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Acute and chronic cardiac and regional haemodynamic effects of the novel bradycardic agent, S16257, in conscious rats

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1 We carried out experiments to assess the cardiac and regional haemodynamic effects of single or repeated injections of the novel bradycardic agent, S16257, (7,8-dimethoxy 3-{3-{[(1S)-(4,5-dimethoxybenzocyclobutan-1-yl)methyl]methylamino}propyl}1,3,4,5-tetrahydro-2*H*-benzapin 2-one), in conscious rats.

2 In the first experiment, male Long Evans rats were chronically instrumented for the measurement of cardiac or regional haemodynamics ($n=9$ in each group), and, on separate experimental days, were randomized to receive i.v. bolus injections of vehicle (5% dextrose) or S16257 at a dose of 1 mg kg^{-1}.

3 In animals instrumented for the measurement of cardiac haemodynamics ($n=9$), following injection of vehicle, there were no immediate changes, and 7-8 h later there were slight reductions in heart rate and mean arterial blood pressure only. Injection of S16257 caused an immediate, transient, pressor effect but thereafter there were reductions in heart rate, mean arterial blood pressure, cardiac index and total peripheral conductance, together with increases in stroke index and peak aortic flow. The integrated decreases in heart rate, mean arterial blood pressure, cardiac index and total peripheral conductance and increases in stroke index, peak aortic flow, dF/dt_{max} and central venous pressure following S16257 were all significantly greater than the changes after vehicle injection. After injection of S16257, the fall in heart rate and fall in cardiac index were not linearly related.

4 In animals instrumented for the measurement of regional haemodynamics ($n=9$). the bradycardic effect of i.v. S16257 was accompanied by reductions in renal, mesenteric and hindquarters blood flows and vascular conductances that were greater than the changes seen following injection of vehicle, but only for the first 1 h. Considering animals instrumented for the measurement of cardiac and regional haemodynamics together, the bradycardic effect of S16257 was greater the higher the resting heart rate.

5 In the second experiment, animals chronically instrumented for the measurement of cardiac or regional haemodynamics ($n=9$ in each group) were given s.c. injections of S16257 (1 mg kg^{-1}) on four consecutive days. The general patterns of change in cardiac and regional haemodynamics following s.c. injection of S16257 were as described above for i.v. injection, although the rates of onset of effects were slower. The bradycardic effect of S16257 was less on the first, than on the subsequent, three days.

6 Overall, these results indicate that the bradycardic action of S16257 is not associated with any signs of negative inotropic action. Only the initial depressor effect of i.v. S16257 is associated with reductions in renal, mesenteric and hindquarters flow and vascular conductance significantly greater than those seen after vehicle injection. With repeated s.c. injection of S16257, there are no signs of desensitization to its bradycardic actions, nor impairment of regional perfusion. If these results extrapolate to the clinical setting, it seems likely that S16257 will have beneficial bradycardic effects, with no concurrent undesirable actions on other aspects of cardiovascular function.

Keywords: Bradycardic agent; S16257; cardiac haemodynamics; regional haemodynamics

Introduction

The development of specific bradycardic agents (Kobinger & Lillie, 1984) offers a new therapeutic strategy in patients with ischaemic heart disease or congestive heart failure, since these drugs can reduce heart rate without impairing cardiac function (e.g., Guth *et al.*, 1987; Krumpl *et al.*, 1988; Van Bogaert *et al.*, 1990; Johnston *et al.*, 1991; Chen & Slinker, 1992; Gout *et al.*, 1992; Furukawa *et al.*, 1992; Breall *et al.*, 1993; Bosmith *et al.*, 1993; Marshall *et al.*, 1993; Wynsen *et al.*, 1994; Rouse & Johnson, 1994; Rouse *et al.*, 1994). One of the first specific bradycardic agents to be developed, namely zatebradine (UL-FS 49; (7,8-dimethoxy 3-{3-{[2-(3,4-dimethoxyphenyl)-ethyl]methylamino}-propyl}1,3,4,5-tetrahydro-2*H*-benzazepin 2-one dihydrochloride) (Kobinger & Lillie, 1984), has structural similarities to verapamil, but causes bradycardia at much lower doses than are needed to reduce contractility of electrically driven atria, or to relax precontracted aorta strips (Kobinger & Lillie, 1984).

Zatebradine has been shown to block the pacemaker current (I_f) in sheep cardiac Purkinje fibres (Van Bogaert *et al.*, 1990), and recently, a new specific bradycardic agent, S16257, (7,8-dimethoxy 3-{3-{[(1S)-(4,5-dimethoxy-benzocyclobutan-1-yl)methyl]methylamino}propyl} 1,3,4,5-tetrahydro-2*H*-benzazepin 2-one), related to zatebradine, was shown to be equipotent with the latter in slowing the diastolic depolarization of rabbit sino-atrial node cells (Thollon *et al.*, 1994). In spite of the profound bradycardic effects these agents can have, little attention has been paid to their integrated cardiac and regional haemodynamic effects *in vivo*, or to the reproducibility of their cardiovascular actions. Although recent studies by Rouse & Johnson (1994) and Rouse *et al.*, (1994) showed that acute central haemodynamic effects of the bradycardic agent, ZD 7288, (which is structurally dissimilar to zatebradine and S16257) were secondary to its bradycardic action in anaesthetized and conscious dogs, there was no assessment of regional haemodynamics, nor of the reproducibility of the effects seen. Therefore, the objectives of the present work were to

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Table 1 Pretreatment resting cardiovascular variable in two groups of Long Evans rats (n = 9 in each): one group was randomized to receive i.v. bolus injections of vehicle or S16257 on different days, the other was given s.c. injections of S16257 on four consecutive days

