Acute Cardiovascular Effects of Sibutramine in Conscious Rats

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ABSTRACT

Sibutramine is a serotonin and norepinephrine reuptake inhibitor, used in the treatment of obesity. In this study, cardiovascular effects of sibutramine (0.9, 3, or 9 mg kg⁻¹ i.p.) were measured in conscious Sprague-Dawley rats, in the absence and presence of β- and/or α-adrenoceptor antagonism (with propranolol and/or phentolamine, respectively). Sibutramine caused pressor and tachycardic effects, with celiac and mesenteric vasoconstrictions, and hyperemic hindquarters vasodilatation. Pretreatment with propranolol inhibited the tachycardic and hindquarters vasodilator effect of sibutramine, whereas phentolamine inhibited the pressor and vasoconstrictor effects of sibutramine. In the presence of phentolamine, sibutramine caused hyperemic mesenteric vasodilatation. In preconstricted, isolated, mesenteric vessels, sibutramine and its metabolites BTS 54 505 (N-desmethylsibutramine) and BTS 54 354 (N-didesmethylsibutramine) (10 μM) produced significant vasodilatations. Neither sibutramine nor BTS 54 505 enhanced vessel sensitivity to norepinephrine, whereas BTS 54 354 produced a significant leftward shift in the concentration-response curve to norepinephrine. Collectively, the results indicate that the overt cardiovascular effects of sibutramine involve α-adrenoceptor-mediated celiac and mesenteric vasoconstrictions, and β-adrenoceptor-mediated hindquarters vasodilatation and tachycardia. The mesenteric vasodilator response to sibutramine, seen in the presence of phentolamine, may be a direct effect of the drug and/or its metabolites, on vessel tone. The cardiovascular effects of sibutramine in vivo may be secondary to inhibition of peripheral and/or central reuptake of monoamines by the metabolites BTS 54 354 and/or BTS 54 505. It remains to explain why BTS 54 354, but not BTS 54 505, enhanced norepinephrine sensitivity in vitro, because both metabolites are potent inhibitors of the norepinephrine transporter.

Obesity is a common disorder associated with a variety of comorbid illnesses (Kissebah and Krakower, 1994; Kopelman, 2000). Currently available pharmacological treatment for obesity is based on a range of drugs with different mechanisms of action, including drugs that affect energy intake and energy expenditure and those preventing dietary fat absorption (for review, see Clapham et al., 2001). Sibutramine hydrochloride (BTS 54 524; N-[1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutyl]-N-dimethylamine HCl monohydrate) is a member of a novel class of drugs that inhibits the reuptake of both serotonin (5-HT) and norepinephrine (NE) (Luscombe et al., 1989, 1990), although details of the interaction between the drug and/or its metabolites and the NE and 5-HT transporter proteins (for review, see Goldberg et al., 2003) are not available. Sibutramine is currently indicated for the management of obesity (for reviews, see Stock, 1997; Luque and Rey, 2002). In animals, sibutramine has been shown to decrease body weight through its actions on food intake and energy expenditure; thus, satiety is enhanced (Fantino and Souquet, 1995; Halford et al., 1998), and thermogenesis in brown adipose tissue is stimulated (Connoley et al., 1999).

In human, the acute administration of a single dose of sibutramine elevated systolic blood pressure and heart rate (HR) for at least 6 h (King and Devaney, 1988). Furthermore, chronic treatment of obese patients with sibutramine (20 mg day⁻¹ for 6 months), caused modest, but significant, increases in mean arterial blood pressure (MAP) (2–3 mm Hg) and HR (3–5 beats min⁻¹) (Fujioka et al., 2000). The pressor and tachycardic effects of sibutramine are attenuated by metoprolol in human (Birkenfeld et al., 2002). The latter study drew attention to the complex actions of sibutramine on autonomic cardiovascular regulation, pointing out that they could be due to a combination

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine, serotonin; NE, norepinephrine; HR, heart rate; MAP, mean arterial blood pressure; PSS, physiological salt solution; BTS 54 505, N-desmethylsibutramine; BTS 54 354, N-didesmethylsibutramine.
of peripheral (stimulatory) effects and central (inhibitory) actions (Birkenfeld et al., 2002).

In spite of the evidence that sibutramine may exert cardiovascular effects, there is little known about its regional hemodynamic actions in humans or animals. This is a potentially important issue, because hypertension may be a concomitant risk factor in obese patients and it is, therefore, critical to obtain a proper understanding of the potential cardiovascular actions of sibutramine (Luque and Rey, 2002). Thus, the overall aims of the present studies were to delineate the regional hemodynamic profile of acute administration of sibutramine and to determine the underlying mechanisms.

