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Serotonin aggravates exercise-induced cardiac ischemia in the dog: effect of serotonin receptor antagonists

Frédérique Guilbert, Pierre Lainée, Brigitte Dubreuil, Gary McCort, Stephen E. O'Connor*, Philip Janiak, Jean-Marc Herbert

Cardiovascular-Thrombosis Research Department, Sanofi-Synthélabo Research, 1 Avenue Pierre Brossolette, 91385 Chilly-Mazarin Cedex, France

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Abstract

We investigated the effects of serotonin (5-HT), SL65.0472 (7-fluoro-2-oxo-4-[2-[4-thieno[3,2-*c*]pyridine-4-yl)piperazin-1-yl]ethyl]-1,2-dihydroquinoline-1-acetamide, a 5-HT_{1B}/5-HT_{2A} receptor antagonist) and ketanserin (a 5-HT_{2A} receptor antagonist) during exercise-induced cardiac ischemia in conscious dogs. Dogs were administered a hypercholesterolemic diet and an inhibitor of nitric oxide synthetase to produce chronic endothelial dysfunction. Myocardial ischemia was induced by a treadmill exercise test associated with limitation of left anterior descending coronary blood flow. Infusion of serotonin during exercise produced dose-related cardiovascular changes (after 10 μg/kg/min; heart rate +27±6 bpm, systolic blood pressure +18±3 mm Hg, left circumflex coronary blood flow +64±8 ml/min, myocardial segment length shortening in the ischemic zone −5.9±1.9%, $P<0.05$). SL65.0472 blocked serotonin-induced increases in blood pressure, rate pressure product and circumflex coronary artery flow (100 μg/kg i.v., $P<0.05$) and reduced serotonin-induced ischemic myocardial segment length shortening (300 μg/kg i.v., $P<0.05$). Ketanserin (30–300 μg/kg i.v.) had no significant effect on any serotonin-induced changes during exercise. Thus, SL65.0472 opposes serotonin-induced myocardial dysfunction in a dog model of exercise-induced ischemia.

Keywords: Serotonin; Exercise-induced cardiac ischemia; 5-HT_{1B} receptor; 5-HT_{2A} receptor; SL65.0472; Ketanserin

1. Introduction

Serotonin (5-HT), released from activated platelets and capable of causing vasospasm and thrombosis, may play a significant role in cardiovascular diseases. In man, following coronary angioplasty, high local concentrations of 5-HT have been identified at the level of the coronary sinus (Golino et al., 1994). Platelet serotonin levels have been demonstrated to be a significant risk factor for coronary artery disease and cardiac events (Vikenes et al., 1999).

Clinical and experimental studies indicate that 5-HT may cause either vasodilation or vasoconstriction depending on endothelial status and the presence or absence of underlying pathologies. 5-HT dilates healthy arteries mostly by an endothelium-dependent mechanism (Cocks and Angus, 1983). Consistently, 5-HT-induced vasoconstriction is favoured in experimental pathological models reproducing endothelial dysfunction, stable angina or atherosclerosis (Woodman, 1990; Geerts et al., 1999; Ishida et al., 2001) and is exacerbated after mechanical endothelial denudation or inhibition of nitric oxide synthetase (Lamping et al., 1985; Woodman and Dusting, 1994). In atherosclerotic monkeys, this enhanced constriction of resistance coronary arteries to 5-HT is believed to be related to endothelial dysfunction of resistance arteries downstream of the atherosclerotic lesions (Heistad et al., 1984). Molecular biological and functional studies have demonstrated that two receptor subtypes, 5-HT_{1B} and 5-HT_{2A} receptors, are present on human and dog coronary arteries (Ishida et al., 1999; Nilsson et al., 1999) and contribute significantly to the coronary vasoconstriction induced by 5-HT in these and

* Corresponding author. Tel.: +33 1 69 79 78 06; fax: +33 1 69 79 78 10.
E-mail address: stephen-eric.o'connor@sanofi-synthelabo.com (S.E. O'Connor).

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other species (Nilsson et al., 1999; Chester et al., 1990; Kaumann et al., 1994). Both 5-HT_{1B} and 5-HT_{2A} receptors have been reported to be upregulated and mediate vascular hyperreactivity to 5-HT under conditions of endothelial dysfunction, ischemia or hypercholesterolemia (Kadokami et al., 1996; Komiyama et al., 2001; Feletou et al., 1994; Janiak et al., 2002).

We chose to investigate the role of 5-HT in a complex, integrated model of cardiac ischemia induced by the combination of treadmill exercise and limiting coronary stenosis in chronically instrumented conscious dogs. The purpose of our study was to determine, in the setting of hypercholesterolemia and endothelial dysfunction, whether 5-HT would aggravate exercice-induced myocardial ischemia, and, if so, whether this detrimental effect could be inhibited by SL65.0472 (7-fluoro-2-oxo-4-[2-[4-thieno[3,2-*c*]pyridine-4-yl)piperazin-1-yl]ethyl]-1,2-dihydro-quinoline-1-acetamide), a mixed 5-HT_{1B}/5-HT_{2A} receptor antagonist (Galzin et al., 2000; O'Connor et al., 2001) by comparison with ketanserin, a selective 5-HT_{2A} receptor antagonist.