	Intravenous		*Subcutaneous*			
	Pre-Vehicle	*Pre-S16257*	*Pre-S16257 Day 1*	*Pre-S16257 Day 2*	*Pre-S16257 Day 3*	*Pre-S16257 Day 4*
Heart rate (beats min^{-1})	358±7	347±8	373±7	372±6	361±6	358±7
Mean blood pressure (mmHg)	101±2	101±2	101±2	98±2	98±2	100±2
Cardiac index (ml min^{-1} 100 g^{-1})	23.2±1.4	23.3±1.1	24.7±1.2	27.2±1.3*	28.5±1.3*	27.9±0.9*
Stroke index (μl min^{-1} 100 g^{-1})	65.3±4.5	67.4±3.8	66.5±3.6	73.6±3.9*	79.1±3.4*	78.2±2.6*
Peak aortic flow (ml min^{-1} 100 g^{-1})	97.2±4.8	96.4±4.8	103.3±4.6	109.4±5.4*	114.8±5.4*	113.9±4.5*
dF/dt_{max} (1 min^{-2} 100 g^{-1})	401±24	398±23	436±19	465±20*	487±23*	484±18*
Total peripheral conductance (μl min^{-1} $mmHg^{-1}$ 100 g^{-1})	230±15	229±9	246±12	278±16*	292±14*	279±8*
Central venous pressure (cmH_2O)	4.3±0.4	4.4±0.2	5.6±0.3	5.0±0.2	4.6±0.2	5.0±0.2

Values are mean±s.e.mean; *$P<0.05$ versus Day 1 for the s.c.group.

assess the cardiac and regional haemodynamic effects of S16257 following a single i.v. injection, and also following repeated s.c. injection (to simulate oral ingestion, but without disturbing the animals).

Methods

All experiments were carried out on male, Long Evans rats (346–450 g) bred in the Biomedical Services Unit (Queen's Medical Centre, Nottingham).

Cardiac haemodynamics

About 8 days before experiments were run, each animal had an electromagnetic flow probe (Skalar, Delft) implanted around the ascending aorta via a transthoracic approach, under sodium methohexitone anaesthesia (40–60 mg kg^{-1}, i.p., supplemented as required) (Gardiner *et al.*, 1990b). Following surgery, animals were given ampicillin (Penbritin, Beecham, 7 mg kg^{-1}, i.m.) and returned to individual home cages with free access to tap water and food (Biosure, GLP grade, 41B (M)). At least 6 days later animals were briefly anaesthetized (sodium methohexitone 40 mg kg^{-1}, i.p.). One group of animals (n=9) had an intra-arterial catheter implanted in the distal abdominal aorta (via the ventral caudal artery), and 3 catheters implanted in the right jugular vein, 2 for administration of S16257 or vehicle, and 1 fashioned and positioned for recording central venous pressure (Gardiner *et al.*, 1990b). The other group (n=9) had an intra-arterial and an intravenous catheter implanted for recording arterial and central venous pressure, respectively, and a s.c. catheter implanted for administering S16257. Animals were allowed to recover for at least 24 h before experiments were begun.

Cardiac haemodynamic data (mean thoracic aortic flow, peak thoracic aortic flow, maximum rate of rise of aortic flow (dF/dt_{max}), instantaneous heart rate, mean arterial pressure, central venous pressure, stroke volume and total peripheral conductance) were digitised by a custom-built microprocessor and stored on disc for off-line analysis (Gardiner *et al.*, 1990b).

All variables, except heart rate, mean arterial and central venous pressures were factored by body weight (i.e., cardiac index = mean thoracic aortic flow 100 g^{-1}; stroke index = stroke volume 100 g^{-1}).

Regional haemodynamics

Animals had miniaturized pulsed Doppler flow probes (Haywood *et al.*, 1981) sutured around the left renal and superior mesenteric arteries, and the distal abdominal aorta (below the level of the ileocaecal artery) to monitor blood flow to the hindquarters (Gardiner *et al.*, 1990b). All surgery was carried out under sodium methohexitone anaesthesia (40–60 mg kg^{-1}, i.p., supplemented as required). Following surgery, animals were given ampicillin (7 mg kg^{-1}, i.m. Penbritin, Beecham) and returned to individual home cages with free access to tap water and food. At least 7 days later, animals were briefly anaesthetized (sodium methohexitone 40 mg kg^{-1}, i.p.). One