In vivo, sibutramine is subject to extensive first-pass metabolism in the liver, predominantly by the cytochrome P450 enzyme CYP3A4. After administration in animals or human, the parent compound is rapidly converted by demethylation first to the secondary amine (BTS 54 354) and then to the primary amine (BTS 54 505) (Luscombe et al., 1989; Hind et al., 1999). In rats, sibutramine is a weak selective NE reuptake inhibitor ($K_i = 283$ nM), whereas BTS 54 505 and BTS 54 354 are $\sim 100$-fold more potent (Cheetham et al., 1996), and in addition, are also potent in vitro inhibitors of 5-HT and dopamine reuptake (Heal and Cheetham, 1997). On this basis, it has been postulated that the majority of sibutramine’s pharmacological action is mediated by its powerful and persistent active metabolites (Luscombe et al., 1989; Hind et al., 1999). In the present study, therefore, the in vitro actions of the metabolites were also examined.

To address the overall aims, experiments were performed to 1) measure the regional hemodynamic changes in response to bolus doses of sibutramine, in conscious, freely moving Sprague-Dawley rats; 2) assess the effects of pretreatment with phenolamine (a nonselective $\alpha$-adrenoceptor antagonist) and propranolol (a nonselective $\beta$-adrenoceptor antagonist), alone or in combination, on the hemodynamic effects of sibutramine; and 3) evaluate whether sibutramine or its metabolites had any effects on isolated, mesenteric small arteries, either directly, or on their responses to NE.

One consequence of the pharmacokinetic profile (see above) is that sibutramine shows no difference in potency or efficacy when administered by the oral or i.p. route. As an example, sibutramine reduces food intake through NE and 5-HT reuptake inhibition, and the ED$_{50}$ values obtained at 2 h are identical when this drug is given orally [ED$_{50}$ = 2.8 mg/kg (95% confidence interval, 1.6–5.0); Jackson et al., 1997] or i.p. [ED$_{50}$ = 2.0 mg/kg (95% confidence interval, 1.0–4.1); Rowley et al., 2000]. Thus, to avoid handling the animals during drug administration, sibutramine was given via chronically implanted i.p. catheters rather than orally by gavage.

Some of these results have been published in abstract form (Woolard et al., 2002).

Materials and Methods

In Vivo Hemodynamic Studies

Animals. Male Sprague-Dawley rats, weighing 250 to 350 g, were obtained from Charles River (Margate, Kent, UK). The holding room temperature was maintained at 21 ± 2°C, with a 12-h light/dark cycle (6:00 AM–6:00 PM). Rats were housed in groups of three to five per cage, but they were kept individually after probe implantation. Standard rat chow (Beekay Feeds, Hull, England), and water was provided ad libitum. At the time of experimentation, all rats weighed approximately 350 to 450 g. All procedures and experiments were approved by The University of Nottingham Local Ethical Review Committee and were under Home Office License authority.

Implantation of Doppler Flow Probes and Catheters. Rats were anesthetized with an i.p. injection of fentanyl and medetomidine (Domitor), 300 $\mu$g kg$^{-1}$ of each (supplemented as required), and miniature, pulsed Doppler flow probes (Crystal Biotech, Holliston, MA) were implanted around the celiac and superior mesenteric arteries, and the distal abdominal aorta, to monitor changes in Doppler shift. At the end of surgery, anesthesia was reversed with atipamezole (1 mg kg$^{-1}$ s.c.) and analgesia provided by nalbuphine (1 mg kg$^{-1}$ s.c.). At least 10 days later, animals were anesthetized (as described above) and had catheters implanted in the distal abdominal aorta, via the ventral caudal artery (for measurement of MAP and HR) and in the jugular vein and peritoneal cavity (for i.v. and i.p. drug administration). Experiments were initiated on the day after catheterization and were continued over the subsequent 3 days.

Cardiovascular Recordings. Under conditions of chronic implantation, the vessels underneath the Doppler probes become “fixed” and hence their diameters are unlikely to change. In these circumstances, changes in blood flow velocity (Doppler shift) can be used as an index of blood flow (Gardiner et al., 1990). All data were collected using the hemodynamics data acquisition system designed and built at the University of Maastricht (Maastricht, The Netherlands). The system sampled every 2 ms and averaged each cardiac cycle and stored data to disc every 5 s. Offline, data were analyzed using software (Datview; University of Maastricht), which provided electronically averaged values over times selected by the analyst.

Experiments. Cardiovascular effects of sibutramine. Three groups of Sprague-Dawley rats were used in this part of the study. Animals were randomized to receive an i.p. injection of either sibutramine [group 1 ($n = 10$) 0.9 mg kg$^{-1}$; group 2 ($n = 9$) 3 mg kg$^{-1}$; group 3 ($n = 8$) 9 mg kg$^{-1}$] or vehicle (0.5 ml of sterile 0.9% saline) on day 1, and the other intervention on day 3. Cardiovascular variables were recorded for a period of 6 h after drug or vehicle administration.