2. Materials and methods

This study was performed in accordance with the European Community Standards on the Care and Use of Laboratory Animals and approved by the Animal Care and Use committee of Sanofi-Synthelabo Recherche.

2.1. Surgical procedure

Female mongrel dogs (Marshall Farm, USA) weighing 19–30 kg were used for these experiments. Surgical implantation was performed under aseptic conditions. Anesthesia was induced with thiopental (25 mg/kg, i.v. bolus), allowing tracheal intubation and artificial ventilation with 1% isoflurane (Forène®, Centravet, France) in a mixture of 1/3 oxygen and 2/3 nitric dioxide. Muscle paralysis was induced with pancurarium bromide (Pavulon®, Organon Teknika - 0.25 mg/kg i.v.). A left thoracotomy was performed in the fifth intercostal space and the heart was suspended in a pericardial cradle. Two Doppler flow probes (10 MHz, Crystal Biotech, Hopkinton, MA, USA) were placed around the left anterior descending and the left circumflex coronary arteries. A 2 mm diameter pneumatic occluder (Ealing, Les Ulis, France) was placed around the left anterior descending coronary artery proximally to the flow probe in order to control coronary flow toward the distal myocardium. A pair of 5 MHz piezoelectric crystals (Coyote Bay, Manchester, NH, USA) was implanted in the myocardium distally to and in the vicinity of the left anterior descending coronary artery flow probe to measure myocardial segment length shortening in the area perfused by the left anterior descending coronary artery. A telemetric implant (Data Sciences International, St. Paul, MN, USA) was inserted in a retrocostal pouch to monitor left ventricular pressure and arterial pressure, via catheters introduced in the apex of the left ventricle and in the thoracic descending aorta and epicardial electrocardiogram (ECG) (leads sutured to myocardium). The left atrium and left jugular vein were catheterized with Silastic® tubing for 5-HT infusions and drug administration, respectively. Muscular layers were sutured, the pneumothorax was evacuated through the last intercostal muscular space and all cables and catheters were exteriorized on the back of the dog.

Post-surgical antibiotic therapy was provided for 10 days by the combination of penicillin (Duphapen LA®, Fort Dodge Santé Animale, France) and gentamycin (Septigen 10®, Shering Plough Santé Animale, France), respectively. Body temperature and weight were checked and catheters flushed every 2 days.

After a 10-day recovery period, dogs instrumented for cardiac hemodynamic measurements were submitted to a hypercholesterolemic diet (JADE 21 Extrude, 5% cholesterol - 17% coco oil) and chronic inhibition of nitric oxide synthase by L-NAME (*N*-nitro-L-arginine-methyl-ester) (10 mg/kg/day s.c.). A 3-week delay was allowed before the start of the experiments.

2.2. Hemodynamic measurements

On the day of the experiment, epicardial ECG, arterial pressure, left ventricular pressure, left anterior descending and left circumflex coronary blood flow velocities, and segment length shortening were recorded continuously (Augury software, Coyote Bay, Manchester, NH, USA). Left ventricular end-diastolic pressure and ST segment depression were derived from the left ventricular pressure and ECG recordings respectively. Heart rate was measured with a cardiotachometer triggered by the left ventricular pressure pulse. Rate-pressure product, an index of oxygen consumption, was defined as the product of systolic arterial pressure and heart rate. The minimal (dP/dt min) and the maximal (dP/dt max) first derivatives of the left ventricular pressure signal were calculated. Mean blood flow was calculated from blood flow velocity by application of a standard formula (mean flow=constant $(1.25)\times\text{radius}^2$ (mm)×velocity (KHz)). The shortening fraction of the ischemic myocardium was defined as ([end diastolic length−end systolic length]/end diastolic length)×100.

2.3. Experimental protocol

Coronary endothelial dysfunction was evaluated by monitoring coronary blood flow after intravenous bolus injection of acetylcholine (0.3 μg/kg) versus nitroglycerin (10 μg/kg) in a subgroup of dogs before and after cholesterol+L-NAME treatment.

A first set of experiments was conducted to evaluate the cardiac effects of 5-HT during exercise-induced ischemia. Myocardial ischemia was induced by the combination of a 5-min treadmill test (7 km/h with a 5% slope) and inflation of the pneumatic occluder to limit left anterior descending coronary blood flow to the baseline value measured at rest. For clarity, future use of the word "exercise" in the context of our studies implies exercise performed with limited coronary blood flow. After the first 2 min of exercise, 5-HT or saline was perfused into the left atrium for the three remaining minutes. Perfusion and exercise were then stopped and the coronary artery occlusion was relieved. Each experiment included a warm-up run, and four successive exercise periods performed with saline, 1, 3 and 10 μg/kg/min 5-HT infusions, respectively. A recovery period of at least 30 min was allowed between each treadmill test.

