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Cardiovascular effects of SL65.0472, a 5-HT receptor antagonist

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Abstract

In this study, we describe the cardiovascular effects of SL65.0472 (7-fluoro-2-oxo-4-[2-[4-(thieno[3,2-*c*] pyridin-4-yl) piperazin-l-yl] ethyl]-1, 2-dihydroquinoline-1-acetamide), a novel 5-hydroxytryptamine (5-HT) receptor antagonist developed for the treatment of cardiovascular disease, in several in vivo models. The haemodynamic profile of SL65.0472 was evaluated in anaesthetised dogs. Following i.v. bolus doses of 0.03 mg/kg i.v. and 0.3 mg/kg, no significant changes in cardiac output, contractility or rate, systemic and pulmonary pressures, regional blood flows and vascular resistances or electrocardiogram were noted. After 1 mg/kg i.v. SL65.0472 significantly reduced arterial blood pressure. In conscious spontaneously hypertensive rats administration of SL65.0472 0.5 mg/kg p.o. had no effect on mean arterial blood pressure or heart rate. Vasoconstriction produced by 5-HT results primarily from the stimulation of two receptor subtypes, 5-HT_{1B} and 5-HT_{2A} receptors. In anaesthetised dogs SL65.0472 antagonised sumatriptan-induced decreases in saphenous vein diameter (5-HT_{1B}-receptor mediated) with an ID_{50} of 10.1 μg/kg i.v. (95% c.l. 8.3–12.4). In anaesthetised pithed rats SL65.0472 inhibited 5-HT pressor responses (5HT_{2A}-receptor mediated) with ID_{50} values of 1.38 μg/kg i.v. (95% c.l. 1.15–1.64) and 31.1 μg/kg p.o. (95% c.l. 22.6–42.6). The duration of the 5-HT_{2A}-receptor antagonist effect of SL65.0472 following oral administration was evaluated in conscious rats. SL65.0472 (0.1 mg/kg p.o.) markedly inhibited 5-HT pressor responses 1 and 6 h after administration. Therefore, in vivo, SL65.0472 potently antagonises vasoconstriction mediated by 5-HT_{1B} and 5-HT_{2A} receptors but has minimal direct haemodynamic effects.

Keywords: SL 65.0472; Haemodynamics; 5-HT_{1B} receptors; 5-HT_{2A} receptors

1. Introduction

5-Hydroxytryptamine (5-HT) has important effects on cardiovascular function and 5-HT receptor antagonists (e.g. ketanserin) have been used to treat cardiovascular disease. 5-HT stimulates platelet aggregation and has a prothrombotic effect which is mediated through activation of 5-HT_{2A} receptors on blood platelets. In addition, 5-HT is a potent vasoconstrictor agent, capable of contracting arterial and venous tissues in vitro and increasing blood pressure and vascular resistance in vivo. Although many of these vasoconstrictor effects are mediated by ketanserin-sensitive 5-HT_{2A} receptors (e.g. increases in blood pressure in the rat, Fozard, 1982; Cohen et al., 1983), it is increasingly recognised that a second subtype, initially classified as 5-HT_1-like, also mediates vasoconstriction in certain vascular tissues (Saxena and Villalon, 1990). The evidence from pharmacological and molecular biological studies indicates that these 5-HT_1-like responses are likely to be mediated via 5-HT_{1B} receptors (previously termed 5-$HT_{1D\beta}$ Hartig et al., 1996). The 5-$HT_{1B/1D}$ receptor agonist sumatriptan causes contraction of several vascular tissues, for example canine saphenous vein (Humphrey et al., 1988), human basilar artery (Parsons et al., 1989) and human coronary artery (Kaumann et al., 1994). In addition, prominent vasoconstrictor responses to sumatriptan can be revealed in other tissues, such as rabbit mesenteric artery (Choppin and O'Connor, 1995) only in the presence of another vasoconstrictor agent. Vascular contraction produced by sumatriptan and related agonists is resistant to the potent 5-HT_{2A} receptor antagonist ketanserin but can be antagonised by 5-$HT_{1B/1D}$ receptor antagonists such as GR 127935 (Clitherow et al., 1994) or GR 125743 (Gupta

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et al., 1999). In addition to blocking 5-HT_{2A} receptors, ketanserin shows significant affinity for the human 5-HT_{1D} subtype but is virtually inactive at human 5-HT_{1B} receptors (Zgombick et al., 1995). This strengthens the 5-HT_{1B} characterisation of these responses to sumatriptan. Most recently, using molecular biological techniques, strong expression of 5-HT_{1B} receptor mRNA (but not 5-HT_{1D} receptor mRNA) has been identified in canine saphenous vein (Sgard et al., 1996), human coronary artery (Nilsson et al., 1999), human temporal artery (Verheggen et al., 1998) and rabbit mesenteric artery (Hinton et al., 1999). The potential importance of vasoconstrictor 5-HT_{1B} receptors is underlined by the observation of increases in blood pressure and vascular resistances following i.v. or s.c. administration of sumatriptan to man (MacIntyre et al., 1992, 1993), although when given by the oral route sumatriptan has proved safe and efficacious when used appropriately.

This paper describes the in vivo cardiovascular effects of SL65.0472 (7-fluoro-2-oxo-4-[2-[4-(thieno[3,2-*c*] pyridin-4-yl) piperazin-l-yl] ethyl]-1, 2-dihydroquinoline-1-acetamide) a new 5-HT receptor antagonist. SL 65.0472 is a potent antagonist of 5-HT_{1B} and 5-HT_{2A} receptors in vitro (Galzin et al., 2000) which is in development for the treatment of cardiovascular disease. SL 65.0472 also inhibits 5-HT induced platelet aggregation and shows potent anti-thrombotic properties in rat and dog thrombosis models (Berry et al., 2000; O'Connor et al., 2000). We have studied the haemodynamic profile of SL 65.0472 in anaesthetised dogs and spontaneously hypertensive rats. In addition, we have evaluated the antagonist effects of SL65.0472 in in vivo models of vasoconstriction mediated by 5-HT_{1B} receptors (Drieu la Rochelle and O'Connor, 1995) and 5-HT_{2A} receptors (Cohen et al., 1983). Certain of these studies have been published in abstract form (O'Connor et al., 2000).