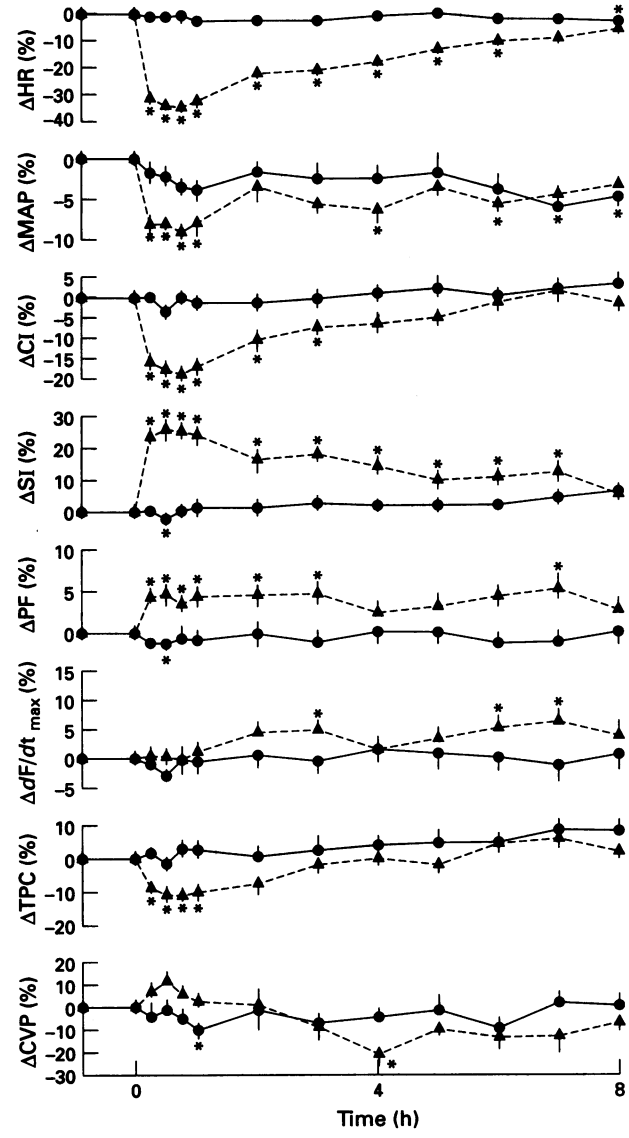

Figure 1 Cardiovascular changes over 8 h following i.v. injection of vehicle (●) or S16257 at 1 mg kg^{-1} (▲) in the same Long Evans (n=9) on 2 experimental days. Values are mean±s.e.mean; *$P<0.05$ versus pre-injection baseline. HR = heart rate; MAP = mean arterial blood pressure; CI = cardiac index; SI = stroke index; PF = peak aortic flow; dF/dt_{max} = maximum rate of rise of aortic flow; TPC = total peripheral conductance; CVP = central venous pressure.

Table 2 Integrated (AOC, $AUC_{0\text{-}1h}$) cardiovascular responses to i.v. vehicle or S16257 in the same conscious Long Evans rats (n = 9)

	Vehicle	*S16257* (1 mg kg^{-1})
Heart rate (AOC; % h)	−3±1	−33±2*
Mean blood pressure (AOC; % h)	−3±1	−8±1*
Cardiac index (AOC; % h)	−3±1	−18±1*
Stroke index (AOC, AUC; % h)	−2±1	+21±2*
Peak aortic flow (AOC, AUC; % h)	−2±1	+4±1*
dF/dt_{max} (AOC, AUC; % h)	−3±1	+2±2*
Total peripheral conductance (AUC, AOC; % h)	+4±1	−10±1*
Central venous pressure (AOC, AUC; % h)	−9±3	+9±2*

Values are mean±s.e.mean. *$P<0.05$ versus vehicle.

Table 3 Pretreatment resting cardiovascular variables in two groups of Long Evans rats ($n=9$ in each): one group was randomized to receive i.v. bolus injections of vehicle or S16257 on different days, the other was given s.c. injections of S16257 on four consecutive days

	Intravenous		*Subcutaneous*			
	Pre-Vehicle	*Pre-S16257*	*Pre-S16257 Day 1*	*Pre-S16257 Day 2*	*Pre-S16257 Day 3*	*Pre-S16257 Day 4*
Heart rate (beats min^{-1})	309±6	314±10	329±8	324±8	321±8	318±9
Mean blood pressure (mmHg)	100±2	97±2	104±2	107±1	105±1	104±2
Renal Doppler shift (kHz)	5.2±0.3	5.1±0.3	6.2±0.5	6.6±0.4	6.7±0.4	6.3±0.4
Mesenteric Doppler shift (kHz)	7.1±0.7	7.5±0.6	6.9±0.5	6.4±0.5	7.2±0.8	7.3±0.7
Hindquarters Doppler shift (kHz)	3.6±0.3	3.7±0.4	4.6±0.3	4.5±0.3	4.6±0.4	4.6±0.4
Renal conductance ([kHz $mmHg^{-1}$]10^3)	52±3	53±3	60±4	61±4	64±5	61±4
Mesenteric conductance ([kHz $mmHg^{-1}$]10^3)	71±6	78±7	66±5	60±6	69±9	71±8
Hindquarters conductance ([kHz $mmHg^{-1}$]10^3)	36±3	38±4	44±3	42±3	44±4	44±4

Values are mean±s.e.mean.

group ($n=9$) of animals had an intra-arterial catheter implanted in the distal abdominal aorta (via the ventral caudal artery) for blood pressure and heart rate recording and 2 catheters implanted in the right jugular vein for S16257 or vehicle administration. The other group of animals ($n=9$) had an intra-arterial and a s.c. catheter (for S16257 administration) implanted. Animals were allowed to recover for at least 24 h before experiments were begun.

In animals instrumented for measurement of regional haemodynamics, continuous recordings (on a Gould ES 1000 system) were made of mean and phasic arterial blood pressure, instantaneous heart rate and mean and phasic Doppler shift signals from renal, mesenteric and hindquarters probes. The latter were monitored to ensure the signals were of an acceptable quality (signal: noise>20:1). Vascular conductance changes were calculated from mean Doppler shift signals and mean arterial blood pressure (Gardiner *et al.*, 1990b).

Responses to i.v. injection of vehicle or S16257

Pilot experiments Pilot experiments were carried out in 2 animals instrumented for measurement of blood pressure and heart rate only. On 5 consecutive days S16257 was given i.v. at doses of 0.1, 0.3, 1.0, 3.0 and 10 mg kg^{-1}. These doses reduced heart rate by a mean of 7, 7, 31, 55 and 56%, respectively. Hence, a dose of 1 mg kg^{-1} S16257 was chosen for the full studies, since this was about the ED_{50} for its bradycardic effect.

Full experiments Animals received vehicle or S16257 (1 mg kg^{-1}, i.v.), on separate experimental days, in random order. Each experimental run began at 07 h 00 min, and i.v. injection was given in a bolus of 100 μl. The times at which measurements were made (0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7 and 8 h) were determined from pilot experiments.