In the second series of experiments, we investigated the ability of ketanserin, a 5-HT_{2A} receptor antagonist, and SL65.0472, a mixed 5-HT_{1B}/5-HT_{2A} receptor antagonist, to counteract the adverse effects of 5-HT infused during exercise. Each experiment included 4 successive exercise periods with a 3 μg/kg/min 5-HT infusion, performed 10 min after i.v. injections of saline, 30, 100 and 300 μg/kg of the antagonist. Dogs underwent three sets of experiments with ketanserin, SL65.0472 or vehicle (NaCl 0.9%) given in a randomized order.

2.4. Drugs

Nitroglycerin (Lenitral®) was purchased from Besins-Iscovesco (France). Acetylcholine, L-NAME and 5-HT were obtained from Sigma (St. Louis, USA). Ketanserin and SL65.0472 (7-fluoro-2-oxo-4-[2-[4-(thieno[3,2-*c*]pyrin-4-yl) piperazin-1-yl]ethyl]-1,2-dihydroquinoline-acetamide) were synthesized at Sanofi-Synthelabo Recherche (Chilly Mazarin, France).

2.5. Statistical analysis

Data are expressed as mean values±S.E.M. Mean values were calculated at rest after a 30-min stabilization period before the treadmill test, after 2 min of exercise and at the end of 5-HT infusion performed during the three remaining minutes of exercise. The changes induced by the injections of acetylcholine and nitroglycerin were tested by unpaired *t*-test. Effects of 5-HT during treadmill tests were compared to saline infusion by one-way analysis of variance for repeated measures. The effects of 5-HT receptor antagonists were evaluated with a two-way analysis of variance for repeated measures. These statistical analyses were performed with a software developed by the Biostatistics service of Sanofi-Synthélabo Research (Chilly Mazarin, France).

3. Results

3.1. Hypercholesterolemia and endothelial dysfunction

Under hypercholesterolemic diet, plasma cholesterol levels were raised by 54% (from 269±29 mg/dl to 415±45 mg/dl, $P<0.05$). Chronic treatment with L-NAME significantly reduced endothelium-dependent relaxation induced by acetylcholine as the coronary blood flow increase was limited to +54±6% in dogs receiving L-NAME versus +124±36% in control dogs ($P<0.05$). The

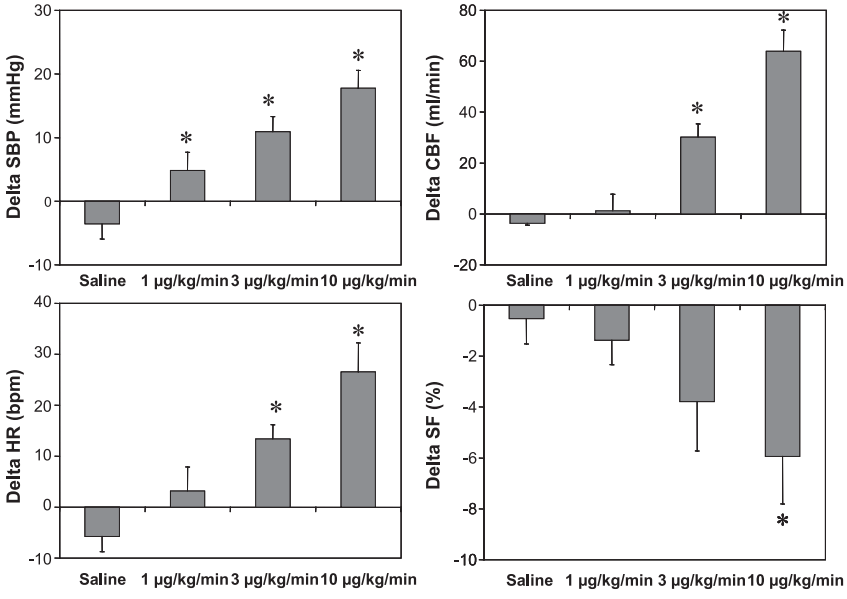

Fig. 1. Changes in systolic blood pressure (SBP), heart rate (HR), coronary blood flow (CBF) in the non-ischemic zone and myocardial shortening fraction (SF) in the ischemic zone, induced by saline or 5-HT (1, 3, 10 μg/kg/min) infused during treadmill exercise in the presence of a limiting coronary stenosis in conscious dogs. *$P<0.05$ versus saline, means±S.E.M., $n=11$.

nitroglycerin-induced increase in coronary blood flow was not significantly modified by L-NAME (+137±11% in L-NAME group versus +108±23% in control group, ns).

3.2. Exercise-induced myocardial ischemia

Exercise produced marked hemodynamic changes which were reproducible each time the protocol was repeated. Representative examples of the changes observed, taken from the first exercise period (5-HT dose-ranging experiments), are described below. Exercise produced rapid and sustained increases in heart rate (156±8 versus 68±5 bpm, $P<0.05$), systolic arterial pressure (153±7 versus 120±5 mm Hg, $P<0.05$), left circumflex coronary blood flow (59±3 versus 28±2 ml/min, $P<0.05$) and dP/dt max (2788±277 versus 2169±217, $P<0.05$). Cardiac work was increased, as assessed by the elevation of the rate-pressure product (23880±1778 versus 8394±826 mmHg.bpm $P<0.05$). Myocardial ischemia resulting from the combination of treadmill exercise plus limitation of left anterior descending coronary artery flow was characterized by a significant reduction of the shortening fraction measured in the hypoperfused area (11.1±1.2% versus 19.2±1.4% at rest $P<0.05$) and an elevation of left ventricular end diastolic pressure (15.1±6.6 versus 4.7±2.1 mm Hg, $P<0.05$).