2. Methods

The animals used in these experiments were treated in accordance with ethical guidelines edited by the European community (EC directive 86/609), the council of Europe (Convention ETS 123) and the French government (decree of 19.10.87).

2.1. Haemodynamic study in anaesthetised dogs

2.1.1. Experimental study plan

This study was performed in 20 male beagle dogs weighing 10.3–13.8 kg. On the day prior to the test dogs were fasted, having access only to drinking water. On the day of dosing, the dog was anesthetized with sodium pentobarbitone 30 mg/kg i.v. A catheter was inserted into the cephalic vein and, throughout the experiment, anesthesia was maintained by means of a sodium pentobarbitone infusion (approximately 0.1 mg/kg/min i.v.). The trachea was intubated with a balloon tube and the animal was placed on an homeothermic blanket. Three electrodes were placed in order to visualize the electrocardiogram (ECG) (lead II). A polyethylene catheter connected to a Statham pressure transducer was inserted into the right femoral artery. A Millar catheter was introduced into the left ventricle via the left carotid artery. The introduction-set was also used to collect arterial samples for blood gases analysis. A Transonic flow probe was fitted around the right carotid artery. A Pulmoball catheter was inserted via the right jugular vein into the pulmonary artery. The dog was then laid on its right side and ventilated artificially with an air/oxygen mixture at a rate of 25 cycles/min and a minute volume of about 0.2 l/kg/min. A left thoracotomy was then performed through the fourth intercostal space. Two Transonic flow probes were placed around the left circumflex coronary artery for measurement of the coronary blood flow and around the aorta just downstream of the subclavian artery, respectively. A fourth Transonic flow probe was fitted around the left renal artery. At the end of this surgical phase, a stabilization period of at least 30 min was given in order to let the animal recover and to check the stability of the measured parameters. Arterial blood gases were also measured. SL65.0472 or the placebo was then injected into the cephalic vein, and the venous catheter was immediately rinsed by injection of 1 ml of isotonic saline solution. All haemodynamic parameters were recorded continuously during the stabilization period and for 120 min after dosing by means of the Po-ne-mah software. When measurements were performed (before treatment and at 1, 3, 5, 10, 15, 30, 60, 75, 90, 105 and 120 min after treatment), a paper recorder was also used. ECG intervals were determined from the paper recording. Samples of arterial blood were collected before treatment for analysis of blood gases and at times 15, 30, 60 and 120 min.

The study involved four groups of five animals, each of which received a single dose of either SL65.0472 (0.03, 0.3 or 1 mg/kg i.v.) or placebo (glucose 5% w/v in water for injection, 0.1 M acetic acid 1% v/v and sodium hydroxide up to pH 4). The allocation of treatments was determined randomly.

The following parameters were measured: systolic left ventricular pressure (LVP) (mm Hg); left ventricular end diastolic pressure (mm Hg); $dLVP/dt(+)$ (mm Hg/s); $dLVP/dt(-)$ (mm Hg/s); cardiac contractility index (mm Hg/s/mm Hg), as defined by $dLVP/dt(+)_{max}$ divided by the LVP at that point; systolic arterial blood pressure (mm Hg); diastolic arterial blood pressure (mm Hg); mean pulmonary artery pressure (mm Hg); mean arterial pressure (mm Hg); heart rate (beats/min); mean carotid blood flow (ml/min); carotid vascular resistance (mm Hg/ml/min); mean aortic blood flow (ml/min); peripheral resistance (mm Hg/ml/min); mean coronary blood flow (ml/min); coronary vascular resistance (mm

Hg/ml/min); mean renal blood flow (ml/min); renal vascular resistance (mm Hg/ml/min); stroke volume (ml). Stroke volume was defined as mean aortic blood flow/heart rate. Mean blood pressures and mean blood flows were calculated by the Po-ne-mah software (as areas under the curve). Vascular resistances were calculated using the formula: Mean arterial pressure/mean blood flow. Mean blood flows were displayed continuously on the flowmeters and were noted on the recording paper at each time of measurement. The following were measured from the ECG: PR interval (ms); QRS interval (ms); QT interval (ms); QTc interval (ms) calculated according to the formula: $QTc = QT/(60/HR)^{1/2}$, in which QT is expressed in ms and heart rate is expressed in beats/min. Arterial blood gases (pH, pO_2, pCO_2) were monitored at regular intervals and kept within normal limits.

2.1.2. Expression and analysis of results

Results were given as mean values ± S.E.M. For each parameter the effects of SL65.0472 were analysed by comparison of the treated group to the control one using a two-way analysis of variance (ANOVA) (treatment × time) with repeated measures on factor time, followed by a test of simple effect of treatment for each level of factor time (Winer analysis) and Dunnett's test if necessary. Statistical significance was assumed for $P < 0.05$.

2.2. Sumatriptan-induced saphenous venoconstriction in the dog

2.2.1. Surgical procedure

This study was performed according to the general technique described in detail previously (Drieu La Rochelle and O'Connor, 1995). Briefly, beagle dogs (12–19 kg) were anaesthetised with sodium pentobarbitone (42 mg/kg i.v. + 6 mg/kg/h i.v.) and artificially ventilated. Saphenous vein diameter was measured instantaneously and continuously by sonomicrometry following suturing of two transducer crystals on opposite sides of the left external saphenous vein. Catheters were placed to enable recording of mean arterial pressure, left ventricular pressures, cardiac contractility and left saphenous vein pressure. Heart rate was recorded from the ECG (lead II). Mean aortic blood flow was measured following left thoracotomy using an electromagnetic flow probe positioned around the ascending aorta.

2.2.2. Experimental protocol

Animals were separated into two experimental groups ($n = 5$ per group). Bolus i.v. doses of sumatriptan were administered in order to determine for each animal, a dose of sumatriptan which reduced saphenous vein diameter by approximately 30%. This response level represents approximately 75% of maximum response obtainable in this model (Drieu La Rochelle and O'Connor, 1995). The mean dose of sumatriptan employed was 29 ± 8.2 μg/kg i.v.

Once chosen, this dose of sumatriptan was repeated until reproducible changes in saphenous vein diameter were obtained. Each animal then received by i.v. injection either SL65.0472 (3, 10 and 30 μg/kg) or vehicle (5% glucose) given in a volume of 10 ml over 2 min. Administration of sumatriptan was repeated 5 min after administration of each dose of compound or vehicle. Saphenous vein diameter was allowed to return to baseline levels before the next administration of antagonist or vehicle (minimum 30 min).