Effects of sibutramine in the presence of phenolamine and/or propranolol. Four groups of rats were used in these experiments. Group 1 rats ($n = 8$) were given saline (0.4 ml kg$^{-1}$ i.v. bolus, 0.4 ml kg$^{-1}$ h$^{-1}$ infusion) to control for the effects of the adrenoceptor antagonists. Group 2 rats ($n = 9$) were given the nonselective, competitive $\alpha$-adrenoceptor antagonist phenolamine (1 mg kg$^{-1}$ i.v. bolus, 1 mg kg$^{-1}$ h$^{-1}$ infusion; Gardiner and Bennett, 1988; Janssen et al., 1991). Group 3 rats ($n = 8$) were given the nonselective $\beta$-adrenoceptor antagonist propranolol (1 mg kg$^{-1}$ i.v. bolus, 0.5 mg kg$^{-1}$ h$^{-1}$ infusion; Gardiner and Bennett, 1988; Janssen et al., 1991). Group 4 rats ($n = 8$) were given a combination of phenolamine and propranolol (doses as described above). Between 30 and 40 min after the onset of adrenoceptor antagonist treatment, when cardiovascular variables were stable, all animals were given a single bolus dose of sibutramine (9 mg kg$^{-1}$ i.p.), and regional hemodynamic variables were monitored over the next 6 h. At the end of the experiments, the cardiovascular responses to methoxamine (60 $\mu$g kg$^{-1}$ i.v.) were abolished in animals receiving phenolamine, and responses to isoprenaline (87 ng kg$^{-1}$) were abolished in animals receiving propranolol.

In Vitro Studies

Preparation of Resistance Arteries. On each experimental day, male Sprague-Dawley rats (Charles River), weighing 350 to 450 g, were killed by stunning, followed by exsanguination. The mesentery was excised and placed in a physiological salt solution (PSS). The superior mesenteric artery was exposed, and second generation mesenteric arteries (200–400 $\mu$m) were isolated from the surrounding tissue (Dunn and Gardiner, 1995). Segments of approximately 5–7 mm in length were transferred to a Halpern pressure-
perfusion myograph (Living Systems Instrumentation, Burlington, VT), as outlined below.

**Pressure Myograph.** Within the organ chamber of the pressure myograph, the ends of each vessel were cannulated with two PSS-filled glass microcannulae and secured in place using single strands (20 μm) of nylon suture (Halpern et al., 1984). One cannula was closed, and the arteriograph was transferred to the stage of an inverted microscope (TMS model; Nikon, Tokyo, Japan), where a pressure transducer, coupled to a peristaltic pump and a 75-ml PSS reservoir, was connected to the open-ended microcannula. The arteriograph contained a 25-ml vessel chamber with both inflow and outflow channels, facilitating continuous superfusion of the vessel segment. The vessel was visualized on a television monitor (TC1914A; Burle Industries, Inc., Lancaster, PA) by a video camera attached to the viewing tube of the inverted microscope. Arterial dimensions were analyzed by a video-dimension analyser (Living Systems Instrumentation), which was connected to the television monitor, a MACLAB data acquisition system (AD Instruments, Hastings, UK), and a Macintosh computer (Leicester Computing Centre, Leicester, UK). The arteriograph was connected to the 75-ml reservoir containing calcium-PSS bubbled with a 5% CO2, 95% O2 gas mixture. A transmural pressure of 60 mm Hg was slowly applied to the vessel, which lengthened as a result; one cannula was, therefore, retracted to remove any buckling of the segment. Using a Masterflex pump (Cole-Palmer, Chicago, IL), vessels were superfused with PSS at a rate of 10 ml min⁻¹, and allowed to equilibrate for a period of 30 min. The pH of the PSS was approximately 7.4, and a temperature probe placed directly into the organ chamber monitored the temperature, which was maintained at 37 ± 0.5°C.

**Measurements.** For all experiments, concentration-response curves were generated by the cumulative addition of the compounds under investigation to the recirculating reservoir. At the end of each experiment, maximal vasodilatation was determined using a calcium-free PSS containing 0.5 mM of a calcium chelating agent, ethylene glycol bis-(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid.

**Experiments.** Effects of sibutramine, BTS 54 505, or BTS 54 354 on vessel diameter. Vessels were preconstricted with 9,11-dideoxy-9α,11α-methanoepoxy prostaglandin F₂α (U46619; dissolved in methyl acetate), in cumulative, 3-fold increments, ranging from 1 nM to 1 μM to decrease diameter by approximately 40 to 60%. U46619 was chosen as a nonadrenoceptor, nonselective contractile agent that provided a partial level of tone against which a vasoconstrictor or vasodilator response could be shown (Dunn et al., 1998). Thereafter, concentration-response curves to sibutramine, BTS 54 505, BTS 54 354, or vehicle were generated, in 3-fold increments, over the concentration range of 10 nM to 10 μM; increasing concentrations were added every 7 min. Effects of sibutramine, BTS 54 505, or BTS 54 354 on responses to NE. In nonpreconstricted segments, sibutramine or vehicle (to provide a time control) was added in a cumulative manner over the concentration range of 10 nM to 3 μM, before a concentration-response curve to NE was generated over the range of 1 nM to 30 μM. The effects of BTS 54 505, BTS 54 354, or their respective vehicles on NE responsiveness were determined in a similar way.