3.3. Effects of 5-HT on exercise-induced myocardial ischemia

The effects of a 5-HT infusion during exercise are shown in Fig. 1. Compared to saline, 5-HT dose-dependently aggravated regional contractility in the ischemic zone as shown by the reduction in shortening fraction. Moreover, 5-HT increased heart rate and systolic arterial pressure after 3 and 10 μg/kg/min, thus inducing an increase in the rate-pressure product. A significant increase in left circumflex coronary blood flow was also observed after 3 and 10 μg/kg/min.

As we found that 5-HT infused at 10 μg/kg/min promoted ventricular arrhythmias in some dogs, the dose of 3 μg/kg/min was selected for the second study designed to evaluate the efficacy of 5-HT receptor antagonists. Hemodynamic changes induced by 5-HT 3 μg/kg/min infusion were similar to those described for the first study. A significant increase in rate pressure product and left

Table 1
Reproducibility of the effects of exercise and 5-HT infusion

		1st exercise	2nd exercise	3rd exercise	4th exercise
HR(bpm)	Baseline	74±6	75±10	71±7	71±7
	Exercise	167±8[a]	168±7[a]	156±6[a]	159±7[a]
	Exercise+5-HT	175±7[a]	179±12[a]	174±7[a]	175±8[a]
SBP (mm Hg)	Baseline	110±3	101±5	109±3	106±6
	Exercise	139±2[a]	140±3[a]	137±3[a]	140±3[a]
	Exercise+5-HT	145±4[a]	149±5[a]	146±5[a,b]	148±3[a]
CBF_{ICX} (ml/min)	Baseline	32±4	38±4	33±4	35±3
	Exercise	62±6[a]	73±5[a]	62±5[a]	65±5[a]
	Exercise+5-HT	83±9[a,b]	97±12[a,b]	86±9[a,b]	87±9[a,b]
SF (%)	Baseline	18.1±1.5	16.5±1.8	17.5±1.5	16.7±1.3
	Exercise	11.3±1.7[a]	11.3±1.1[a]	11.6±1.3[a]	11.4±1.1[a]
	Exercise+5-HT	6.8±1.7[a,b]	9.0±2.3[a]	7.6±1.5[a,b]	7.7±1.3[a,b]
ST segment (mV)	Baseline	−0.03±0.18	−0.12±0.23	0.02±0.18	−0.05±0.18
	Exercise	−0.39±0.13	0.51±0.15	0.39±0.12	−0.42±0.12
	Exercise+5-HT	−0.91±0.28[a]	−0.89±0.35[a]	−0.90±0.28[a]	−0.91±0.32[a]
dP/dt max (mm Hg/s)	Baseline	2384±59	2324±194	2318±96	2173±134
	Exercise	3315±43[a]	3319±78[a]	3367±107[a]	3380±86[a]
	Exercise+5-HT	3307±78[a]	3234±69[a]	3447±111[a]	3354±113[a]
dP/dt min (mm Hg/s)	Baseline	−1928±72	−1809±170	−1856±98	−1781±150
	Exercise	−2890±113[a]	−2862±157[a]	−2750±118[a]	−2807±88[a]
	Exercise+5-HT	−3059±131[a]	−3043±165[a]	−3052±188[a,b]	−2992±123[a]
LVEDP (mm Hg)	Baseline	7.3±1.8	8.3±3.0	6.6±2.2	7.1±2.1
	Exercise	18.8±4.2[a]	23.0±6.3[a]	16.5±3.6[a]	21.4±5.2[a]
	Exercise+5-HT	28.1±8.1[a]	29.1±10.4[a]	25.3±7.7[a]	28.2±8.5[a]
RPP (mm Hg bpm)	Baseline	8136±749	7783±1362	7767±819	7581±1006
	Exercise	23,152±1298[a]	23,377±996[a]	21,278±1021[a]	22,288±1071[a]
	Exercise+5-HT	25,497±1400[a]	26,670±1992[a]	25,432±1371[a,b]	25,826±1406[a,b]

Hemodynamic parameters measured at baseline and during exercise-induced ischemia before and at the end of 5-HT (3 μg/kg/min) infusion. The same protocol was performed four times separated by a recovery period of at least 30 min.

HR: heart rate; SBP: systolic blood pressure; SF_{lad}: shortening fraction; CBF_{ICX}: left circumflex coronary artery blood flow; dP/dt max: maximal first derivative of left ventricular pressure; dP/dt min: minimal first derivative of left ventricular pressure; LVEDP: left ventricular end-diastolic pressure; RPP: rate-pressure product. Saline was administered i.v. 10 min before each exercise/5HT protocol. Means±S.E.M. n=7.

[a] $P<0.05$ versus baseline.

