



# Sphingosine-1-phosphate reduces rat renal and mesenteric blood flow *in vivo* in a pertussis toxin-sensitive manner

\*<sup>1</sup>Angela Bischoff, <sup>1</sup>Peter Czyborra, <sup>2</sup>Dagmar Meyer zu Heringdorf, <sup>2</sup>Karl H. Jakobs & <sup>1</sup>Martin C. Michel

<sup>1</sup>Department of Medicine, Universitätsklinikum Essen, 45122 Essen, Germany and <sup>2</sup>Institute of Pharmacology, Universitätsklinikum Essen, 45122 Essen, Germany

**1** Sphingolipids such as sphingosine-1-phosphate (SPP) and sphingosylphosphorylcholine constrict isolated rat intrarenal and mesenteric microvessels *in vitro*. The present study investigates their effects on the cardiovascular system *in vivo* in anaesthetized rats.

**2** The animals were given intravenous or intrarenal arterial bolus injections of sphingolipids (0.1–100  $\mu\text{g kg}^{-1}$ ) with subsequent measurements of mean arterial pressure, heart rate and renal and mesenteric blood flows (RBF, MBF) using a pressure transducer and electromagnetic flow probes, respectively.

**3** Intravenous injection of SPP rapidly (within 30 s), transiently and dose-dependently reduced RBF (maximally  $-4.0 \pm 0.3 \text{ ml min}^{-1}$ ) and MBF (maximally  $-1.4 \pm 0.2 \text{ ml min}^{-1}$ ), without affecting mean arterial pressure or heart rate. Other sphingolipids had no significant effect.

**4** Intrarenal arterial SPP administration caused greater blood flow reductions (maximally  $-6.4 \pm 0.3 \text{ ml min}^{-1}$ ) than systemic administration. Upon intrarenal administration, sphingosylphosphorylcholine also lowered RBF (maximally  $-2.8 \pm 0.6 \text{ ml min}^{-1}$ ), while the other sphingolipids remained without effect.

**5** Pretreatment with pertussis toxin (PTX, 10  $\mu\text{g kg}^{-1}$ ) 3 days before the acute experiment abolished the SPP-induced reductions of RBF and MBF.

**6** These data demonstrate, that SPP is a potent vasoconstrictor *in vivo*, particularly in the renal vasculature, while the other structurally related sphingolipids had little if any effects. The PTX-sensitivity strongly suggests that the effects of SPP on renal and mesenteric blood flow are mediated by receptors coupled to  $G_i$ -type G-proteins.

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**Abbreviations:** BSA, bovine serum albumine; HR, heart rate; MAP, mean arterial pressure; MBF, mesenteric blood flow; PTX, pertussis toxin; RBF, renal blood flow; SPP, sphingosine-1-phosphate; SPPC, sphingosylphosphorylcholine

## Introduction

Sphingosine-1-phosphate (SPP) has been identified in human serum and plasma, where it occurs in concentrations of  $484 \pm 82$  and  $191 \pm 79 \text{ nmol l}^{-1}$ , respectively (Yatomi *et al.*, 1997a). One source of extracellular SPP are platelets, which release the lipid upon activation (Yatomi *et al.*, 1995; 1997a). SPP and related sphingolipids can affect the function of cultured cells in many ways *via* receptor-mediated and receptor-independent mechanisms (Hla *et al.*, 1999; Meyer zu Heringdorf *et al.*, 1997; Van Brocklyn *et al.*, 1998). Furthermore, it has been demonstrated that SPP, sphingosylphosphorylcholine (SPPC) and other sphingolipids can constrict mesenteric and intrarenal microvessels *in vitro* in a concentration-dependent manner (Bischoff *et al.*, 2000). The vasoconstrictor effect of SPPC on mesenteric microvessels was stereospecific, sensitive to pertussis toxin (PTX) and dependent on the influx of extracellular  $\text{Ca}^{2+}$  but not on the presence of a functionally intact endothelium (Bischoff *et al.*, 2000). Despite a large number of recent studies of sphingolipid effects on cultured cells (Bornfeldt *et al.*, 1995; Goetzl & An, 1998; Im *et al.*, 1997; MacDonell *et al.*, 1998; Mattie *et al.*, 1994; Meyer zu Heringdorf *et al.*, 1996; van Koppen *et al.*, 1996), little is known about their effects *in vivo*, particularly with regard to

the cardiovascular system. Therefore, the cardiovascular responses to SPP and other sphingolipids in anaesthetized rats *in vivo* have been investigated.