**Data Analysis.** For the in vivo experiments, changes relative to baseline values within each group were analyzed by Friedman’s test (Theodorsson-Norheim, 1987). Integrated responses (areas under or over curves) to vehicle and sibutramine in the same group were compared using the Wilcoxon signed ranks test. Differences between groups were compared using the Mann-Whitney U test (two groups) or the Kruskal-Wallis test (multiple comparisons); significance was accepted at P ≤ 0.05.

For the in vitro experiments, vasoconstrictor responses were calculated as a percentage change in diameter. Vasodilator responses were calculated as a percentage of the maximum range for dilatation (i.e., the difference between the preconstricted diameter and maximally vasodilated diameter in calcium-free PSS). Results are expressed as mean ± S.E.M. Within-group analysis of data was by Friedman’s test, to determine changes relative to baseline values (Theodorsson-Norheim, 1987). EC₅₀ values between groups were compared using the Mann-Whitney U test; significance was accepted at P ≤ 0.05.

**Drugs.** Sibutramine and its metabolites (BTS 54 505 and BTS 54 354) were obtained from Knoll Ltd. Research and Development (Nottingham, UK). For the in vivo studies, sibutramine was dissolved in 0.5 ml of sterile, isotonic 0.9% saline (154 mM sodium chloride) for i.p. injection; the vehicle consisted of 0.5 ml of sterile 0.9% saline (i.p.), buffered to pH 5.5 (i.p.). Phentolamine mesylate, (+)-propranolol HCl, methoxamine HCl, and (-)-isoprenaline HCl were obtained from Sigma Chemical (Poole, Dorset, UK). Drugs were dissolved in 0.5 ml of sterile, 0.9% saline (154 mM sodium chloride) and injected i.v.

For the in vitro experiments, the chemicals used included U46619 (Cayman Chemical, Ann Arbor, MI), and NE bitartrate (Sigma Chemical). Sibutramine, BTS 54 505, and BTS 54 354 were prepared on the day of the experiment and dissolved in 0.5 ml of 2% dimethyl sulfoxide and 0.5 ml of deionized, distilled water. Successive dilutions were made using deionized, distilled water. Vehicles for sibutramine and both metabolites were prepared using 0.5 ml of 2% dimethyl sulfoxide and 0.5 ml of deionized, distilled water; successive dilutions were made using deionized, distilled water. NE was dissolved in a 23 mM EDTA solution, which was also used for dilutions. In addition, EDTA (23 mM) was added to the buffer solution for all experiments involving the addition of NA, to act as an antioxidant. The composition of the PSS was 119 mM NaCl, 24 mM NaHCO₃, 4.7 mM KCl, 1.17 mM MgSO₄·7H₂O, 1.25 mM CaCl₂, 5.5 mM glucose.

**Results**

**In Vivo Hemodynamic Studies**

**Cardiovascular Effects of Sibutramine (Fig. 1).** The baseline values for resting cardiovascular variables, i.e., before the administration of sibutramine or vehicle, for the three groups of Sprague-Dawley were compared. They were not significantly different (data not shown; P > 0.05, Kruskal-Wallis test). Although recordings were made continuously over 6 h, the most marked hemodynamic changes after the administration of sibutramine (0.9, 3, or 9 mg kg⁻¹ i.p.) took place within the first 10 min of drug administration. Therefore, a detailed examination of the events occurring within this time period was undertaken, and these are the results shown in Fig. 1. Effects of sibutramine over the 6-h period after administration can be seen in Figs. 2 to 4.

Relative to baseline values, sibutramine at doses of 0.9, 3, or 9 mg kg⁻¹ i.p. produced significant increases in HR (44 ± 7, 48 ± 12, and 62 ± 12 beats min⁻¹, respectively) and MAP (17 ± 2, 20 ± 3, and 18 ± 3 mm Hg, respectively) 2 min after drug administration. These events were associated with significant reductions in celiac (−26 ± 4, −26 ± 4, and −21 ± 5%, respectively) and mesenteric (−31 ± 5, −46 ± 5, and −35 ± 7%, respectively) vascular conductances, and increases in hindquarters vascular conductance (27 ± 4, 18 ± 7, and 26 ± 10%, respectively). As a result, sibutramine caused decreases in celiac and mesenteric Doppler shifts (an index of blood flow) and an increase in hindquarters Doppler shift, apparent at each dose of the drug. All of the hemodynamic changes outlined above were significantly different from those seen after vehicle administration, but there were no significant differences between the integrated (0–10-min) hemodynamic responses to the different doses of sibutramine.
Effects of Sibutramine in the Presence of Phentolamine and/or Propranolol (Figs. 2–4). The baseline values for resting cardiovascular variables, i.e., before the administration of any drugs, for the four groups of Sprague-Dawley rats were not significantly different (data not shown; $P > 0.05$, Kruskal-Wallis test).