2.2.3. Data and statistical analysis

Haemodynamic data were recorded on a multichannel electrostatic recorder (ES 1000, Gould) and digitized at a sampling rate of 2 kHz per channel at full 12-bit resolution using HEM software (Notocord Systems). Digitized data were filtered and stored at 500 Hz on the hard disk of a PC and used in parallel for calculation and display in real time of derived parameters.

The peak change in saphenous vein diameter induced by sumatripan was measured and expressed as a percentage of the pretreatment control value. Values were expressed as means ± S.E.M. SL65.0472-induced inhibition of changes in saphenous vein diameter mediated by sumatriptan were compared with the corresponding vehicle group. ID_{50} values were calculated by linear regression analysis (RS1). Haemodynamic parameters were monitored at 0.5, 1, 2, 4, 10, 20 and 30 min after administration of SL65.0472. Results are expressed as means ± S.E.M. (absolute values). Statistical significance was evaluated by using ANOVA followed by a Dunnett's test. A value of $P < 0.05$ was considered to be significant.

2.3. Pressor responses to 5-HT in anaesthetised pithed rats

2.3.1. Surgical procedure

Male Sprague–Dawley rats (200–300 g) were anaesthetised with sodium pentobarbitone (60 mg/kg i.p.). After tracheotomy, animals were pithed via the orbital sinus, artificially ventilated and placed on a heated table. Arterial blood pressure was measured using a catheter introduced into the carotid artery. The second carotid artery was ligated and the vagus nerves were sectioned. A catheter was introduced into the femoral vein.

Arterial blood pressure was measured via a pressure transducer (Gould P23ID) linked to a Grass 7P1 preamplifier. Mean arterial pressure was integrated by the preamplifier and displayed on a Grass 7D recorder.

2.3.2. Experimental protocol

Five groups of rats were used for the oral administration study, comprising one control group which received vehicle (0.9% saline) and four treatment groups each of which was administered a single oral dose of SL65.0472 (3, 10, 30 or 100 μg/kg p.o., $n = 6–9$ per group). Oral dosing was performed 60 min before anaesthesia in a volume of

0.1 ml per 100 g body weight. After surgical preparation, each animal received two bolus doses of 5-HT (30 μg/kg i.v.) at 75 and 80 min after oral administration.

In the intravenous administration study rats each received two bolus doses of 5-HT (30 μg/kg i.v.) 10 and 5 min before drug treatment. SL65.0472 (0.3, 1, 3 and 10 μg/kg i.v.) was administered cumulatively, a 20-min interval being applied between each dose. Injections of 5-HT were repeated 5 and 10 min after each dose of SL65.0472.

2.3.3. Results analysis

The pressor response to the administration of 5-HT was calculated as the peak increase in mean arterial pressure following injection. Following oral administration of SL65.0472, the percentage inhibition of 5-HT induced pressor responses was calculated by comparing the response in each drug-treated animal with the mean value of the corresponding vehicle-treated animals. Following i.v. administration, the antagonist activity of SL65.0472 was evaluated for each dose tested by calculation of the percentage inhibition of the pressor response to 5-HT by reference to the pre-treatment value. ID_{50} values were calculated by linear regression analysis (RS-1).

2.4. 5-HT-induced pressor responses in conscious rats

The duration of action of SL65.0472 following oral administration was evaluated by measuring its inhibitory effect on 5-HT-induced increases in mean arterial pressure in conscious rats pre-treated with atropine and propranolol to eliminate cardiac autonomic reflex function.

At least 48 h before the planned study, male Wistar rats (200–300 g) were anaesthetised with pentobarbitone 60 mg/kg i.p. and catheters introduced into the right femoral artery and the left femoral vein. The catheters were exteriorised in the extrascapular region and the animals were allowed to recover from the anaesthetic.

On the day of the experimental study, the animals were housed in individual cages and the arterial catheter was linked to a swivel allowing 360° rotation such that the animals were able to move freely. Recording of changes in arterial blood pressure was performed using an Ailtech MS20 pressure transducer linked to a Gould amplifier. Values of mean arterial pressure were analysed with the aid of a Notocord HEM 3.1 data analysis programme.

After a stabilisation period each rat received propranolol 1 mg/kg i.v. and atropine 1 mg/kg i.v. to block cardiac autonomic tone. Twenty minutes afterwards a control pressor response to 5-HT 30 μg/kg i.v. was performed prior to the drug treatment (vehicle or SL65.0472 0.1 mg/kg p.o. $n = 8$ per group). Administration of 5-HT was repeated 1, 6 and 24 h after drug treatment. For the 6- and 24-h readings, administration of 5-HT was preceded by repeat administration of atropine and propranolol to ensure the maintenance of autonomic blockade.

2.4.1. Data and statistical analysis

Increases in mean arterial pressure induced by 5-HT administration were measured at pre-treatment, 1, 6 and 24 h post-treatment in vehicle and drug treated groups and expressed as means ± S.E.M. Statistical comparisons were made between each treatment time and the pre-treatment value using ANOVA with a Dunnett's test and at the same time point between SL65.0472 and vehicle group using a Dunnett's test. A value of $P < 0.05$ was considered to be significant.

Values of mean arterial pressure and heart rate were measured just before each administration of 5-HT in order to identify a possible haemodynamic effect of SL65.0472. Comparisons were made between vehicle and drug-treated groups by ANOVA. A value of $P < 0.05$ was considered significant.

2.5. Effects on blood pressure and heart rate in conscious spontaneously hypertensive rats

Male spontaneously hypertensive rats aged between 18 and 20 weeks weighing 275–375 g were obtained from Charles River. Blood pressure was recorded from a catheter implanted in the right femoral artery by the method described above.

After a stabilisation period animals received one of the following treatments in randomised order: vehicle (5% glucose, $n = 7$), SL65.0472 0.5 mg/kg p.o. ($n = 8$) or prazosin 1 mg/kg p.o. ($n = 8$). Mean arterial pressure and heart rate were recorded at 1, 2, 3, 4, 5, 6 and 24 h after treatment and expressed as means ± S.E.M. Statistical comparisons were analysed using two-way ANOVA with repeated measurements of the time factor. A value of $P < 0.05$ was considered to be significant.