## Methods

### *Animal surgery and experimental protocol for anaesthetized rat experiments*

All animal studies were performed in accordance with the NIH guidelines for the care and use of laboratory animals following approval by the state animal welfare board. Male Wistar rats (strain: Hsd/Cpb:WU; 272–430 g in study 1; 300–496 g in study 2; 320–470 g in study 3) were obtained from Harlan (Borchum, Germany). In studies 1 and 2 naive rats were used. In study 3 rats were pretreated with PTX (10  $\mu\text{g kg}^{-1}$ ) or its vehicle 3 days before the experiment. For this the rats were injected with PTX or its vehicle under a light ketamine anaesthesia (100  $\text{mg kg}^{-1}$  i.p.) *via* the jugular vein. For haemodynamic measurements, the rats were prepared as previously described (Bischoff *et al.*, 1996) with minor modifications. Briefly, the rats were anaesthetized with a single dose of thiobutobarbitone (Inactin<sup>®</sup>, 100  $\text{mg kg}^{-1}$  i.p.). The animals were placed on a heating pad to maintain the body temperature at 37°C. Following tracheotomy to facilitate

\*Author for correspondence at: Nephrologisches Labor, Universitätsklinikum, Hufelandstr. 55, 45122 Essen, Germany; E-mail: angela.bischoff@uni-essen.de

ventilation, the left femoral artery was cannulated for monitoring mean arterial pressure (MAP) *via* a Statham pressure transducer. The femoral vein was catheterized for systemic infusion and bolus injections. Following an abdominal midline incision, the connective tissue was carefully dissected from the right renal artery and the cranial mesenteric artery, and electromagnetic blood flow sensors (Skalar MDL 1401, Föhr Medical Instruments GmbH, Seeheim/Oberbeerbach, Germany) were placed on the vessels for monitoring heart rate (HR), renal blood flow (RBF), and mesenteric blood flow (MBF). In some experiments, a catheter was placed into the suprarenal artery for intrarenal bolus injections. The signals from the flow sensors and the pressure transducer were continuously recorded online using the HDAS haemodynamic data acquisition system (Dept. of Bioengineering, Rijksuniversiteit Limburg, Maastricht, The Netherlands).

Following completion of the preparation, the animals were allowed a 120 min recovery period, during which 0.9% saline was infused *via* the femoral vein (study 1) or femoral vein plus suprarenal artery (study 2) at a rate of  $60 \mu\text{l min}^{-1}$ . In study 3, the rats were allowed 65 min of recovery. Thereafter, the effectiveness of the PTX treatment was tested with three bolus injections of neuropeptide Y (1, 3 and  $10 \mu\text{g kg}^{-1}$ ) in 5 min intervals 60 min before the start of the SPP bolus injections. The fluid substitution was started immediately after completion of the surgery and maintained until the end of the experiment except during systemic bolus injections.

Systemic ( $1-100 \mu\text{g kg}^{-1}$ ) and intrarenal bolus injections ( $0.1-100 \mu\text{g kg}^{-1}$ ) of sphingolipids were administered in a volume of  $100 \mu\text{l}$  per  $100 \text{g}$  body weight and injected within 30 s. Bolus injections were given in 40 min intervals in studies 1 and 2 and in 3 min intervals in study 3. During the experimental period, MAP, HR, RBF and MBF were monitored every min from 3 min before until 10 min (studies 1 and 2) or 3 min (study 3) after the bolus injection. The maximum response to sphingolipid injection occurred within 20 s. At the end of the experiment, the rats were killed with an overdose of anaesthetic.