Group 1, saline (0.4 ml kg$^{-1}$ i.v. bolus, 0.4 ml kg$^{-1}$ h$^{-1}$ infusion i.v.). The cardiovascular responses to sibutramine (9 mg kg$^{-1}$) in the presence of saline were as described above, namely, tachycardia, a rise in MAP, vasoconstriction in the celiac and mesenteric vascular beds, and hyperemic vasodilatation in the hindquarters (Figs. 2–4).

Group 2, phentolamine (1 mg kg$^{-1}$ i.v. bolus, 1 mg kg$^{-1}$ h$^{-1}$ infusion i.v.). Thirty minutes after the onset of phentolamine administration, there was tachycardia, hypotension, decreases in celiac and mesenteric Doppler shift, and increases in hindquarters Doppler shift and vascular conductance (Fig. 2). In the presence of phentolamine, sibutramine caused no further tachycardia and no further hindquarters vasodilatation, but there was a fall in MAP associated with celiac and mesenteric vasodilatations (Fig. 2). The mesenteric vasodilator response to sibutramine under these conditions seemed to be biphasic with an early, marked increase (0–20 min) and a later, gradual rise (120–360 min) (Fig. 2).

Group 3, propranolol (1 mg kg$^{-1}$ i.v. bolus, 0.5 mg kg$^{-1}$ h$^{-1}$ infusion i.v.). Thirty minutes after the onset of propranolol administration, there was bradycardia, a modest rise in MAP, and mesenteric vasoconstriction (Fig. 3). In the presence of propranolol, sibutramine had little effect on HR but caused a significant increase in MAP, associated with reductions in celiac and mesenteric Doppler shifts and vascular conductances, and a slight increase in hindquarters Doppler shift and vascular conductance (Fig. 3).

Group 4, phentolamine plus propranolol (1 mg kg$^{-1}$ i.v. bolus, 1 mg kg$^{-1}$ h$^{-1}$ + 0.5 mg kg$^{-1}$ h$^{-1}$ infusion i.v.). Thirty minutes after the onset of combined administration of phentolamine and propranolol, there was modest bradycardia, hypotension, mesenteric vasoconstriction, and hindquarters vasodilatation (Fig. 4). In the presence of phentolamine and propranolol, sibutramine caused a modest tachycardia and depressor response, accompanied by variable changes in celiac and mesenteric Doppler shift, but substantial increases in celiac and mesenteric vascular conductances (Fig. 4). In the hindquarters, sibutramine caused an increase in vascular conductance, but had little effect on Doppler shift (Fig. 4).

Intergroup Comparisons. Intergroup comparisons were made on the integrated (0–10 min) responses to sibutramine in the various conditions (Table 1).

In the presence of phentolamine, or propranolol, or the combination of phentolamine plus propranolol, the sibutramine-induced tachycardia was less. Phentolamine, alone or with propranolol, converted the sibutramine-induced pressor response into a depressor response, and under these conditions, there was vasodilatation, rather than vasoconstriction, in the celiac and mesenteric vascular beds. The celiac vasodilatation was greater in the presence of phentolamine plus propranolol than in the presence of phentolamine alone, whereas the mesenteric vasodilatation was greater with phentolamine alone than in the presence of phentolamine...
plus propranolol (Table 1). The integrated increase in hindquarters vascular conductance in response to sibutramine was not significantly affected by phentolamine alone, or by phentolamine plus propranolol, but it was inhibited by propranolol alone. However, only in the presence of saline was the hindquarters vasodilator effect of sibutramine associated with a substantial increase in flow.

**In Vitro Studies**

There were no statistical differences in baseline vessel diameter measurements, either before the addition of U46619, or in the absolute maximal responses to U46619, or after maximal dilatation with calcium-free PSS (data not shown).

**Effects of Sibutramine, BTS 54 505, or BTS 54 354 on Vessel Diameter.** Before the addition of sibutramine (n = 6), BTS 54 505 (n = 7), BTS 54 354 (n = 7), or vehicle (n = 6), vascular diameter was reduced by 43 ± 3.55 ± 4.51 ± 5, and 49 ± 4% of the initial diameter with U46619, respectively. The concentration of U46619 required to produce this reduction in diameter varied between 0.1 and 0.3 μM.