2.6. Drugs

SL65.0472, sumatriptan and prazosin were synthesised by the Department of Cardiovascular Chemistry, Sanofi-Synthélabo, Chilly-Mazarin, France. Other drugs used were propranolol, 5-HT (Sigma, St Louis, MO, USA), atropine (Prolabo, Paris, France) and pentobarbitone (Sanofi, Libourne, France).

3. Results

3.1. Haemodynamic profile in anaesthetised dogs

There were no significant differences in baseline values of any of the parameters studied between vehicle and SL65.0472-treated groups.

Table 1 shows the main haemodynamic parameters in the different treatment groups measured 30 min after administration of vehicle or SL65.0472 0.03, 0.3 and 1 mg/kg i.v. Fig. 1 illustrates the evolution of nine key

Table 1
Haemodynamic profile in anaesthetised dogs. Main cardiovascular parameters measured 30 min after administration i.v. of vehicle or SL65.0472

		Vehicle	SL65.0472 (0.03 mg/kg)	SL65.0472 (0.03 mg/kg)	SL65.0472 (1 mg/kg)
Systolic left ventricular pressure	(mm Hg)	144 ± 11	144 ± 9	135 ± 8	108 ± 4
	(Δ%)	−3.8 ± 2.5	−3.0 ± 2.3	−6.7 ± 2.1	−15.7 ± 3.3
			NS	NS	S
Left ventricular end diastolic pressure	(mm Hg)	7.4 ± 1.4	6.6 ± 1.2	7.8 ± 0.5	6.0 ± 0.4[a]
	(Δ%)	+3.5 ± 6.6	−5.6 ± 3.5	−12.5 ± 4.4	−1.1 ± 17.2
			NS	NS	NS
Contractility index	(mm Hg/s/mm Hg)	36.6 ± 2.2	37.6 ± 3.1	32.8 ± 2.1	33.8 ± 1.2
	(Δ%)	−0.8 ± 3.2	+4.2 ± 2.8	+7.4 ± 1.0	+4.4 ± 2.9
			NS	NS	NS
Systolic arterial blood pressure	(mm Hg)	174 ± 15	174 ± 11	162 ± 11	129 ± 8
	(Δ%)	−3.1 ± 2.4	−3.1 ± 2.8	−7.3 ± 2.0	−18.2 ± 3.1
			NS	NS	S
Mean pulmonary artery pressure	(mm Hg)	13.6 ± 1.0	16.6 ± 1.3	12.2 ± 0.9	14.0 ± 1.1
	(Δ%)	−4.1 ± 2.7	+2.3 ± 2.6	−1.9 ± 3.1	−2.9 ± 3.4
			NS	NS	NS
Heart rate	Beats/min	174 ± 7	179 ± 12	174 ± 11	153 ± 8
	(Δ%)	−3.0 ± 1.7	+1.4 ± 0.9	+2.7 ± 0.6	−3.0 ± 2.0
			NS	NS	NS
Aortic blood flow	(ml/min)	1535 ± 73	1497 ± 112	1312 ± 138	1391 ± 182
	(Δ%)	+9.6 ± 11.1	+6.1 ± 2.3	+12.8 ± 7.8	+16.7 ± 4.5
			NS	NS	NS
Stroke volume	(ml)	8.9 ± 0.5	8.4 ± 0.4	7.6 ± 0.8	9.0 ± 1.5
	(Δ%)	+12.9 ± 11.1	+4.9 ± 2.5	+10.4 ± 7.3	+17.1 ± 4.8
			NS	NS	NS
Peripheral resistance	(mm Hg/ml/min)	0.082 ± 0.004	0.087 ± 0.006	0.097 ± 0.009	0.075 ± 0.008
	(Δ%)	−9.6 ± 9.5	−9.0 ± 3.9	−15.2 ± 5.3	−28.8 ± 4.1
			NS	NS	NS
Coronary blood flow	(ml/min)	14.5 ± 0.9	12.5 ± 0.9	13.6 ± 2.8	9.6 ± 1.9
	(Δ%)	−6.9 ± 2.5	−9.5 ± 6.1	0.0 ± 10.4	−17.1 ± 9.3
			NS	NS	NS
PR interval	(ms)	99 ± 6	98 ± 4	101 ± 4	102 ± 3
	(Δ%)	−0.8 ± 0.8	−0.8 ± 0.8	−3.8 ± 2.8	+0.9 ± 1.6
			NS	NS	NS
QTc interval	(ms)	336 ± 6	339 ± 7	354 ± 4	337 ± 6
	(Δ%)	−0.5 ± 0.6	+1.5 ± 0.7	+0.6 ± 1.5	−1.1 ± 1.5
			NS	NS	NS

Mean ± S.E.M., $n = 5$/group Δ%: percentage change from the baseline value. NS: not significantly different, S: significantly different from the vehicle-treated group, $P < 0.05$.
[a] $n = 4$/group.

parameters in the 120-min period following vehicle or drug treatment.

SL65.0472 0.03 mg/kg and 0.3 mg/kg i.v. did not produce statistically significant changes in any cardiovascular parameter measured up to 120 min after drug administration. At a dose of 1 mg/kg i.v. SL65.0472 significantly reduced systolic LVP; systolic blood pressure and mean arterial pressure. These hypotensive effects appeared soon after drug administration and were of relatively long duration. Maximum changes did not exceed a reduction of 20% with respect to pre-treatment values. There was also a tendency for an increase in aortic blood flow and for decreases in peripheral resistance and carotid vascular resistance which did not reach statistical significance. The PR and QTc intervals of the ECG were unchanged by drug treatment and no arrhythmias were observed.

3.2. Sumatriptan-induced saphenous venoconstriction in the dog

Basal saphenous vein diameter was 2.9 ± 0.1 mm ($n = 5$) in vehicle treated dogs and 2.8 ± 0.1 mm ($n = 5$) in dogs treated with SL65.0472. Bolus injection of sumatriptan produced marked saphenous venoconstriction of relatively short duration. Fig. 2 shows a typical recording of sumatriptan-induced saphenous vein diameter changes in a dog treated with SL65.0472. Pre-treatment reductions in saphenous vein diameter following sumatriptan administration were equivalent in vehicle (−31.2 ± 2.6%, $n = 5$) and SL65.0472 (−34.3 ± 1.6%, $n = 5$) treated groups of animals.