#### Data analysis

The averages of the haemodynamic parameters during the last 3 min before the first bolus injection were taken as baseline values (see Results); basal values remained stable throughout the experiments (data not shown). All other data are expressed as alterations relative to the baseline values. Data are shown as mean  $\pm$  s.e. mean for  $n$  experiments. Statistical significance of differences was determined by unpaired two-tailed *t*-tests when two groups were compared, e.g. for baseline values. When multiple groups were compared, one-way analysis of variance followed by a multiple comparison-corrected Dunnett test was performed. Statistical significance of the overall treatment effect (time or dose) relative to control animals was determined by a two-way analysis of variance. All statistical calculations were performed with the Prism program (GraphPAD Software, San Diego, CA, U.S.A.) and  $P < 0.05$  was considered significant.

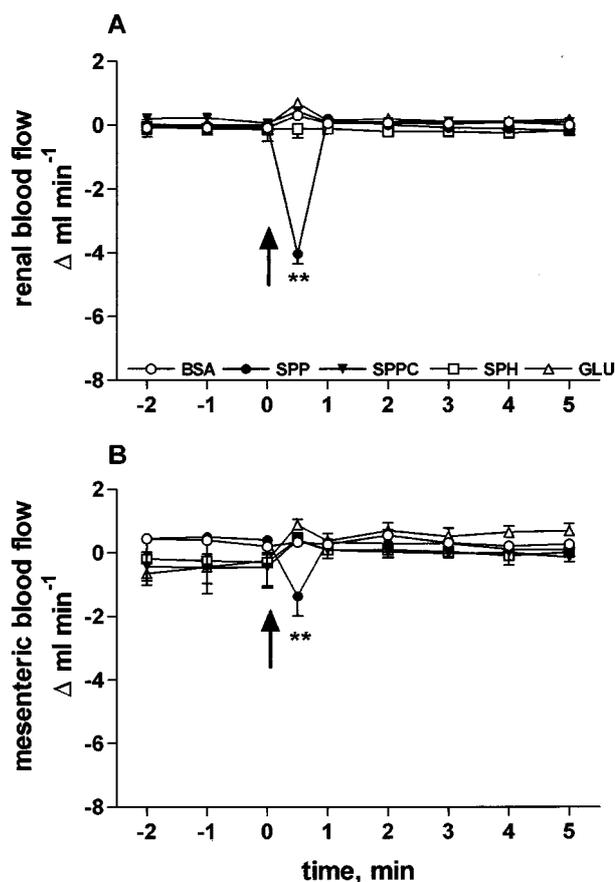
#### Chemicals

SPP and SPPC were obtained from Biomol (Hamburg, Germany); sphingosine and glucopsychosine were obtained from Matreya (Bad Homburg, Germany). For all studies, sphingolipids were dissolved in methanol, dried in a SpeedVac concentrator and redissolved in  $1 \text{ mg ml}^{-1}$  bovine serum albumine (BSA). Thiobutabarbitone (Inactin<sup>®</sup>) was obtained from RBI (Natick, MA, U.S.A.), and PTX was from ICN

(Eschwege, Germany). PTX was dissolved in a phosphate buffer ( $\text{mol l}^{-1}$ ):  $0.5 \text{ NaCl}$ ,  $0.1 \text{ NaH}_2\text{PO}_4$  and  $0.1 \text{ Na}_2\text{HPO}_4$ ; this buffer served as vehicle.