Figure 5 shows the mean data for the responses of pressurized mesenteric small arteries to either sibutramine, BTS 54 505, BTS 54 354, or vehicle. Over a concentration range of 10 nM to 3 μM, neither sibutramine nor its metabolites had any effect on vessel diameter. However, at the highest concentration used (10 μM), sibutramine, BTS 54 505, and BTS 54 354 produced significant vasodilatations (38 ± 11, 39 ± 9, and 32 ± 9% of the maximum relaxed diameter, respectively), which were not observed in vessels exposed to vehicle (Fig. 5).

**Effects of Sibutramine, BTS 54 505, or BTS 54 354 on Responses to NE.** Sibutramine (n = 7), BTS 54 505 (n = 6), BTS 54 354 (n = 7), and their respective vehicles, over a concentration range of 10 nM to 3 μM, had no direct vasoconstrictor effects in mesenteric small arteries. Sibutramine (3 μM) had no effect on responses of mesenteric small arteries to NE (EC40 values of 6.6 ± 0.1 and 6.5 ± 0.2 for the sibutramine and its vehicle group, respectively; Fig. 6). In vessels exposed to either BTS 54 505 (3 μM) or vehicle, NE produced similar responses (EC40 values of 6.8 ± 0.2 and 6.8 ± 0.2, respectively; Fig. 6). In the presence of BTS 54 354 (3 μM), responses to NE were enhanced and produced a significant leftward shift in the cumulative concentration response curve to NE, compared with vehicle (EC40 values of 7.0 ± 0.1 and 6.2 ± 0.3, respectively; Fig. 6).

**Discussion**

The results showed that, in conscious rats, i.p. administration of sibutramine produced significant tachycardic and pressor effects, together with vasoconstriction in the celiac and mesenteric vascular beds and vasodilatation in the hindquarters. Further experiments showed that the overt cardio-
vascular effects of sibutramine were inhibited by adrenoceptor antagonists, suggesting an indirect involvement of adrenoceptors in the effects observed. The effects of NE reuptake inhibitors on sympathetic activity vary depending on their site of action. Thus, a central effect causes sympathoinhibition (clonidine-like), whereas a peripheral effect enhances sympathetically-mediated actions (Birkenfeld et al., 2002; Schroeder et al., 2002; Tank et al., 2003). Hence, the overall effect would be determined by the balance between these opposing actions. The present results, showing adrenoceptor-mediated tachycardic, pressor, and vasoconstrictor effects of sibutramine are most consistent with a predominance of inhibition of peripheral NE reuptake by sibutramine through its metabolites BTS 54 354 and BTS 54 505 (Cheetham et al., 1996). However, in this context, the β-adrenoceptor-mediated hyperemic vasodilator actions of sibutramine in the hindquarters are noteworthy. This vascular bed is particularly well endowed with β₂-adrenoceptors, which can mediate marked hyperemic vasodilator effects (Gardiner et al., 1990, 1991). However, it has been suggested that NE released from postganglionic terminals supplying the hindquarters vascular bed does not cause overt activation of the β₂-adrenoceptors in the rat (Guimaraes and Moura, 2001). Hence, we speculate our findings may indicate that the marked hyperemic vasodilator effect of sibutramine in the hindquarters may have been due to adrenomedullary epinephrine release. The tachycardic effect of sibutramine observed here is also likely to have involved enhancement of the effects of NE and/or epinephrine, in accordance with recent data from Birkenfeld et al. (2002), who demonstrated that, in humans, the tachycardic effects of sibutramine were abolished by metoprolol. Interestingly, Birkenfeld et al. (2002) found that metoprolol also inhibited the pressor effect of sibutramine in human, whereas we showed that in the presence of propranolol, the pressor and vasoconstrictor effects of sibutramine were still present. Whether this is a species difference, or due to some other aspect of experimental design (timing, route, and level of dosing) is not known.

If the above-mentioned interpretation is correct, it would be expected that combined inhibition of α-and β-adrenoceptor-mediated processes would abolish the cardiovascular changes after sibutramine. But, in the presence of propranolol and phentolamine, sibutramine administration still caused some hindquarters vasodilatation, suggesting that the latter may not all be β-adrenoceptor-mediated. However, examination of the flow (Doppler shift) changes in the different conditions showed that, in the presence of adrenoceptor antagonism, the short-lived hindquarters vasodilatation caused by sibutramine was not associated with a consistent increase in flow, whereas, in the absence of adrenoceptor antagonism, there was a clear hindquarters hyperemic response to sibutramine. Thus, it is feasible that the modest, short-lived, hindquarters vasodilatation after sibutramine, which occurred in the presence of phentolamine and propranol-
was an autoregulatory response to the fall in blood pressure caused by hyperemic vasodilatations in the splanchnic circulation (see below).