The effects of SL65.0472 and vehicle on sumatriptan-induced saphenous venoconstriction are shown in Fig. 2. SL65.0472 (3–30 μg/kg i.v.) produced dose-related inhi-

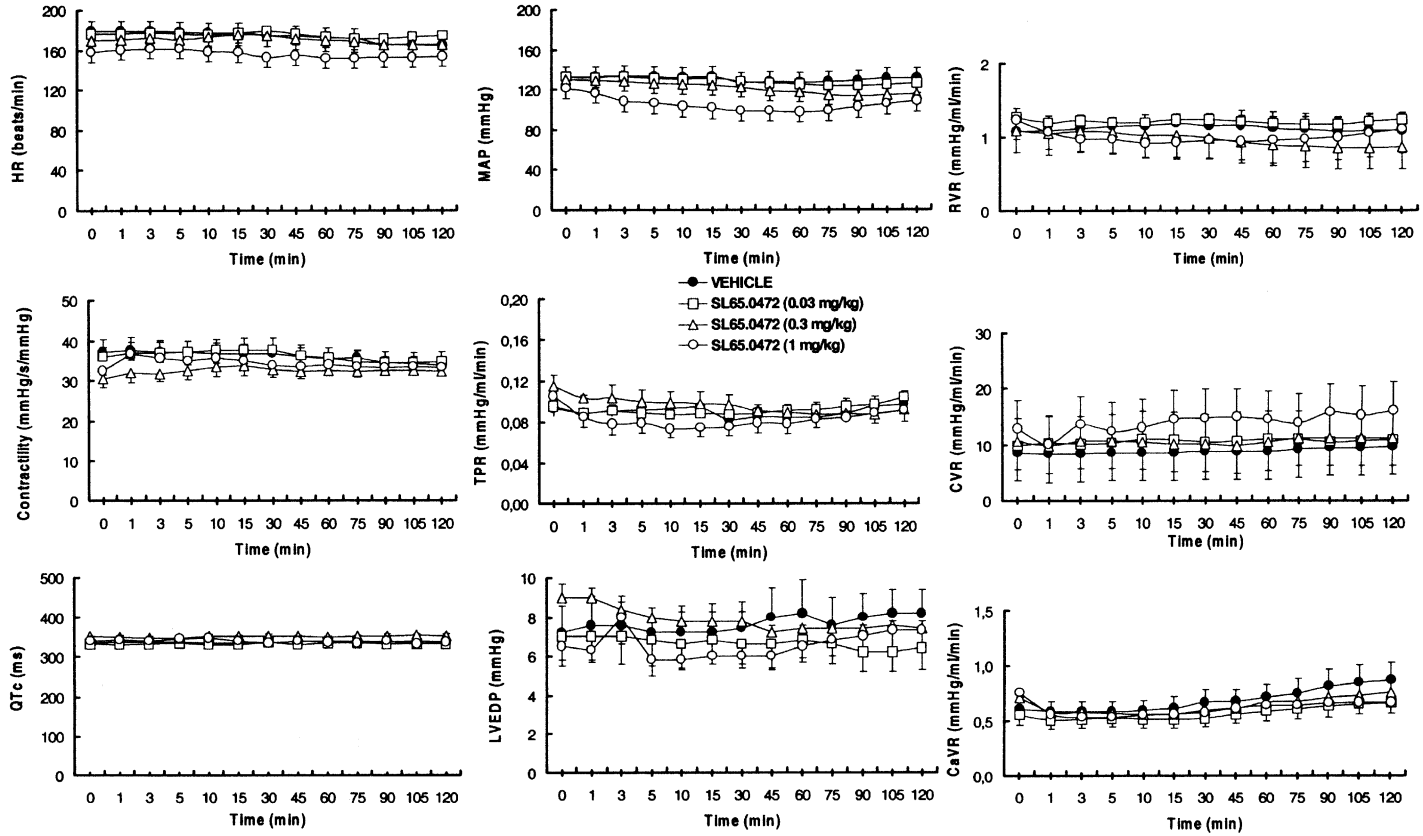

Fig. 1. Haemodynamic profile of SL65.0472 (0.03–1 mg/kg i.v.) or its vehicle in anaesthetised dogs. Parameters shown are mean arterial pressure (MAP), total peripheral vascular resistance (TPR), left ventricular end-diastolic pressure (LVEDP), heart rate (HR), cardiac contractility, QTc interval of the ECG, renal vascular resistance (RVR), coronary vascular resistance (CVR) and carotid vascular resistance (CaVR). Each parameter was monitored for 120 min following administration of SL65.0472 or vehicle. Values are means ± S.E.M., $n = 5$ (except LVEDP, SL65.0472 1 mg/kg i.v. group, $n = 4$). $P < 0.05$ for SL65.0472 1 mg/kg i.v. versus vehicle group for MAP 15 min after administration. All other data points—NS.