Responses to s.c. injection of S16257

Pilot experiments Pilot experiments were carried out in 2 animals instrumented for measurement of blood pressure and heart rate only. These animals received s.c. injections of S16257 (1 mg kg^{-1}) at 07 h 00 min on 4 consecutive days, and mean arterial blood pressure and heart rate were measured every 1 h for 8 h. This experiment demonstrated that the maximum bradycardic effect of S16257 given s.c. occurred between 2–3 h after injection, as opposed to about 1 h when the same dose was given i.v., but the magnitude was the same as that seen after i.v. dosing.

Full experiments Animals received S16257 at 1 mg kg^{-1} s.c. on 4 consecutive experimental days. Each experimental run began at 07 h 00 min, and s.c. injection was given in a bolus of 100 μl. The times at which measurements were made (1, 2, 3, 4, 5, 6, 7 and 8 h) were determined from the pilot experiments.

Data analysis

All calculations (mean±s.e.mean, % changes, areas over or under curves) were done using a Pascal Turbo programme (that also performed the within-run and between-run analyses using Friedman's test (Theodorsson-Norheim, 1987)); unpaired comparisons were by Mann-Whitney U tests; a P value<0.05 was taken as significant. Correlations were assessed by the Spearman ranks test.

Drugs

S16257 was supplied by Institut de Recherches Internationales Servier (France). S16257 was dissolved in sterile isotonic (5%) dextrose.

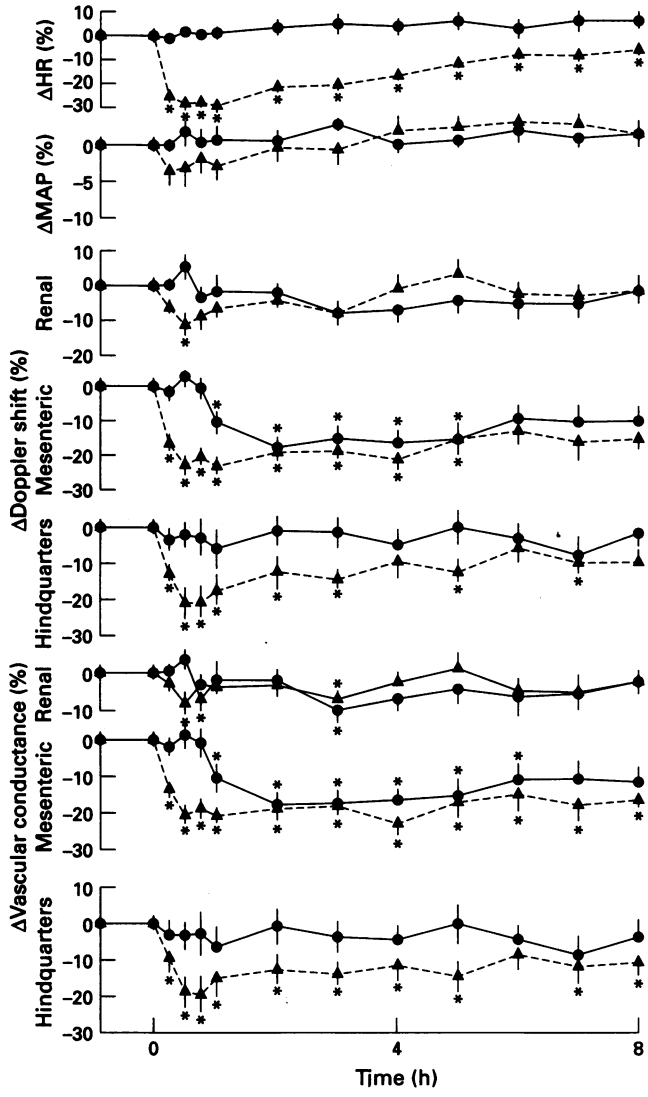

Figure 2 Cardiovascular changes over 8 h following i.v. injection of vehicle (●) or S16257 at 1 mg kg^{-1} (▲) in the same Long Evans rats (n=9) on 2 experimental days. Values are mean ± s.e.mean; *P<0.05 versus pre-injection baseline. HR = heart rate; MAP = mean arterial blood pressure.

Results

Responses to i.v. injection of vehicle or S16257

Cardiac haemodynamics Resting values for cardiovascular variables in animals instrumented for the measurement of cardiac haemodynamics are shown in Table 1.

Cardiovascular changes following injection of vehicle There were no acute changes following injection of vehicle (Figure 1). However, 7 and 8 h after injection of vehicle there were slight reductions in mean arterial blood pressure and heart rate, but no change in other variables (Figure 1).

Cardiovascular changes following injection of S16257 at 1 mg kg^{-1} Injection of S16257 caused an immediate, but slight and transient pressor effect, as also seen in the animals instrumented for regional haemodynamics; thereafter, there was a modest fall in mean arterial blood pressure, but a marked bradycardia (Figure 1). The latter was accompanied by a significant reduction in cardiac index, although stroke index showed a sustained increase (Figure 1); there was a modest rise in peak aortic flow, and dF/dt_{max} was maintained or increased slightly (Figure 1). The higher the resting heart rate, the greater was the bradycardic effect of S16257, but the fall in heart rate and the fall in cardiac index were not linearly related (data not shown). Over the first 1 h following injection of S16257 there was a slight reduction in total peripheral conductance and a tendency for central venous pressure to rise (Figure 1). Subsequently, central venous pressure fell slightly (Figure 1).