An additional point to note was that, in the presence of phentolamine, the tachycardic effect seen with sibutramine seemed to be blunted. If, as discussed above, the tachycardic response to administration of sibutramine was catecholamine-mediated, secondary to inhibition of NE reuptake, then it would not be expected that phentolamine would inhibit this process. Indeed, the reverse might be anticipated, due to antagonism of prejunctional α2-adrenoceptor-mediated autoinhibitory events. Hence, the most likely explanation for our finding is that the marked tachycardic effect of phentolamine itself resulted in there being little scope for a further increase in heart rate with sibutramine.

Interestingly, in the combined presence of phentolamine and propranolol, and in the presence of phentolamine alone, when the vasoconstrictor responses to sibutramine in the celiac and mesenteric vascular beds were blocked, hyperemic vasodilatations were uncovered. It was notable that the pattern of mesenteric vasodilatation after sibutramine in the presence of phentolamine and propranolol differed from that in the presence of phentolamine alone. In the latter condition, there was an early and a late phase, whereas in the

<table>
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<th>Values (mean ± S.E.M.) are areas under or over the curve (0–10 min). Superscript letters indicate significant (P &lt; 0.05; Kruskal-Wallis test) difference from the corresponding column.</th>
<th>Saline + Sibutramine</th>
<th>Phentolamine + Sibutramine</th>
<th>Phentolamine + Propranolol + Sibutramine</th>
<th>Propranolol + Sibutramine</th>
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<tr>
<td>HR (beats)</td>
<td>+390 ± 108&lt;sup&gt;BCD&lt;/sup&gt;</td>
<td>+197 ± 78&lt;sup&gt;AD&lt;/sup&gt;</td>
<td>+104 ± 33&lt;sup&gt;AD&lt;/sup&gt;</td>
<td>-115 ± 52&lt;sup&gt;ABC&lt;/sup&gt;</td>
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<tr>
<td>MAP (mm Hg)</td>
<td>+150 ± 11&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>-87 ± 7&lt;sup&gt;AD&lt;/sup&gt;</td>
<td>-115 ± 32&lt;sup&gt;AD&lt;/sup&gt;</td>
<td>+152 ± 28&lt;sup&gt;BC&lt;/sup&gt;</td>
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<td>Celiac Doppler shift (% min)</td>
<td>-142 ± 37&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>-74 ± 40&lt;sup&gt;AD&lt;/sup&gt;</td>
<td>+170 ± 42&lt;sup&gt;AD&lt;/sup&gt;</td>
<td>-150 ± 39&lt;sup&gt;BC&lt;/sup&gt;</td>
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<tr>
<td>Mesenteric Doppler shift (% min)</td>
<td>-150 ± 24&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>+419 ± 77&lt;sup&gt;ACD&lt;/sup&gt;</td>
<td>+105 ± 40&lt;sup&gt;AD&lt;/sup&gt;</td>
<td>-141 ± 34&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hindquarters Doppler shift (% min)</td>
<td>+446 ± 98&lt;sup&gt;BCD&lt;/sup&gt;</td>
<td>-162 ± 34&lt;sup&gt;ACD&lt;/sup&gt;</td>
<td>+66 ± 20&lt;sup&gt;AD&lt;/sup&gt;</td>
<td>+166 ± 47&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>Celiac vascular conductance (% min)</td>
<td>-231 ± 38&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>+140 ± 40&lt;sup&gt;ACD&lt;/sup&gt;</td>
<td>+346 ± 50&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>-232 ± 37&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mesenteric vascular conductance (% min)</td>
<td>-231 ± 27&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>+525 ± 77&lt;sup&gt;ACD&lt;/sup&gt;</td>
<td>+284 ± 82&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>-213 ± 43&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hindquarters vascular conductance(% min)</td>
<td>+281 ± 3&lt;sup&gt;D&lt;/sup&gt;</td>
<td>+148 ± 89&lt;sup&gt;D&lt;/sup&gt;</td>
<td>+231 ± 54&lt;sup&gt;D&lt;/sup&gt;</td>
<td>-92 ± 41&lt;sup&gt;ABC&lt;/sup&gt;</td>
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former condition (phentolamine plus propranolol) there was only a relatively short-lived early response, which was smaller than in the presence of phentolamine alone. One interpretation of these findings is that \(\beta\)-adrenoceptor-mediated vasodilatation contributed to the early response and was solely responsible for the later phase. However, there is little evidence for overt \(\alpha\)-adrenoceptor-mediated effects in the mesenteric vasculature (Gardiner et al., 1991). Moreover, in the celiac vascular bed, the situation was different, inasmuch as the sibutramine-induced vasodilatation was greater with combined \(\alpha\)- and \(\beta\)-adrenoceptor antagonism than with \(\alpha\)-adrenoceptor antagonism alone. It is feasible that direct and/or indirect activation of the renin-angiotensin system after sibutramine administration resulted in angiotensin-mediated vasoconstriction that served to limit the vasodilatation in the celiac vascular bed and that propranolol suppressed renin release (Buhler et al., 1972). If this was the case, however, it is not clear why such an influence was restricted to the celiac vascular bed, because it is known that angiotensin II is a very potent mesenteric vasoconstrictor (Gardiner et al., 1993). Clearly, this is speculation and would require further experimentation to support or refute.