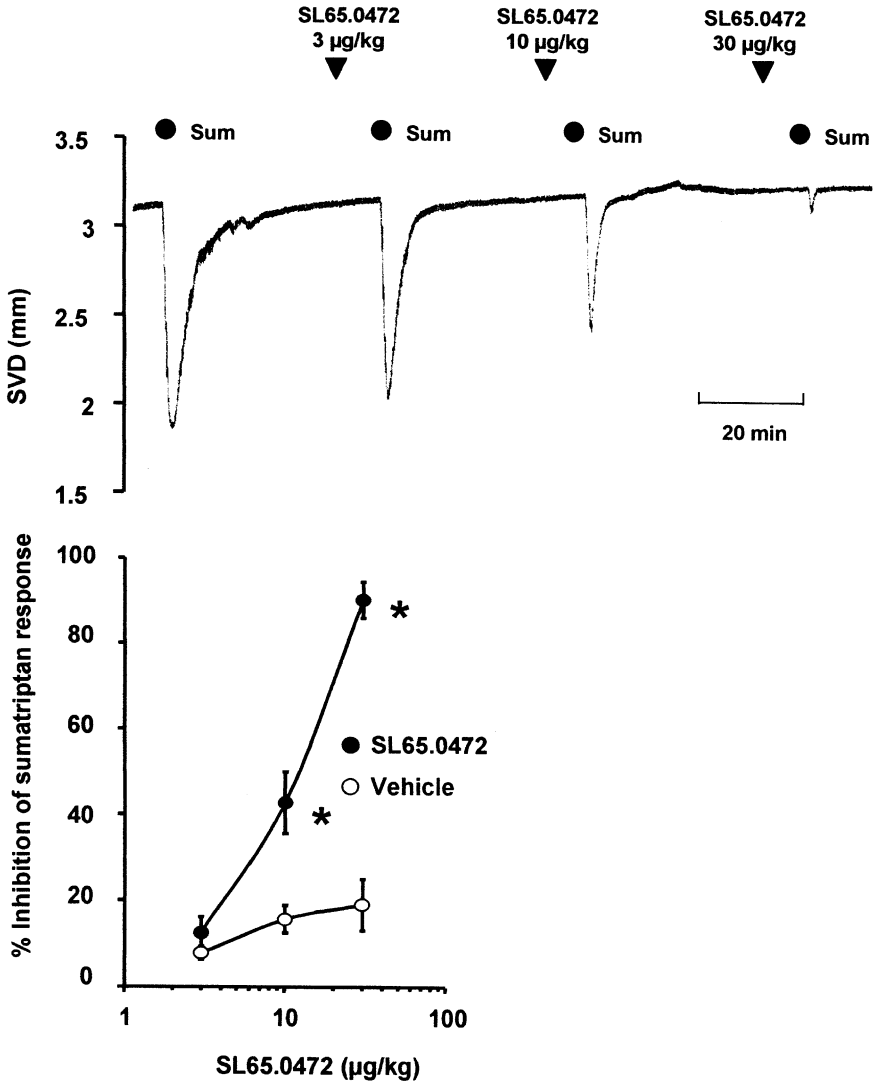

Fig. 2. 5-HT_{1B} receptor mediated saphenous venoconstriction in anaesthetised dog. Upper Panel—Bolus i.v. injection of sumatriptan (SUM, 20 μg/kg) causes marked but relatively short-lived saphenous venoconstriction (reductions in saphenous vein diameter, SVD) in an anaesthetised dog. Sumatriptan-induced saphenous venoconstriction is antagonised in a dose-dependent fashion by administration of SL65.0472. Lower Panel—Effects of SL65.0472 (3–30 μg/kg i.v.) or its vehicle on sumatriptan-induced decreases in saphenous vein diameter in the anaesthetised dog. Values are means ± S.E.M. ($n = 5$). * $P < 0.05$ versus vehicle group. Responses are expressed as a percentage inhibition of the pretreatment saphenous venoconstrictor response to sumatriptan.

bition of sumatriptan-induced decreases in saphenous vein diameter with an ID_{50} of 10.1 μg/kg i.v. (95% c.l. 8.3–12.4). 90.2 ± 4.3% inhibition was achieved following 30 μg/kg SL65.0472. Statistically significant differences versus vehicle group were observed at the two highest doses ($P < 0.05$).

Regardless of the dose used, the administration of SL65.0472 produced only minor changes in haemodynamic parameters which did not reach statistical significance (data not shown). After the highest dose used (30 μg/kg i.v.) maximum drug-induced variations in mean arterial pressure, heart rate, dP/dt_{max}, left ventricular end diastolic pressure, saphenous vein diameter and saphenous vein pressure did not exceed 5%. One min after administration, total peripheral resistance was reduced by 10.9 ± 2.5% and aortic blood flow increased by 10.5 ± 3.6%. It was notable that baseline saphenous vein diameter was unaffected by administration of SL65.0472 (Fig. 2).

3.3. Pressor response to 5-HT in anaesthetised pithed rats

In vehicle-treated rats administration of 5-HT, 30 μg/kg i.v., 75 min after oral dosing caused an increase in mean arterial pressure of 46 ± 3 mm Hg ($n = 9$). Fig. 3 shows the effects of oral administration of SL65.0472. Over the dose-range 3–100 μg/kg p.o., SL65.0472 produced dose-related inhibition of 5-HT pressor responses with an ID_{50} value of 31.1 μg/kg (95% c.l. 22.6–42.6).

In rats prepared for i.v. administration of SL65.0472, pre-treatment increases in mean arterial pressure in response to 5-HT 30 μg/kg i.v. were 53 ± 4 mm Hg ($n = 6$). As shown in Fig. 3, SL65.0472 0.3–10 μg/kg

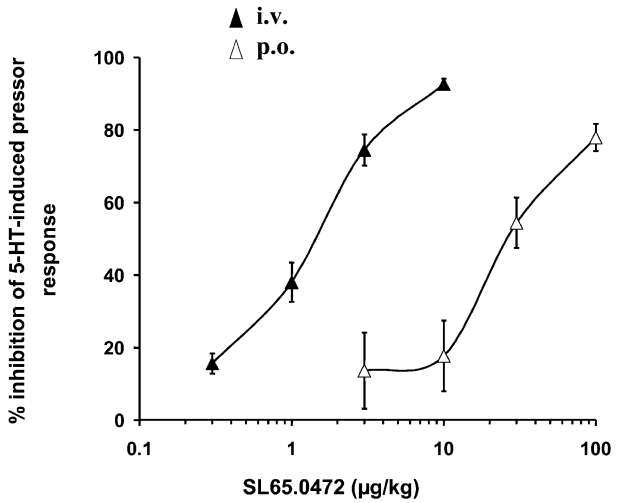

Fig. 3. Effects of SL65.0472 administered by i.v. or oral routes on pressor responses to 5-HT in anaesthetised, pithed rats. Pressor responses were measured as increases in mean arterial pressure following bolus i.v. administration of 5-HT 30 μg/kg and are expressed as percentage inhibition. Values shown are means ± S.E.M., $n = 6–9$.