Comparison of integrated responses to vehicle and S16257 The temporal drift in some of the variables in the vehicle-injected group tended to obscure the immediate differences between the effects of treatment with vehicle and treatment with S16257 (Figure 1) if integrated responses were considered over the whole 8 h post-injection period. Therefore, the analysis of integrated responses was confined to the 1 h following injection, when the effects of S16257 were most marked (Figure 1). Relative to the effects of vehicle, S16257 caused significantly greater reductions in heart rate, mean arterial blood pressure, cardiac index, and total peripheral conductance, together with significantly greater increases in stroke index, peak aortic flow, dF/dt_{max} and central venous pressure (Table 2).

Regional haemodynamics Resting values for cardiovascular variables in animals instrumented for the measurement of regional haemodynamics are shown in Table 3.

Cardiovascular changes following injection of vehicle There were no acute changes following injection of vehicle (Figure 2), but between 1–5 h later, there were significant reductions in mesenteric flow and conductance (Figure 2). This is a phenomenon we have described previously (Gardiner *et al.*, 1990a; 1994); it is likely it represents a waning of the hyperaemic vasodilatation in the gut induced by food ingestion during the dark cycle (rats are nocturnal feeders). There was also a slight fall in renal vascular conductance (see Gardiner *et al.*, 1990a; 1994), but no significant changes in mean arterial blood pressure, heart rate, or hindquarters haemodynamics (Figure 2).

Cardiovascular changes following injection of S16257 at 1 mg kg^{-1} There was a slight, transient pressor response immediately following injection of S16257, but thereafter there

Table 4 Integrated (AOC, $AUC_{0\text{-}1h}$) cardiovascular responses to i.v. vehicle or S16257 in the same conscious Long Evans rats (n = 9)

	Vehicle	*S16257* (1 mg kg^{-1})
Heart rate (AOC; %h)	−2±1	−28±2*
Mean blood pressure (AUC, AOC; %h)	+2±2	−5±1*
Renal flow (AOC; %h)	−3±1	−10±1*
Mesenteric flow (AOC; %h)	+5±2	−20±2*
Hindquarters flow (AOC; %h)	−8±2	−19±3*
Renal conductance (AOC; %h)	−3±1	−7±2*
Mesenteric conductance (AOC; %h)	−6±2	−17±2*
Hindquarters conductance (AOC; %h)	−8±2	−17±3*

Values are mean ± s.e.mean. *P<0.05 versus vehicle.

were no significant changes in mean arterial blood pressure (Figure 2). However, there was a substantial and sustained bradycardia (Figure 2). There were slight reductions in renal flow and vascular conductance, and larger reductions in mesenteric and hindquarters flow and vascular conductance (Figure 2).

Comparison of integrated responses to vehicle and S16257 As with animals instrumented for the measurement of cardiac haemodynamics (see above), the temporal drift in cardiovascular variables following injection of vehicle (Figure 2) meant that consideration of integrated responses over the full 8 h recording period obscured the clear, early differences between the effects of vehicle and S16257. However, analysis of the changes over the 1 h following injection showed significantly greater reductions in heart rate, mean arterial blood pressure, and renal, mesenteric and hindquarters blood flows and vascular conductances after S16257, than after vehicle (Table 4).

Responses to s.c. injection of S16257

Cardiac haemodynamics Resting values for cardiovascular variables in animals instrumented for the measurement of cardiac haemodynamics are shown in Table 1. Cardiac index, stroke index, peak aortic flow, dF/dt_{max} and total peripheral conductance were slightly lower on Day 1 than on subsequent days.

Subcutaneous injection of S16257 caused no immediate effects, in contrast to the animals given S16257 by i.v. injection (see above).

Table 5 Cardiovascular changes following s.c. injection of S16257 in the same conscious, Long Evans rats ($n = 9$) on 4 consecutive days

		1 h	*2 h*	*3 h*	*4 h*	*5 h*	*6 h*	*7 h*	*8 h*	*Integrated response* (% h)
Δ Heart rate (%)	Day 1	−19±3*	−27±2*	−26±2*	−18±2*	−14±2*	−11±2*	−9±2*	−7±2*	−128±12
	Day 2	−22±2*	−32±2*	−30±1*	−27±1*	−21±1*	−18±2*	−14±2*	−13±2*	−170±7†
	Day 3	−16±2*	−32±2*	−30±2*	−28±1*	−20±2*	−16±1*	−16±2*	−16±1*	−164±5†
	Day 4	−12±2*	−29±1*	−31±2*	−29±2*	−22±1*	−20±1*	−18±2*	−13±2*	−166±8†
Δ Mean blood pressure (%)	Day 1	−2±2	−5±2*	−8±2*	−5±2*	−3±2	−3±2	−4±2	−3±2	−36±11
	Day 2	−3±2	−4±1*	−8±1*	−4±1*	−3±2	−6±2	−4±1	−3±1	−34±7
	Day 3	−2±1	−3±2	−5±1*	−6±2*	−4±2	−2±1	−5±1*	−4±1*	−31±7
	Day 4	−2±2	−3±1	−4±2	−6±3	−7±2*	−5±2	−6±1*	−5±1*	−38±8
Δ Cardiac index (%)	Day 1	−5±2*	−6±2*	−3±4	1±3	4±3	1±4	4±4	6±2*	−28±8
	Day 2	−12±2*	−15±2*	−13±1*	−9±1*	−6±2*	−5±3	1±2	−3±2	−64±9†
	Day 3	−8±2*	−15±2*	−13±2*	−14±2*	−8±1*	−7±2*	−6±2*	−6±2*	−75±10†
	Day 4	−4±3	−12±3*	−14±3*	−10±3*	−6±3	−6±3	−6±3	3±3	−70±13†
Δ Stroke index (%)	Day 1	19±5*	30±5*	31±5*	25±5*	21±4*	14±5*	15±3*	14±3*	162±29
	Day 2	15±3*	25±3*	25±3*	24±2*	18±3*	16±4*	18±3*	12±4*	147±17
	Day 3	9±2*	25±3*	24±2*	20±3*	15±2*	11±2*	11±2*	11±2*	123±9
	Day 4	9±3*	24±4*	25±4*	26±3*	20±3*	17±3*	14±2*	11±3*	141±19

Values are mean±s.e.mean; *$P<0.05$ for change; †$P<0.05$ versus Day 1 response (Friedman's test).