[b] $P<0.05$ versus exercise.

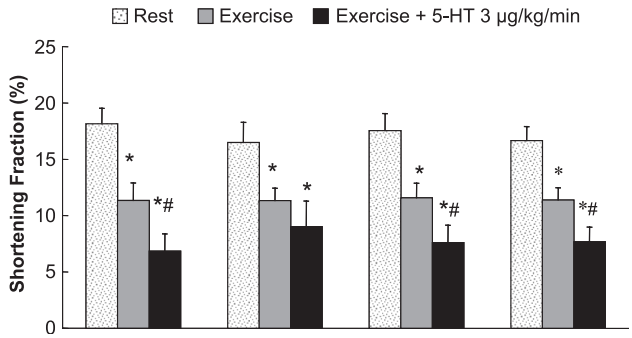

Fig. 2. Changes in myocardial shortening fraction measured in the zone perfused by the left anterior descending coronary artery in conscious dogs under three different experimental conditions; at rest, during treadmill exercise (with a limiting coronary stenosis) and during exercise performed in association with an infusion of 5-HT (3 μg/kg/min). This protocol was performed four times with a recovery period of at least 30 min between each exercise test. Values are means±S.E.M., n=7; *P<0.05 versus rest; #P<0.05 versus exercise.

circumflex coronary flow associated with a significant decrease in shortening fraction was observed (Table 1). Consistent with the presence of regional contractile dysfunction, a significant ST segment depression was observed after 5-HT infusion as compared to baseline resting values.

No ischemic preconditioning was observed over the repeated treadmill tests, as cardiac ischemia, reflected by a significant decrease in shortening fraction and a ST segment shift was identical during each of the 4 successive exercises performed in the vehicle experiment (Table 1, Fig. 2). The deleterious cardiovascular effects of 5-HT infusion (3 μg/kg/min) on shortening fraction were also reproducible over four repeated administrations (Table 1, Fig. 2).

3.4. Effects of 5-HT receptor antagonists

At rest and without coronary stenosis, ketanserin, but not SL65.0472, produced a slight increase in the rate-pressure product, which reached significance only after 100 μg/kg. This effect was due to a moderate tachycardia, statistically significant at 100 and 300 μg/kg, arterial pressure remaining unchanged at these doses (Table 3). Neither ketanserin nor SL65.0472 altered shortening fraction, left ventricular end diastolic pressure, ST segment and coronary blood flow. dP/dt max was significantly increased after ketanserin or SL65.0472 (100 and 300 μg/kg) (Table 2).

Administration of ketanserin or SL65.0472 during the exercise period did not modify myocardial ischemia, as measured by the variations in shortening fraction and of

Table 2
Hemodynamic effects of ketanserin and SL65.0472

		Baseline value	30 μg/kg i.v. Δ	100 μg/kg i.v. Δ	300 μg/kg i.v. Δ
HR (bpm)	Vehicle	74±6	−3±3	−3±3	−3±2
	Ketanserin	77±6	5±5	19±8[a]	25±2[a]
	SL65.0472	75±5	−2±4	7±4	14±16
SBP (mm Hg)	Vehicle	110±3	−8±5	−1±2	−4±5
	Ketanserin	118±5	2±3	5±5	−5±6
	SL65.0472	118±5	1±6	6±3	2±2
CBF_{lcx} (ml/min)	Vehicle	32±4	2±2	1±2	3±2
	Ketanserin	38±7	1±3	3±2	1±8
	SL65.0472	36±4	2±1	3±1	2±4
SF (%)	Vehicle	18.1±1.4	−1.1±0.5	−0.5±0.9	−1.3±0.6
	Ketanserin	16.3±1.9	0.7±0.8	1.2±1.0	2.0±1.7
	SL65.0472	16.9±1.6	0.6±1.0	1.4±0.8	1.5±1.4
ST segment (mV)	Vehicle	−0.03±0.18	−0.07±0.04	0.00±0.02	−0.03±0.03
	Ketanserin	−0.14±0.19	−0.02±0.02	−0.06±0.05	−0.06±0.05
	SL65.0472	−0.07±0.18	−0.03±0.03	−0.01±0.02	−0.06±0.09
dP/dt max (mm Hg/s)	Vehicle	2384±59	−115±221	−75±93	−217±127
	Ketanserin	2542±125	166±100	367±72[a]	284±176[a]
	SL65.0472	2493±170	126±123	245±90[a]	152±96[a]
dP/dt min (mm Hg/s)	Vehicle	−1928±72	130±115	51±72	126±103
	Ketanserin	−2157±100	−137±44	−187±51	−204±156[a]
	SL65.0472	−2198±152	−104±145	−254±92	−276±121[a]
LVEDP (mm Hg)	Vehicle	7.3±1.8	0.4±1.2	−0.1±2.1	0.4±1.0
	Ketanserin	8.4±1.1	−0.4±0.9	1.0±0.8	−3.5±2.1
	SL65.0472	7.8±1.0	−1.0±0.7	−0.7±0.6	−1.4±1.3
RPP (mm Hg bpm)	Vehicle	8136±749	−761±601	−382±430	−568±513
	Ketanserin	9106±866	815±681	2847±1237[a]	2286±661
	SL65.0472	8854±729	−148±796	1187±579	1854±1868