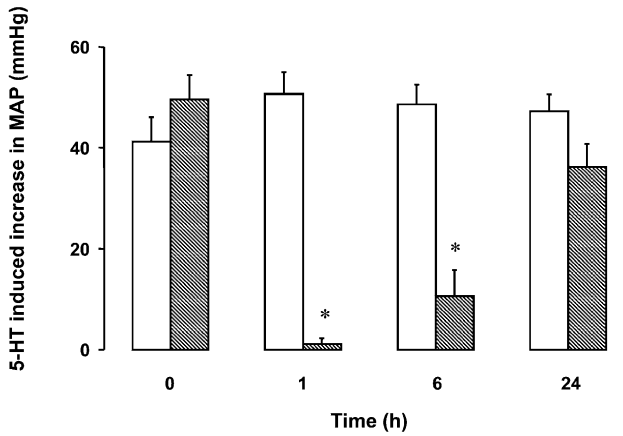

Fig. 4. Effect of SL65.0472 (0.1 mg/kg p.o. hatched columns) or its vehicle (open columns) on increases in mean arterial blood pressure produced by injection of 5-HT (30 μg/kg i.v.) in conscious rats. Readings were taken at time 0 (pretreatment) and 1, 6 and 24 h post-treatment. Values are means ± S.E.M. ($n = 8$). $^{*}P < 0.05$ versus pretreatment values. All animals were pretreated with the combination of atropine and propranolol in order to eliminate cardiac autonomic reflex tone.

i.v. produced dose-related inhibition of 5-HT induced increases in mean arterial pressure with an ID_{50} of 1.38 μg/kg (95% c.l. 1.15–1.64).

3.4. 5-HT pressor responses in conscious rats

Pretreatment increases in mean arterial pressure produced by administration of 5-HT 30 μg/kg were 50 ± 5 mm Hg in the SL65.0472 group ($n = 8$) and 41 ± 5 mm Hg in the vehicle group ($n = 8$).

Neither SL65.0472 0.1 mg/kg p.o. nor vehicle modified mean arterial pressure and heart rate throughout the experiment. For example in the SL65.0472 treated group mean arterial pressure values were 106 ± 4 mm Hg pretreatment and 102 ± 4 mm Hg at 1 h. Corresponding heart rate values were 402 ± 7 beats/min and 404 ± 8 beats/min ($n = 8$).

The results of this study are shown in Fig. 4. In vehicle treated animals, the pressor responses to 5-HT were maintained at a similar level to the pre-treatment value. By contrast, SL65.0472 produced marked inhibition of the 5-HT-induced increase in mean arterial pressure 1 h after administration (96 ± 3%, $n = 8$, $P < 0.05$) and 6 h after administration (80 ± 10%, $n = 8$, $P < 0.05$). A residual inhibition was observed at 24 h (26 ± 8%) which was significant with respect to pre-treatment value ($P < 0.05$) but not with respect to the control group ($P = 0.073$).

3.5. Effects on blood pressure and heart rate in conscious spontaneously hypertensive rats

Pretreatment values of mean arterial pressure and heart rate were similar in all three treatment groups, i.e. mean arterial pressure vehicle group 164 ± 7 mm Hg, SL65.0472 group 162 ± 3 mm Hg, prazosin group 165 ± 2 mm Hg, heart rate vehicle group 346 ± 9 beats/min, SL65.0472

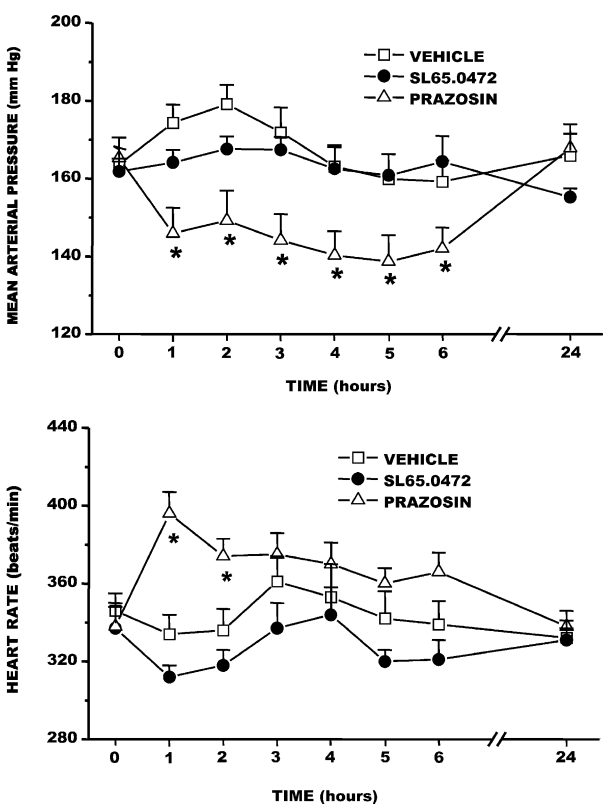

Fig. 5. Effects of SL65.0472 (0.5 mg/kg p.o.), prazosin (1 mg/kg p.o.) or vehicle on mean arterial blood pressure and heart rate of conscious spontaneously hypertensive rats. Parameters were recorded at regular intervals during the 24-h period following administration. Values are means ± S.E.M., $n = 7–8$. $^{*}P < 0.05$ versus vehicle group.

group 337 ± 13 beats/min, prazosin group 337 ± 10 beats/min. Administered at a dose of 0.5 mg/kg p.o., SL65.0472 did not modify either mean arterial pressure or heart rate of spontaneously hypertensive rats during the 24 h period following oral dosing. In contrast, prazosin 1 mg/kg p.o. lowered mean arterial pressure between 1 and 6 h, these effects being associated with an increase in heart rate (Fig. 5), demonstrating the sensitivity of the model.

4. Discussion

A full haemodynamic profile of SL65.0472 was performed in anaesthetised dogs. No statistically significant modifications were observed in any of the multiple parameters of cardiac or vascular function monitored at 0.3 mg/kg i.v., which is 10 times greater than the dose necessary to produce maximal pharmacological activity in anaesthetised dogs (present study and O'Connor et al., 2000). At 1 mg/kg i.v., SL65.0472 produced moderate hypotensive effects which appeared to be due to systemic vasodilation since peripheral resistance tended to be reduced and cardiac output was maintained. Throughout the dose range studied SL65.0472 did not modify the ECG and notably did not change the QTc interval which measures cardiac repolarisation. Ketanserin, the prototype 5-HT_2 receptor antagonist, slows cardiac repolarisation in man (Nademanee et al., 1987). Indeed, this effect may limit the clinical utility of ketanserin because a harmful interaction with diuretics and antiarrhythmic agents has been demonstrated which reduced the effectiveness of ketanserin in preventing atherosclerotic complications in subjects with peripheral arterial disease (Prevention of Atherosclerotic Complications with Ketanserin (PACK) Trial Group, 1989). Animal data is consistent with an effect of ketanserin on repolarisation since this drug prolongs action potential duration in canine Purkinje fibre preparations and increases QT interval in paced anaesthetised dogs starting from 0.2 mg/kg i.v. (Zaza et al., 1989).