Table 6 Cardiovascular changes following s.c. injection of S16257 in the same conscious, Long Evans rats ($n = 9$), on 4 consecutive days

		1 h	*2 h*	*3 h*	*4 h*	*5 h*	*6 h*	*7 h*	*8 h*	*Integrated response* (% h)
Δ Peak flow (%)	Day 1	3±2	7±2*	9±2*	9±3*	9±3*	6±3*	7±3*	6±2*	55±17
	Day 2	1±2	4±2*	6±1*	7±1*	6±1*	7±1*	8±1*	5±1	44±8
	Day 3	1±1	5±2	7±2*	6±2*	6±1*	4±1*	4±1*	2±1	37±7
	Day 4	3±2	5±2	6±2*	8±2*	6±2*	5±1*	4±2*	3±2	42±10
Δ dF/dt_{max} (%)	Day 1	9±1	5±2	7±3*	10±3*	10±3*	6±4	8±4*	6±3	55±20
	Day 2	−2±2	1±2	3±2	6±2*	6±1*	6±2*	9±1*	4±2	39±9
	Day 3	0±2	2±3	7±3	3±3	8±3*	6±2*	6±2*	2±2	42±12
	Day 4	3±3	3±4	5±3	6±3	7±3	5±2*	4±3	4±3	46±16
Δ TPC (%)	Day 1	−3±2	0±3	6±5	7±4	8±5	5±5	9±5	10±4	−19±8
	Day 2	−8±3*	−11±3*	−5±1*	−6±2*	−3±4	2±4	5±2	1±3	−42±11
	Day 3	−6±2*	−12±3*	−9±2*	−8±4*	−4±2	−5±2	−1±3	−1±3	−54±11
	Day 4	−1±4	−10±4*	−10±4	−3±5	1±4	0±5	0±4	2±4	−49±12
Δ CVP (%)	Day 1	−1±4	−6±5	−12±5*	−13±5*	−16±5*	−13±5*	−16±3*	−12±4*	−90±27
	Day 2	5±2*	4±3	−3±5	2±5	−1±6	2±4	−2±5	2±6	48±17†
	Day 3	10±4	11±6	8±6	9±6	12±4*	11±8	10±7	−1±2	102±24†
	Day 4	10±5	12±4	14±7	7±5	6±4	2±4	3±3	−3±7	74±21†

Values are mean±s.e.mean; *$P<0.05$ for change; †$P<0.05$ versus Day 1 response (Friedman's test). TPC = total peripheral conductance; CVP = central venous pressure.

On the first day, injection of S16257 caused a bradycardia that was maximal 2–3 h after injection (Table 5). Although slower in onset than the bradycardic response to i.v. injection of S16257, the nadirs in heart rate following both routes of administration were similar. There was a slight, but significant, bradycardia even 8 h after s.c. injection of S16257 (Table 5), but by 24 h after injection resting heart rate was not different from the pre-injection value.

On the three subsequent days, injection of S16257 evoked bradycardias that were not significantly different from each other, but were significantly greater than that seen on the first experimental day (Table 5).

On the first day, S16257 caused only a slight fall in cardiac index, but on the subsequent three days, the integrated falls in cardiac index were similar to each other and significantly greater than on day 1 (Table 5). Generally, the falls in heart rate and cardiac index on each experimental day were not linearly related (data not shown).

The patterns of change in the other variables were similar on all four experimental days (Tables 5 and 6).

Regional haemodynamics Resting values for cardiovascular variables in animals instrumented for the measurement of regional haemodynamics are shown in Table 3. Subcutaneous injection of S16257 caused no immediate effects, in contrast to the animal given i.v. injections (see above). The patterns of change in heart rate on the four days following S16257 were similar to those described above, i.e., the integrated response was significantly less on the first day than on the subsequent days (Tables 7 and 8). All other variables, with the exception of

Table 7 Cardiovascular changes following s.c. injection of S16257 in the same conscious, Long Evans rats ($n = 9$) on 4 consecutive days