## Results

In study 1, the haemodynamic effects of systemically administered SPP, SPPC, sphingosine and glucopsychosine were tested. At the end of the equilibration period, basal MAP, HR, RBF and MBF were  $112 \pm 3 \text{ mmHg}$ ,  $353 \pm 6 \text{ b.p.m.}$ ,  $7.8 \pm 0.3 \text{ ml min}^{-1}$  and  $9.0 \pm 0.3 \text{ ml min}^{-1}$ , respectively ( $n = 31$  each). During constant saline infusion, these values remained stable throughout the experimental period (data not shown). Five groups of 4–8 rats received nine systemic bolus injections (1, 3, 5, 10, 20, 40, 60, 80 and  $100 \mu\text{g kg}^{-1}$ ) of either SPP, SPPC, sphingosine, glucopsychosine or their vehicle (BSA  $1 \text{ mg ml}^{-1}$ ). Intravenous bolus injections of SPP caused rapid, transient (Figure 1) and dose-dependent (Figure 2) reductions of RBF and MBF by up to 50 and 16%, respectively (in the time course experiments  $P < 0.01$  for SPP vs vehicle at the 30 s time point for either RBF or MBF; in the dose-response experiments  $P < 0.01$  for the overall treatment effect of SPP vs vehicle for either RBF or MBF). Half-maximal and maximal reductions in both RBF and MBF were seen at about 5 and  $60 \mu\text{g kg}^{-1}$  of SPP, respectively (Figure 2). The reductions



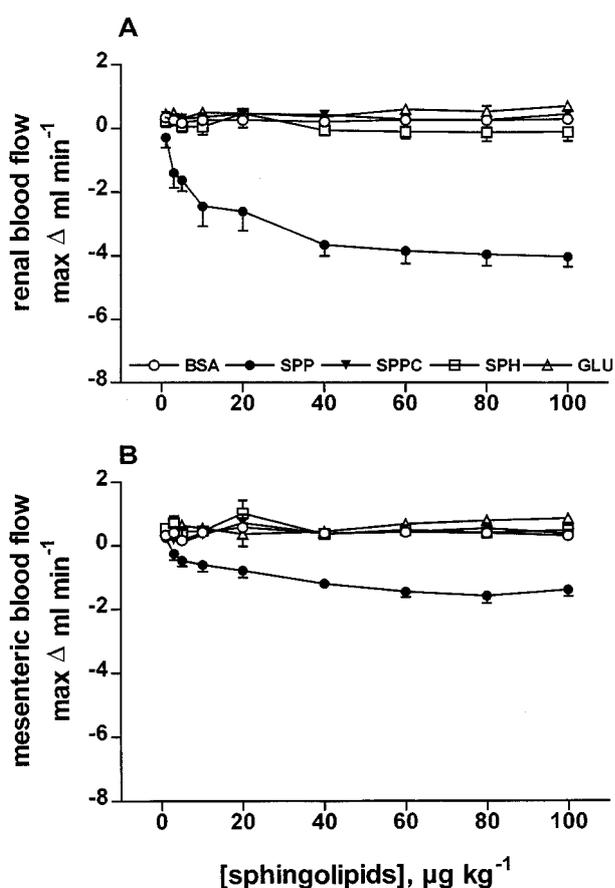
**Figure 1** Effects of  $100 \mu\text{g kg}^{-1}$  intravenously injected sphingolipids on RBF and MBF in anaesthetized rats. Data are mean  $\pm$  s.e. mean ( $n = 4-8$ ) of alterations relative to baseline values. Start of the bolus injections was indicated by an arrow. RBF (A) and MBF (B) were rapidly and transiently reduced by injection of  $100 \mu\text{g kg}^{-1}$  of SPP ( $4.0 \pm 0.3$  and  $1.4 \pm 0.2 \text{ ml min}^{-1}$ , respectively,  $** P < 0.01$  in a one-way analysis of variance). BSA, SPPC, sphingosine (SPH) or glucopsychosine (GLU) had no effect.

occurred immediately after termination of the injection, and were reversible within the next minute and were not accompanied by detectable alterations of MAP or HR (Figure 3). In contrast to SPP, intravenous injections of SPPC, sphingosine or glucopsychosine in doses up to  $100 \mu\text{g kg}^{-1}$  or vehicle did not alter RBF or MBF (Figures 1 and 2).