The mechanism(s) underlying the vasodilator effect of sibutramine seen in the presence of phentolamine and propranolol is (are) unknown. Although it could be suggested that it was a local, possibly toxic, effect of i.p. injection of sibutramine, this seems unlikely for two reasons. First, the effect was more marked in the mesenteric than in the celiac vascular bed, whereas both would have been exposed to any local effects of sibutramine. Second, it seems unlikely that a toxic action would have given rise to the biphasic, differential changes in mesenteric and celiac hemodynamics. To determine whether sibutramine and/or its metabolites had any direct vascular effects in vitro studies were performed on rat isolated, mesenteric small arteries.

At high concentrations, sibutramine and its metabolites produced an increase in vessel diameter, in preconstricted arteries. Thus, the mesenteric vasodilator effect of sibutramine uncovered in vivo in the presence of adrenoceptor antagonism could have been a direct action of the drug or its metabolites affecting vascular smooth muscle tone by an, as yet, unidentified process. This effect is not likely to have been the result of an action of sibutramine or its pharmacologically active metabolites directly at \(\alpha\)- or \(\beta\)-adrenoceptors because these compounds have no affinity for these receptor subtypes (Heal and Cheetham, 1997).

The finding that sibutramine did not cause a vasoconstrictor response, either in the absence of constrictor tone, or in

**Fig. 5.** Responses to sibutramine \((n = 6)\), BTS 54 505 \((n = 7)\), BTS 54 354 \((n = 7)\), or vehicle \((n = 6)\) in mesenteric small arteries preconstricted with U46619. Each point represents mean \(\pm\) S.E.M. and is expressed as a percentage of the maximum possible vessel relaxation \((*, P < 0.05\) versus baseline, Friedman’s test).

**Fig. 6.** Responses to NA in mesenteric small arteries after exposure to either sibutramine (closed symbols; \(n = 7)\) or vehicle (open symbols; \(n = 8)\) (A), or BTS 54 505 (closed symbols; \(n = 6)\) or vehicle (open symbols; \(n = 6)\) (B), or BTS 54 354 (closed symbols; \(n = 7)\) or vehicle (open symbols; \(n = 6)\) (C). Each point represents mean \(\pm\) S.E.M. and is expressed as a percentage reduction in vessel diameter \((*, P < 0.05,\) Mann-Whitney U test).
the presence of U46619-induced tone, supports the conclusions drawn from the in vivo experiments, i.e., that the vasoconstrictor effect of sibutramine in vivo was an indirect effect of the drug. Given the known pharmacology of sibutramine as a weak inhibitor of NE reuptake (see Introduction), and the present finding that sibutramine did not enhance responses to NE in isolated mesenteric arteries, it seems most likely that the effects observed in vivo were due to the actions of one or more of its metabolites. However, the observation that only BTS 54 354 potentiated the vasoconstrictor effects of NE is puzzling, because the metabolites have almost equal potencies as reuptake inhibitors of NE, i.e., $K_i$ values of 4.9 nM for BTS 54 505 and 2.5 nM for BTS 54 354 (Cheetham et al., 1996).

In summary, the findings from this study are consistent with adrenergic activity playing an important role in the regional hemodynamic responses after administration of sibutramine. The underlying mechanism for the overt cardiovascular changes after sibutramine is most likely to be inhibition of the peripheral NE transporter by its metabolites, causing $\alpha$-adrenergic-mediated celiac and mesenteric vasoconstrictions, and $\beta$-adrenergic-mediated hindquarters vasodilatation and tachycardia. In addition, although it is feasible that central sympatholytic (clonidine-like) actions of sibutramine are involved (Birkenfeld et al., 2002), it is not obvious why these should be unmasked by phentolamine, which antagonizes $\alpha_2$-adrenoceptors. Therefore, we suggest that the mesenteric vasodilator response to sibutramine, seen in the presence of phentolamine, is due to a direct effect of the drug and/or its metabolites on vessel tone. Moreover, although it is feasible that some of the residual actions of sibutramine seen in the presence of adrenergic antagonists are due to inhibition of 5-HT reuptake, we believe this is unlikely because they are not like the cardiovascular actions of 5HT in our experimental model (Gardiner et al., 2002). In this context, it is of interest that the NE reuptake inhibitor nisoxetine resembles sibutramine in causing mesenteric and celiac vasoconstriction (Woolard et al., 2001).

The present work considered only the acute effects of sibutramine, and its cardiovascular actions might differ with chronic administration, as used in a clinical context. Furthermore, it remains to be determined whether the hemodynamic and antiappetitive effects of sibutramine and/or its metabolites can be separated, such that effects on obesity can be achieved without raising blood pressure.

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References

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