 µg kg \(^{-1}\)) did not affect the 5-HT-induced systemic haemodynamic changes. The above results suggest that hypotension induced via 5-HT7 receptor activation was exclusively caused by vasodilatation of the systemic vasculature, confined to skeletal muscle, carcass, mesentery/pancreas and adrenal vascular beds. Furthermore, this study shows that blockade of vaso-relaxant 5-HT7 receptors by lisuride is stereoselective.

Key words 5-HT · 5-HT7 receptor · Cardiac output · GR127935 · Hypotension · Lisuride · Regional blood flow · Serotonin

Introduction

The complexity of cardiovascular effects (bradycardia or tachycardia, hypotension or hypertension and vasodilatation or vasoconstriction) produced by serotonin (5-hydroxytryptamine; 5-HT) has been explained by its capacity to interact with specific receptors (see Saxena and Villalón 1990, 1991; Martin 1994; Jones et al. 1995). These receptors include 5-HT1 (5-HT1A, 5-HT1B and/or 5-HT1D subtypes), 5-HT2 (5-HT2A, 5-HT2B/2C), 5-HT3, 5-HT4 as well as 5-HT7 receptors (Hoyer et al. 1994; Villalón et al. 1997b; Saxena et al. 1998).

The cardiovascular response induced by 5-HT in the rat serves as an example to illustrate the above complexity. Thus, i.v. administration of 5-HT produces a triphasic blood pressure response in anaesthetised rats with intact vagus nerves. This response consists of an initial hypotension associated with a brief, but intense, bradycardia via the von Bezold-Jarisch reflex (mediated by 5-HT3 receptors located on cardiac vagal afferents), followed by a vasopressor effect (mediated by vascular 5-HT2A receptors) and, finally, a longer lasting hypotension (Hoyer et al. 1994; Villalón et al. 1997b; Saxena et al. 1998). After bilateral vagosympathectomy and pretreatment with ketanserin or cyproheptadine (5-HT2A receptor antagonists), 5-HT exclusively produces the late depressor response (Saxena and Lawang 1985). Although this 5-HT-induced hypotensive response had initially been ascribed to an action at vascular 5-HT1-like receptors (Martin et al. 1987; Saxena and Villalón 1990), it was subsequently concluded that these receptors closely resemble the pharmacological profile of the 5-HT7 receptors (De Vries et al. 1997b). This conclusion was based, amongst other findings, on: (1) the inactivity of the 5-HT1B/1D receptor agonist, sumatriptan; (2) the blockade by mesulergine and clozapine, compounds that do not...
interact with the 5-HT\textsubscript{1} receptor family (Hoyer et al. 1994) as well as by lisuride; and (3) the resistance to blockade by GR127935 (N-[methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2' methyl-(5-methyl-1,2,4-oxadiazol-3-yl),[1,1-biphenyl]-4-carboxamide hydrochloride), a selective 5-HT\textsubscript{1B/D} receptor antagonist (Skingle et al. 1996). In the light of these findings, the present study was designed to further analyse this late hypotensive response, with particular emphasis on ascertaining the regional blood flows responsible for the 5-HT-induced increase in systemic vascular conductance in the rat. For this purpose, the distribution of cardiac output to the different tissues during 5-HT-induced increase in systemic vascular conductance was determined using the radioactive microsphere technique (Saxena et al. 1980). In order to confirm the involvement of 5-HT\textsubscript{7} receptors, we decided to use: (1) the stereoisomers of lisuride \([R(+)] \) and \([S(-)]\) to investigate whether the blockade of 5-HT\textsubscript{7} receptors by this ergot derivative is stereoselective; and (2) GR127935, which fails to antagonise 5-HT-induced hypotension in rats (De Vries et al. 1997b). Preliminary results of this investigation have been communicated to the British Pharmacological Society (De Vries et al. 1998a).