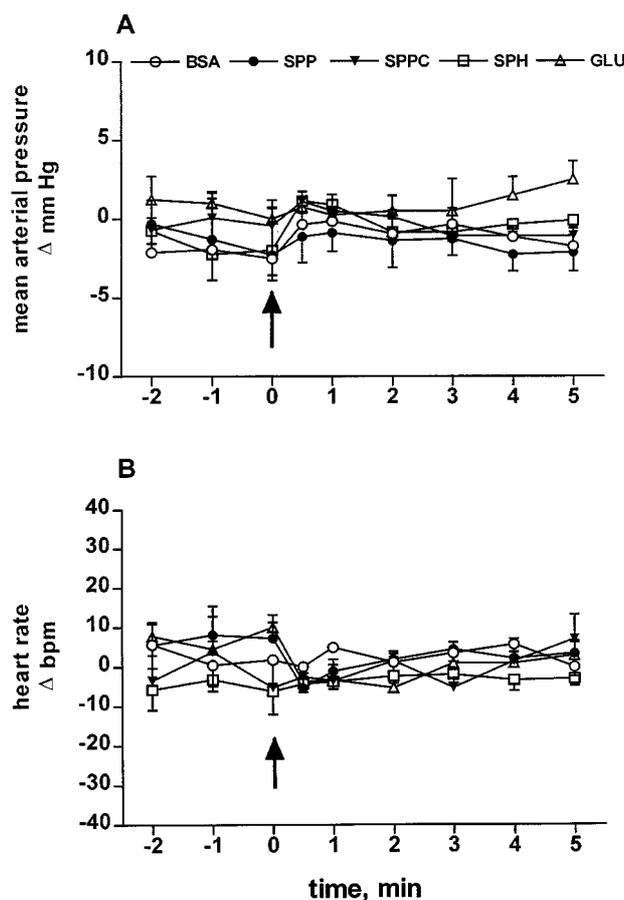
To test whether the lack of effect of SPPC, sphingosine and glucopsychosine was related to dilution in the systemic circulation, the sphingolipids were administered intrarenally in study 2. Despite the more extensive preparation of the rats, basal values of MAP ( $113 \pm 3$  mmHg), HR ( $345 \pm 6$  b.p.m.), RBF ( $7.2 \pm 0.3$  ml  $\text{min}^{-1}$ ), and MBF ( $8.3 \pm 0.5$  ml  $\text{min}^{-1}$ ;  $n=25$  each) were very similar to the values in study 1 and remained stable throughout the experiment. Four groups of 5–7 rats received seven intrarenal bolus injections of sphingolipids (0.1, 0.3, 1, 3, 10, 30 and  $100 \mu\text{g kg}^{-1}$ ). Intrarenal injection of SPP rapidly, transiently (Figure 4) and dose-dependently (Figure 5) lowered RBF and MBF (in the time course experiments  $P < 0.01$  for SPP and SPPC vs vehicle at the 30 s time point for RBF and for SPP vs vehicle for MBF; in the dose-response experiments  $P < 0.01$  for the overall treatment effect of SPP vs vehicle for either RBF or MBF). In comparison with the effects of systemic injection, maximal reductions of RBF lasted longer, were almost twice as large (maximum reduction  $4.0 \pm 0.3$  vs  $6.4 \pm 0.3$  ml  $\text{min}^{-1}$ ,  $P = 0.0001$ ) and appeared to occur with smaller half-maximally effective SPP

doses (approximately  $0.3 \mu\text{g kg}^{-1}$ , Figure 5). Upon intrarenal administration, RBF reductions also became detectable for SPPC, while sphingosine and BSA remained without effect (Figure 4). None of the sphingolipids affected MAP or HR upon intrarenal administration (data not shown).