		1 h	*2 h*	*3 h*	*4 h*	*5 h*	*6 h*	*7 h*	*8 h*	*Integrated response* (% h)
Δ Heart rate (%)	Day 1	−22±2*	−22±3*	−22±1*	−18±2*	−14±2*	−11±2*	−10±3*	−9±2*	−124±11
	Day 2	−25±2*	−25±1*	−27±1*	−21±2*	−17±2*	−17±2*	−13±2*	−11±2*	−150±10†
	Day 3	−20±3*	−29±2*	−27±2*	−26±1*	−20±1*	−18±2*	−16±2*	−14±2*	−164±8†
	Day 4	−18±2*	−29±3*	−30±2*	−28±2*	−21±2*	−19±2*	−18±2*	−14±3*	−168±13†
Δ Mean blood pressure (%)	Day 1	−2±1	−3±1*	−2±1	−2±1	0±2	−1±1	−1±1	1±1	−16±6
	Day 2	−4±1*	−6±1*	−5±1*	−6±1*	−4±1*	−4±1*	−3±2	−4±1*	−35±8
	Day 3	−3±1*	−5±1*	−4±1*	−5±1*	−6±1*	−2±1	−5±1*	−3±1*	−32±7
	Day 4	−3±1*	−5±2*	−8±1*	−6±1*	−5±1*	−4±2*	−5±2*	−3±2	−39±8
Δ Renal flow (%)	Day 1	−1±2	−7±5	−4±3	−4±3	−5±3	−3±3	−6±5	−4±3	−45±13
	Day 2	−6±3	−13±4	−6±5	−6±2	−3±4	−3±5	−9±4	−5±3	−59±16
	Day 3	−4±4	−9±4	−9±4	−9±4	−7±4	−4±5	−10±3	−6±3	−65±20
	Day 4	−1±4	−9±3	−8±5	−11±2	−4±3	−6±3	−5±2	−7±3	−55±11
Δ Mesenteric flow (%)	Day 1	−11±4	−19±1*	−20±2*	−17±2*	−14±3*	−13±3*	−11±5*	−11±5*	−115±16
	Day 2	−14±3*	−20±4*	−21±3*	−15±4*	−14±5*	−19±4*	−16±4*	−16±4*	−128±23
	Day 3	−11±2*	−21±4*	−21±4*	−19±4*	−17±4*	−15±4*	−10±6*	−11±5*	−124±20
	Day 4	−11±2*	−23±4*	−21±4*	−22±4*	−18±3*	−14±4*	−16±3*	-10±4*	−129±15
Δ Hindquarters flow (%)	Day 1	−13±4*	−12±5*	−14±4*	−13±5*	−7±3	−8±4	−12±7	−10±6	−90±26
	Day 2	−13±4*	−13±4*	−22±9*	−14±4*	−9±4*	−12±5*	−4±6	−8±5	−99±27
	Day 3	−5±4	−15±4*	−15±4*	−15±4*	−14±3*	−12±4*	−12±4*	−10±4*	−97±18
	Day 4	−9±5	−19±5*	−17±4*	−15±4*	−10±5*	−11±5	−5±6	−7±6	−101±25

Values are mean ± s.e.mean; *$P < 0.05$ for change; †$P < 0.05$ versus Day 1 (Friedman's test).

Table 8 Cardiovascular changes following s.c. injection of S16257 in the same conscious, Long Evans rats ($n = 9$) on 4 consecutive days

		1 h	*2 h*	*3 h*	*4 h*	*5 h*	*6 h*	*7 h*	*8 h*	*Integrated response* (% h)
Δ Renal conductance (%)	Day 1	1±2	−4±4	−1±3	−1±3	5±3	−2±4	−6±4	−5±3	−38±13
	Day 2	−2±4	−8±4	−1±5	−1±3	2±4	1±5	−6±4	−1±3	−38±12
	Day 3	−1±4	−4±4	−5±3	−4±3	−1±4	−2±5	−5±4	−3±3	−45±16
	Day 4	2±4	−4±2	0±5	−5±4	2±4	−2±4	0±2	−3±4	−32±11
Δ Mesenteric conductance (%)	Day 1	−9±4	−17±1*	−18±2*	−15±2*	−14±4*	−12±3*	−11±4	−12±5	−106±14
	Day 2	−10±4	−16±3*	−16±4*	−10±4*	−10±4*	−16±4*	−13±4*	−13±4*	−102±21
	Day 3	−8±2	−17±4*	−17±3*	−14±5*	−12±4*	−13±4*	−5±6	−8±6	−100±19
	Day 4	−8±3	−18±4*	−14±4*	−16±5*	−13±3*	−10±5	−11±5	−7±4	−99±17
Δ Hindquarters conductance (%)	Day 1	−11±5	−9±5	−12±4	−11±5	−7±4	−6±5	−11±7	−11±6	−84±27
	Day 2	−9±4	−8±5	−18±10	−8±5	−5±4	−8±6	0±6	−4±6	−78±26
	Day 3	−1±5	−10±5	−11±4*	−10±4*	−9±3	−10±4*	−8±5	−7±4	−73±18
	Day 4	−7±5	−14±5	−10±4	−10±4	−5±5	−7±6	1±7	−3±8	−78±22

Values are mean ± s.e.mean; *$P < 0.05$ for change (Friedman's test).

mesenteric flow and conductance, showed only slight and variable changes (Tables 7 and 8). The pattern of change in mesenteric haemodynamics was similar to that seen after vehicle (see above).

Discussion

Although the electrophysiological and other cardiac effects of specific bradycardic agents have been delineated (see Introduction), the acute cardiovascular consequences of administering these drugs *in vivo* are less well known, and somewhat contradictory. For example, Kobinger & Lillie (1984) found that zatebradine (0.3 mg kg^{-1}, i.v., 1 min before) caused marked bradycardia and a slight reduction in diastolic blood pressure (about 10 mmHg) in chloralose-anaesthetized cats. However, in conscious dogs, zatebradine (1 mg kg^{-1}, i.v.) caused bradycardia with no change in blood pressure. The magnitude of the bradycardia was greater the higher the resting heart rate. Subsequently, Krumpl *et al.* (1988) found that zatebradine (0.5 mg kg^{-1} infused over 5 min) in conscious dogs caused bradycardia but no significant change in cardiac output or stroke volume. However, Johnston *et al.* (1991) reported that a single dose of zatebradine (0.3 mg kg^{-1} in 3 ml given i.v. over 5 min) in anaesthetized, closed-chest dog, caused bradycardia and a reduction in cardiac output, accompanied by a rise in total peripheral resistance, but no change in stroke volume or mean arterial blood pressure 30 min later. Marshall *et al.* (1993) observed that oral dosing (0.1 – 10 mg kg^{-1}) of dogs with another specific bradycardic agent, ZD 7288, unrelated to zatebradine, had no effect on resting heart rate, whereas in conscious rats there was a dose-dependent (1 – 100 mg kg^{-1}) bradycardia, but no change in systemic arterial blood pressure averaged over the 6 h following dosing. Subsequently, Rouse & Johnson (1994) and Rouse *et al.* (1994) produced evidence that the acute haemodynamic effects of ZD 7288 (0.02 – 1 mg kg^{-1}, i.v.) were secondary to the bradycardia. In contrast, Adachi (1994) found that i.v. infusion (30 – 300 μg kg^{-1} min^{-1}) of the structurally unrelated bradycardic agent, E4080, caused dose-dependent reductions in mean aortic blood pressure in conscious dogs, but with no change in heart rate. Adachi (1994) suggested that E4080 was exerting an anti-tachycardic action under these conditions, since the occurrence of reflex sympathetic activation was apparent from dose-dependent increases in plasma noradrenaline.