Materials and methods

General procedures. Experiments were carried out in 26 male Wistar rats (300–330 g). After initial anaesthesia with ether, the trachea was cannulated and a catheter was placed in the right external jugular vein. At this point, ether anaesthesia was stopped and the animals received i.v. bolus injections of pentobarbital (30–40 mg kg\textsuperscript{-1}). Subsequently, the rats were artificially ventilated with a mixture of oxygen and room air using a respiratory pump (Infant ventilator MK3; Hoeckloos, The Netherlands) at a rate of 40 strokes min\textsuperscript{-1} (volume: 20 ml kg\textsuperscript{-1}). Bilateral vagosympathectomy was performed to avoid the bradycardia and hypotension caused by the von Bezold-Jarisch reflex (Paintal 1973). The right common carotid artery was exposed and a catheter, connected to a pressure transducer (Combitrans disposable pressure transducer; Braun, Melsungen, Germany) was guided through the carotid artery into the left ventricle. The presence of the tip of the catheter in the left ventricle was confirmed by the observation of the sudden switch from an arterial to a ventricular pressure profile. Additionally, a catheter was placed into the left femoral artery and connected to another pressure transducer for the recording of blood pressure and for the withdrawal of reference blood samples. Heart rate was measured with a tachograph (CRW; Erasmus University, Rotterdam, The Netherlands) triggered from electrocardiogram signals. Both blood pressure and heart rate were recorded simultaneously on a polygraph (CRW; Erasmus University, Rotterdam, The Netherlands). The right external jugular vein was used for the administration of drugs.

Distribution of cardiac output. The distribution of aortic blood flow was determined with 15.5±0.1 (SD) μm diameter microspheres labelled with \(^{141}\text{Ce}, ^{113}\text{Sn}, ^{89}\text{Ru} \) or \(^{46}\text{Sc}\) (NEN Dupont, Boston, Mass., USA). For each measurement about 200,000 microspheres, suspended in 0.2 ml physiological saline and labelled with one of the isotopes, was mixed and injected into the left ventricle over a 15-s period; the catheter was thoroughly flushed with 0.5 ml saline. Starting 10 s before microsphere injection and lasting 70 s, an arterial reference blood sample was drawn from the left femoral artery at a constant rate of 0.5 ml min\textsuperscript{-1}, using a withdrawal pump (Model 55; Harvard Apparatus, Natick, Mass., USA). At the end of the experiment the animal was sacrificed with an overdose of pentobarbital and all tissues and organs were dissected out, weighed and put in vials. The following tissues were studied: skeletal muscle, carcass (consisting of bone with skeletal muscle residue, fat, tail, eyes and urogenital tract), mesentery/pancreas (for practical reasons, these two tissues were not studied separately), adrenals, lungs, kidneys, skin, heart, liver, brain, gastrointestinal tract and spleen. The radioactivity in the reference blood samples and the tissues was counted for 5 min in a \(\gamma\)-scintillation counter (Packard, MiniMax Auto-Gamma 5000 series), using suitable windows discriminating different isotopes. All data were processed by a set of specially designed computer programs (Saxena et al. 1980).

The cardiac output was calculated by multiplying the ratio of total and arterial blood sample radioactivity by the withdrawal rate of the arterial reference blood sample (0.5 ml min\textsuperscript{-1}). Accordingly, tissue blood flow was calculated by multiplying the ratio of tissue and total radioactivity by cardiac output (Saxena et al. 1980). Conductances were calculated as blood flow divided by the mean arterial blood pressure and expressed as 10\textsuperscript{2} ml mmHg\textsuperscript{-1} min\textsuperscript{-1}.

Experimental protocols. The experiments were started after a stabilisation period of about 30 min. At this point, all animals were systemically pretreated with ritanserin (50 µg kg\textsuperscript{-1}, i.v.), which has been shown to be sufficient to block 5-HT\textsubscript{2A} receptor-mediated vasopressor responses (Villalón et al. 1993; De Vries et al. 1997a,b). The animals were then divided into five groups. In the first group (n=6), the effects of three consecutive 10-min i.v. infusions of vehicle (physiological saline; 0.1 ml min\textsuperscript{-1}) on heart rate (HR; beats min\textsuperscript{-1}), cardiac output (CO; ml min\textsuperscript{-1}), mean arterial blood pressure (MAP; mmHg), diastolic arterial blood pressure (DAP; mmHg), stroke volume (SV; 10\textsuperscript{-2} ml), systemic vascular conductance (SVC; 10\textsuperscript{12} ml mmHg\textsuperscript{-1} min\textsuperscript{-1}; upper panel) and skeletal muscle (Mus), car- cass (Car), mesentery/pancreas (M+P), adrenal (Adr), lung (Lung) and kidney (Kid) vascular conductances (10\textsuperscript{12} ml mmHg\textsuperscript{-1} min\textsuperscript{-1}; lower panel) in anaesthetised rats systematically pretreated with ritanserin (50 µg kg\textsuperscript{-1}, i.v.). Values are presented as the means±SEM. *P<0.05 vs. baseline. For the sake of clarity, adrenal vascular conductance values have been multiplied by a factor of 10.