I.v. administration of sumatriptan produces marked saphenous venoconstriction in the anaesthetised dog, indeed it seems that this activity is amongst the most potent in vivo effects reported for this drug (Drieu la Rochelle and O'Connor, 1995) and probably occurs at lower doses than the well characterised constriction of the carotid vascular bed observed in the dog (Feniuk et al., 1989) and in the pig (Den Boer et al., 1991). In our initial characterisation of this model we demonstrated that sumatriptan-induced decreases in saphenous vein diameter were unaffected by ketanserin (0.3 mg/kg i.v.) but were significantly antagonised by the non-selective 5-HT_1 receptor antagonist, methiothepin (0.3 mg/kg i.v.) (Drieu la Rochelle and O'Connor, 1995). Reverse transcriptase polymerase chain reaction studies using canine saphenous veins obtained from our own laboratory have demonstrated abundant expression of 5-HT_{1B} receptor mRNA but an absence of 5-HT_{1D} receptor mRNA (Sgard et al., 1996), thus supporting the 5-HT_{1B} receptor characterisation of this model. SL65.0472 antagonises sumatriptan-induced saphenous vasoconstriction over the dose range 3–30 μg/kg i.v. Although direct comparisons are not possible (because of differences in the protocol) these data suggests that SL65.0472 is at least as potent as methiothepin to block 5-HT_{1B} receptors in vivo in this model.

5-HT causes a prominent pressor response in anaesthetised pithed rats which is antagonised by 5-HT_{2A} receptor antagonists such as ketanserin (Fozard, 1982). SL65.0472 is a potent antagonist of 5-HT induced pressor responses in this model following i.v. and oral administration. In addition, its duration of action is long by the oral route because following administration of SL65.0472 (0.1 mg/kg p.o.) to conscious rats, 5-HT pressor responses were substantially reduced at 6 h and still modestly inhibited 24 h after administration. Taking account of data from canine saphenous vein diameter and rat blood pressure models it is apparent that SL65.0472 is a potent antagonist of both 5-HT_{1B} receptors and 5-HT_{2A} receptors in vivo. Direct comparison of the ID_{50} values in the two models (10.1 μg/kg i.v. versus 1.4 μg/kg i.v.) suggests a modest preference for 5-HT_{2A} receptors, however, in view of the differences between the two models (species, protocol, etc) it would be unwise to overemphasise this. In addition, we have demonstrated previously (O'Connor et al., 2000) that in an anaesthetised dog coronary thrombosis model SL65.0472 (30 μg/kg i.v.) produces a near maximal antithrombotic effect (which is almost certainly 5-HT_{2A} receptor mediated). In the present study, in the dog saphenous venoconstriction model, the same dose of SL65.0472 produces near-maximal inhibition of 5-HT_{1B} responses.

During studies in the anaesthetised dog saphenous vein diameter and pithed rat models SL65.0472 did not produce significant haemodynamic effects, even at doses capable of blocking 5-HT_{1B} or 5-HT_{2A} responses almost maximally. This suggests that in the animal models used there is no significant endogenous serotoninergic cardiovascular tone mediated via 5-HT_{1B} and 5-HT_{2A} receptors. This was largely anticipated since intravascular 5-HT release usually only occurs locally as a result of platelet activation at sites of vascular damage. More significantly, the administration of SL65.0472 had no direct effect on saphenous vein diameter in the anaesthetised dog or on mean arterial pressure in the pithed rat suggesting that it is devoid of partial agonist properties. This is in contrast to the 5-$HT_{1B/1D}$ receptor antagonist GR 127935 which has been reported to have some intrinsic activity (Watson et al., 1995) and which following i.v. administration in vivo increases porcine carotid vascular resistance (De Vries et al., 1996).

SL65.0472 0.5 mg/kg p.o., a dose which is 5-fold greater than that necessary to produce near-maximal inhibition of 5-HT_{2A}-mediated vasoconstriction in the rat, did not reduce the blood pressure of spontaneously hyperten-

sive rats. This result is consistent with the majority of the literature which demonstrates that 5-HT_2 receptor antagonism does not translate into an antihypertensive effect in spontaneously hypertensive rats (Cohen et al., 1983; Pettersson et al., 1985). Although ketanserin lowers blood pressure in animals and man (Van Nueten et al., 1981) it seems likely that this activity is related to the moderate α-adrenoceptor antagonist properties of ketanserin (Fozard, 1982; Pettersson et al., 1985).

There is accumulating evidence that 5-HT_{1B} receptors mediate vasoconstriction in man. In addition to human isolated tissue studies demonstrating their role in the contraction of coronary (Kaumann et al., 1994) or pulmonary arteries (MacLean et al., 1996), administration of the $5HT_{1B/1D}$ agonist sumatriptan causes increases in blood pressure, pulmonary and systemic vascular resistance and reduces coronary artery diameter in man (MacIntyre et al., 1992, 1993). By virtue of its ability to block vasoconstriction and thrombotic episodes mediated through stimulation of both 5-HT_{1B} and 5-HT_{2A} receptors, SL65.0472 could prove to be useful in the treatment of coronary artery disease. SL65.0472 is a potent antagonist of 5-HT-induced contractions of human coronary arteries (Galzin et al., 2000) and shows antithrombotic activity in animal models (Berry et al., 2000). Patients with unstable angina or those undergoing coronary artery bypass grafts, thrombolysis or angioplasty, risk suffering cardiac events as a result of coronary artery thrombosis or vasospasm at the site of ruptured atherosclerotic plaques or damaged vascular endothelium. In man, coronary sinus plasma concentrations of 5-HT are substantially elevated following coronary angioplasty (Golilno et al., 1994). It is probable that 5-HT, released locally in the coronary circulation in subjects with acute coronary syndromes, activates 5-HT_{1B} and 5-HT_{2A} receptors resulting in vasospasm and coronary artery thrombosis and, thereby, may contribute to the incidence of cardiac events. A recent analysis of risk factors in coronary artery disease patients supports a significant role of 5-HT (Vikenes et al., 1999).

In conclusion, we have demonstrated that SL65.0472, a novel 5-HT receptor antagonist, shows minimal direct haemodynamic effects and potently antagonises vasoconstriction mediated by 5-HT_{1B} and 5-HT_{2A} receptors in vivo.

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