Absolute changes in hemodynamic parameters measured at rest after ketanserin, SL65.0472 or vehicle (saline) administration.
HR: heart rate; SBP: systolic blood pressure; SF_{lad}: shortening fraction; $SBFI_{CX}$: left circumflex coronary artery blood flow; dP/dt max: maximal first derivative of left ventricular pressure; dP/dt min: minimal first derivative of left ventricular pressure; LVEDP: left ventricular end-diastolic pressure; RPP: rate-pressure product. Means±S.E.M., n=6–11.
[a] P<0.05 versus control group.

the ST segment (data not shown). Heart rate during exercise was increased after ketanserin administered at 100 μg/kg (177±6 versus 156±6 bpm in the control group, $P<0.05$) and at 300 μg/kg (191±9 versus 159±7 bpm, $P<0.05$). Similarly, a higher heart rate was recorded during exercise at 300 μg/kg in the SL65.0472 group (186±5 versus 159±7 bpm in the control group, $P<0.05$). No significant changes were observed in systolic arterial pressure, left circumflex coronary flow, left ventricular pressure, dP/dt max or dP/dt min recorded during exercise in ketanserin- or SL65.0472-treated groups as compared to the control group.

SL65.0472 inhibited 5-HT-mediated aggravation of exercise-induced myocardial ischemia since it reduced, in a dose-dependent manner, the ischemia-related change of the myocardial shortening fraction (Table 3, Fig. 3). The 5-HT-induced increase in left circumflex coronary blood flow was significantly antagonized by SL65.0472 at 100 and 300 μg/kg. The elevation of systolic arterial pressure and rate-pressure product induced by 5-HT were limited by SL65.0472, but this effect reached significance only after 100 μg/kg (Table 3, Fig. 3).

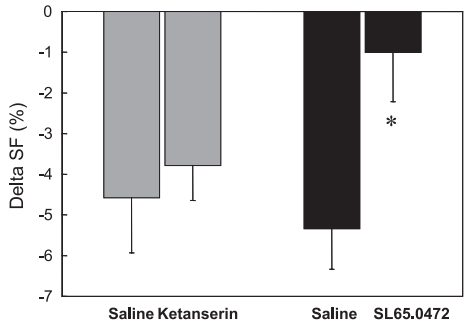

Fig. 3. Effect of ketanserin (300 μg/kg i.v.), SL65.0472 (300 μg/kg i.v.) or their saline controls on 5-HT (3 μg/kg/min)-induced changes in myocardial shortening fraction (SF) measured in conscious dogs during treadmill exercise and limiting coronary stenosis. Values are means± S.E.M., n=6; *$P<0.05$ versus saline administration performed during the same experiment.

In contrast, ketanserin did not prevent the deterioration of regional contractility (shortening fraction) induced by 5-HT in the hypoperfused myocardium. 5-HT induced increases in left circumflex coronary blood flow, heart rate and rate-pressure product were not significantly modified by ketanserin (Table 3, Fig. 3).

Table 3
Effects of SL65.0472 (30–300 μg/kg i.v.), ketanserin (30–300 μg/kg i.v.) or vehicle (saline) on hemodynamic changes induced by intra-atrial infusion of 5-HT (3 μg/kg/min) during exercise

		Hemodynamic changes produced by 5-HT 3 μg/kg/min			
Dose of antagonist		Saline	30 μg/kg i.v.	100 μg/kg i.v.	300 μg/kg i.v.
HR (bpm)	Vehicle	9±5	12±7	18±6	16±6
	Ketanserin	16±6	23±7	32±9	2±12
	SL65.0472	21±5	24±4	13±7	12±5
SBP (mm Hg)	Vehicle	7±3	9±3	10±3	8±4
	Ketanserin	9±2	2±2	0±5	0±5
	SL65.0472	12±3	5±5	0±2[a]	2±3
CBF_{ICX} (ml/min)	Vehicle	21±5	24±8	24±6	22±5
	Ketanserin	28±9	30±9	24±6	15±6
	SL65.0472	31±7	19±5	5±3[a]	3±2[a]
SF (%)	Vehicle	−4.5±1.1	−2.3±1.7	−4.0±1.1	−3.7±1.2
	Ketanserin	−4.6±1.4	−3.7±1.3	−5.1±1.3	−3.8±0.9
	SL65.0472	−5.3±1.0	−3.3±1.5	−2.9±1.1	−1.0±1.2[a]
ST segment (mV)	Vehicle	−0.52±0.23	−0.38±0.22	−0.36±0.23	−0.48±0.23
	Ketanserin	−0.25±0.17	−0.24±0.19	−0.37±0.17	−0.77±0.23
	SL65.0472	−0.32±0.14	−0.26±0.23	−0.21±0.12	−0.19±0.04
dP/dt max (mm Hg/s)	Vehicle	−8±68	−85±31	95±57	−26±109
	Ketanserin	73±60	−11±27	−77±83	−190±100
	SL65.0472	10±58	−19±78	−45±28	−9±67
dP/dt max (mm Hg/s)	Vehicle	−169±65	−181±77	−301±73	−185±81
	Ketanserin	−132±26	−224±85	−105±101	1±85
	SL65.0472	−197±43	−164±117	−65±48	−61±85
LVEDP (mmHg)	Vehicle	9.3±4.2	6.1±4.7	8.8±4.8	6.8±3.6
	Ketanserin	7.4±1.3	4.3±2.5	6.2±2.4	1.1±1.0
	SL65.0472	6.3±2.3	2.2±2.3	1.4±1.6	−0.1±2.3
RPP (mm Hg bpm)	Vehicle	2345±882	3293±1192	4154±997	3538±952
	Ketanserin	4060±1206	3971±1209	4739±1759	432±1440
	SL65.0472	5342±1225	4342±1242	1652±1075[a]	2118±988