Against this background we considered it useful to establish the *in vivo* cardiac and regional haemodynamic responses to the specific bradycardic agent, S16257 (Thollon *et al.*, 1994), following single i.v. injection to quantify its acute effects, and following repeated s.c. injection to establish its chronic effects. Although, in a clinical setting, S16257 would be given by mouth, repeated administration by gavage to conscious, chronically-instrumented rats would have made undisturbed haemodynamic measurements impossible. Therefore, in order to stimulate the slower drug absorption following oral, compared to i.v., administration, the chronic dosing of S16257 was achieved through a catheter implanted s.c.

Responses to single i.v. injection of S16257

The present experiments showed that, relative to vehicle, i.v. injection of S16257 caused marked reduction in heart rate. As observed by Kobinger & Lillie (1984) for zatebradine, the higher the resting heart rate, the greater was the bradycardic effect of S16257. This is consistent with S16257 acting directly on sino-atrial node cells to inhibit the pacemaker current (I_f), and hence fix the rate of diastolic depolarization (Thollon *et al.*, 1994) irrespective of the level of sympathetic and vagal tone. Although cardiac index fell with S16257, the percentage reduction was half that seen in heart rate, due to a substantial increase in stroke index. The early fall in mean arterial blood pressure was less than would have been expected from the fall in cardiac index because vascular conductance was reduced, particularly in the mesenteric and hindquarters vascular beds, and less so in the kidney. It is likely these early regional vasoconstrictor responses were due to reflex and neurohumoral activation subsequent to arterial baroreceptor unloading, as a result of the fall in cardiac index (Charlton & Baertschi, 1982), but with the substantial increase in stroke index and in diastolic interval, it is not immediately obvious how the pattern of baroreceptor discharge would have changed. Such reflex mechanisms would usually involve activation of the sympathoadrenal and renin-angiotensin systems, and release of vasopressin (Gardiner & Bennett, 1985; Schadt & Ludbrook, 1991; see Adachi, 1994), and hence it would be of interest to know how therapeutic agents that interfere with these mechanisms influence the responses to S16257.

Although total peripheral conductance fell, and this, alone, might have reduced venous return, S16257 caused a rise in central venous pressure. While it is feasible this was due to active venoconstriction (involving the mechanisms mentioned above), it is probable that the prolongation of diastole caused by S16257 contributed importantly to the rise in central venous pressure. The latter effect would normally be accompanied by activation of cardiopulmonary receptors, the influence of which might oppose those elicited by unloading of arterial baroreceptors (see Thorén, 1979, for review). Hence, the degree of peripheral vasoconstriction following i.v. S16257 probably reflected this interaction. Since S16257 is related to zatebradine, which is structurally similar to verapamil, and can cause vasorelaxation at high doses (Kobinger & Lillie, 1984), any direct vascular effect of S16257 would have been expected to cause dilatation. Such an action would have opposed any reflex vasoconstriction, and hence should have enhanced the fall in blood pressure resulting from the fall in cardiac index. The finding that the hypotensive effect of S16257 was slight indicates that any putative vasodilator action it was exerting was minimal. These observations also argue against S16257 exerting any marked effect on central nervous mechanisms at the dose used, although we cannot exclude such an action.

In association with the effects of S16257 discussed above, it was clear that the compound did not reduce peak aortic flow or dF/dt_{max}. Considering that afterload increased (i.e. total peripheral conductance fell), then this is good evidence for the absence of a negative inotropic effect of S16257 (De Wildt & Sangster, 1983).

Responses to repeated s.c. injections of S16257

Although after S16257 was given by s.c injection, the rate of onset of its bradycardic effect was slower, the maximal effect and its duration were similar to those after i.v. injection. More notably, repeated administration of S16257 on four consecutive experimental days produced no signs of desensitization to its bradycardic effects. Indeed, the integrated response on the first day was less than on the subsequent three days, although the overall pattern of response was remarkably similar. It is feasible that pharmacokinetic factors accounted for the differences between the response to S16257 on the first day, compared to subsequent days.

The somewhat lesser bradycardic effect of S16257 on the first experimental day was accompanied by a smaller fall in cardiac index and total peripheral conductance, consistent with the latter being secondary to the fall in cardiac index (see above). As after i.v. injection, however, the marked bradycardic action of s.c. S16257 was associated with a lesser reduction in cardiac index, due to the substantial increase in stroke index. This effect, which was very reproducible over the four experimental days, was accompanied by slight increases in peak aortic flow and dF/dt_{max}, indicating that S16257 was devoid of negative inotropic effects, even with repeated administration. The fact that s.c. S16257 had only slight, variable hypotensive effects on any of the experimental days is consistent with the absorption of S16257 being sufficiently slow to allow the reflex reduction in total peripheral conductance more effectively to oppose the fall in cardiac index.

In conclusion, it is clear that S16257 can have marked and reproducible, bradycardic effects without any signs of negative inotropic action, or detrimental effects on regional haemodynamics, even with chronic exposure over four days. If these results extrapolate to the clinical setting, it seems likely that S16257 will have beneficial bradycardic effects, with no concurrent undesirable actions on other aspects of cardiovascular function.

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