HR: heart rate; SBP: systolic blood pressure; SF: shortening fraction; CBF_{ICX}: left circumflex coronary artery blood flow; dP/dt max: maximal first derivative of left ventricular pressure; dP/dt min: minimal first derivative of left ventricular pressure; LVEDP: left ventricular end-diastolic pressure; RPP: rate-pressure produce. The 5-HT infusion (+exercise) protocol was performed four times each separated by a recovery period of at least 30 min. Saline or antagonist were administered i.v. 10 min before 5-HT+exercise. Means±S.E.M., n=6–11.

[a] $P<0.05$ versus 5-HT effects after saline injection evaluated at the beginning of the same experiment.

4. Discussion

In this study, treadmill exercise in the presence of flow-limiting stenosis of the left anterior descending coronary artery induced myocardial ischemia characterized by a reduction of shortening fraction and a rise of left ventricular end diastolic pressure, which occurred in conjunction with tachycardia and increases in systolic arterial pressure and left circumflex coronary blood flow. Coronary stenosis required to maintain left anterior descending coronary blood flow constant during exercise promotes, at the distal level, ischemic vasodilatation of coronary resistance arterioles and mobilization of collateral vessels. The combination of coronary stenosis and chronic treatment with a nitric oxide synthetase inhibitor should tend to diminish the maximal coronary vasodilatory reserve. Under these experimental conditions, intra-atrial infusion of 5-HT during exercise aggravated myocardial ischemia as exemplified by a further reduction in shortening fraction, an additional rise in left ventricular end diastolic pressure, a ST segment shift, and hemodynamic changes consistent with increased oxygen demand. In addition, 5-HT accentuated significantly the increase in left circumflex coronary blood flow associated with the treadmill exercise. These cardiovascular effects of 5-HT were dose-dependent and reproducible. These results are consistent with previous studies in dogs indicating that 5-HT infusion causes constriction of large epicardial coronary arteries and collateral vessels, and vasodilation of arterioles (Wright et al., 1992), the latter action being responsible for the observed increase of the coronary blood flow in the normally perfused cardiac area. Collateral vessels have been documented to show high sensitivity to vasoconstriction by 5-HT in models of ischemia performed in rats, rabbits, cats and dogs (Hollenberg, 1992; Bauters et al., 1995). In a rat model of hindlimb ischemia, we have previously reported that ischemic territories exhibited an exacerbated vasoconstriction in response to i.v. injection of 5-HT as compared to the normal contralateral limb (Janiak et al., 2002). This oversensitivity to 5-HT was associated with a sustained upregulation of 5-HT_{1B} and 5-HT_{2A} receptor mRNA in the ischemic limb. The fact that 5-HT administration produced ischemic symptoms such as ST segment depression and regional contractile dysfunction, in parallel with an increase in the left circumflex coronary blood flow irrigating the non-ischemic zone, suggests the existence of a coronary steal phenomenon. Although we did not measure regional coronary blood flow, our data are consistent with those of Bache et al. (1992) who reported that, during exercise performed in the presence of a coronary stenosis, 5-HT worsened the hypoperfusion of the subendocardium although subepicardial perfusion tended to improve. Alternatively, the effects of 5-HT observed in our study could equally be explained by an increase in oxygen consumption secondary to increased cardiac work, associated with coronary collateral vessel constriction.

Since 5-HT_{1B} and 5-HT_{2A} receptor subtypes are involved in coronary vasoconstriction (Nilsson et al., 1999; Chester et al., 1990; Kaumann et al., 1994), antagonism of both receptors may be necessary in the treatment of cardiac ischemia. SL65.0472 is a mixed 5-HT_{1B}/5-HT_{2A} receptor antagonist, orally active with a long duration of action and a low cerebral penetration (Galzin et al., 2000; O'Connor et al., 2001). In vivo, SL65.0472 inhibits vasoconstriction induced by activation of 5-HT_{1B} and 5-HT_{2A} receptors and inhibits thrombus formation in various models of arterial and venous thrombosis via its 5-HT_{2A}-receptor antagonist properties (O'Connor et al., 2001; Berry et al., 2001). More recently, we have reported a beneficial effect of SL65.0472 on distal perfusion after lower limb ischemia in obese diabetic rats (Janiak et al., 2002) and demonstrated that SL65.0472, unlike ketanserin, blocks 5-HT induced vasoconstriction in a dog hindlimb ischemia model (Barbe et al., 2003). The antagonist dose range (30, 100, 300 μg/kg i.v.) selected for this study was based on previous data obtained in the dog and was intended to enable discrimination of a 5-HT_{1B}-receptor mediated effect in vivo. At these doses SL65.0472 causes marked antagonism of 5-HT_{1B} and 5-HT_{2A} responses (O'Connor et al., 2001; Berry et al., 2001; Barbe et al., 2003) whereas the potent 5-HT_{2A} receptor antagonist ketanserin does not modify 5-HT_{1B}-receptor mediated responses in the dog at 300 μg/kg i.v. (Drieu la Rochelle and O'Connor, 1995; Barbe et al., 2003). In animal models, as in man, both 5-HT_{2A} and 5-HT_{1B} receptors are involved in coronary constriction that occurs in the presence of atherosclerosis or endothelial dysfunction (Chester et al., 1990; Kaumann et al., 1994; Kadokami et al., 1996; Feletou et al., 1994). In clinical studies, administration of the 5-HT_{1B} receptor agonist sumatriptan produces coronary, pulmonary and systemic vasoconstriction (Macintyre et al., 1992, 1993). SL65.0472 is a potent antagonist of 5-HT contraction of human isolated coronary arteries (Galzin et al., 2000). In our study it is difficult to establish with certainty the contribution of 5-HT_{2A} receptors to the adverse effects of 5-HT, since the 5-HT_{2A} receptor antagonist, ketanserin, produced no statistically significant inhibition. However, a strong trend was observed with both ketanserin and SL65.0472 to limit 5-HT induced increases in left ventricular end-diastolic pressure and systolic arterial pressure which may indicate a 5-HT_{2A}-receptor mediated component. In constrast to ketanserin, SL65.0472 antagonised 5-HT induced impairement of regional contractility in the ischemic zone. There are several possible explanations for this activity of SL65.0472. It may represent inhibition of 5-HT_{1B} mediated collateral vessel constriction, possibly associated with a more favourable redistribution of coronary blood flow since 5-HT-induced vasodilatation of the non-ischemic region perfused by the left circumflex coronary artery was also blocked by SL65.0472. The tachycardic effect evoked by 5-HT was

not significantly antagonized either by SL65.0472 or by ketanserin. This observation is consistent with Wilson et al. (1990), who reported in conscious dogs that tachycardia induced by 5-HT was mediated through activation of the 5-HT_3 receptor subtype. Neither SL65.0472 (unpublished observations) nor ketanserin (Akuzawa et al., 1995) has any affinity for 5-HT_3 receptors. However, in spite of this, SL65.0472, but not ketanserin, reduced the increase in rate-pressure product caused by 5-HT. This suggests that the capacity of SL65.0472 to limit 5-HT induced myocardial dysfunction in the ischemic zone could be due in part to a limitation of the increased cardiac work and oxygen consumption.

In our experimental setting which combined hypercholesterolemia, chronic inhibition of NOS, and transient coronary stenosis, endogenous 5-HT did not seem to participate to the ischemic process since neither of the 5-HT receptor antagonists alleviated myocardial ischemia induced by limiting left anterior descending coronary blood flow during exercise. The severity and/or duration of the stenosis might not have been sufficient to generate appropriate amounts of 5-HT from activated platelets. Indeed, in the coronary bed, platelet activation induced by rapid atrial pacing occurred only in patients with atherosclerotic stenosis exceeding 50% of lumen size (Diodati et al., 1992). In theory, this phenomenon could contribute to the pathogenesis of stable coronary artery disease. In this context, sarpogrelate, a 5-HT_{2A} receptor antagonist, increased the blood flow reserve of the coronary collateral vessels and hence improved the exercise capacity of patients with stable effort angina, suggesting that endogenous 5-HT released from activated platelets constricted the collaterals perfusing the ischemic myocardium (Tanaka et al., 1998). Besides stable angina, endogenous 5-HT is also recognized to play a major role in animal models of unstable angina. Mechanical damage of the endothelium associated with a fixed coronary stenosis in dogs is known to induce cyclic flow variations, in which high levels of 5-HT released locally at the site of the stenosis promote thrombus formation and vasospasm. Blockade of 5-HT_{2A} receptors by ketanserin or SL65.0472 reduced markedly the frequency of the coronary cyclic flow variations (Berry et al., 2001; Bush, 1987). Although our dogs were chronically submitted to diet-induced hypercholesterolemia, nitric oxide synthetase inhibition and transient limiting coronary stenosis, cyclic blood flow variations were not observed in the present study.

In conclusion, we have characterised the adverse effects of superimposing 5-HT administration on exercise-induced regional myocardial ischemia in conscious dogs fed on a hypercholesterolemic diet and with endothelial dysfunction. Some of these effects of 5-HT are blocked by the mixed 5-HT_{1B}/5-HT_{2A} antagonist SL65.0472, but not by ketanserin, suggesting an involvement of vascular 5-HT_{1B} receptors.

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