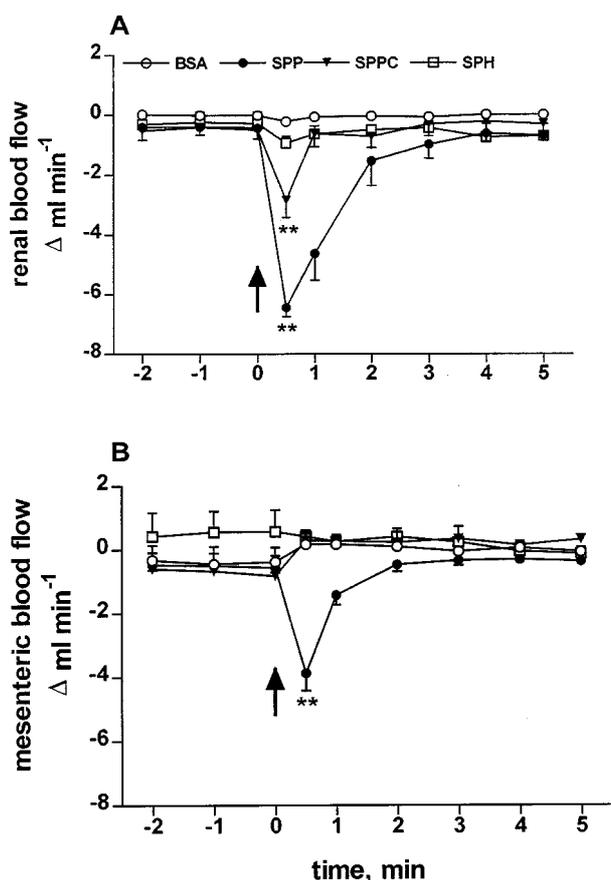
In study 3, two groups of rats ( $n=5-6$  each) were investigated, one pretreated with vehicle and the other pretreated with PTX ( $10 \mu\text{g kg}^{-1}$ ) 3 days before the acute experiment. Pretreatment with PTX did not significantly alter basal values of MAP ( $101 \pm 10$  vs  $98 \pm 4$  mmHg), HR ( $374 \pm 8$  vs  $359 \pm 11$  b.p.m.), RBF ( $6.2 \pm 0.2$  vs  $7.2 \pm 0.5$  ml  $\text{min}^{-1}$ ) or MBF ( $5.5 \pm 0.9$  vs  $4.7 \pm 1.0$  ml  $\text{min}^{-1}$ ). On the other hand, reduction of RBF caused by  $10 \mu\text{g kg}^{-1}$  neuropeptide Y was abolished in PTX-treated rats ( $1.9 \pm 0.4$  vs  $0.3 \pm 0.1$  ml  $\text{min}^{-1}$ ,  $n=5-6$ ,  $P = 0.0022$ ). SPP administered in seven consecutive systemic bolus injections (1, 3, 5, 10, 20, 30 and  $100 \mu\text{g kg}^{-1}$ ) via the femoral vein, did not markedly alter MAP (Figure 6A) or HR (data not shown) in vehicle- and PTX-pretreated rats and dose-dependently reduced RBF and MBF in vehicle-treated control rats ( $P < 0.0001$  for SPP vs vehicle for RBF and MBF; Figure 6B,C). In contrast, in PTX-pretreated rats SPP was without effects on RBF and MBF at any studied dose. To demonstrate the lack of non-specific effects of the experimental conditions of PTX treatment on vascular reactivity, a second group of rats was treated with the same PTX dose or vehicle ( $n=6$  each). Following equilibration these rats received intravenous bolus doses of methoxamine ( $40 \mu\text{g kg}^{-1}$ ) and



**Figure 2** Dose-response curves of intravenously injected sphingolipids on RBF and MBF in anaesthetized rats. Data are mean  $\pm$  s.e.mean ( $n=4-8$ ) of alterations relative to baseline values. RBF (A) and MBF (B) were dose-dependently reduced by SPP ( $P < 0.0001$  in a two-way analysis of variance) but not by BSA, SPPC, sphingosine (SPH) or glucopsychosine (GLU).



**Figure 3** Effects of  $100 \mu\text{g kg}^{-1}$  intravenously injected sphingolipids on MAP and HR in anaesthetized rats. Data are mean  $\pm$  s.e.mean ( $n=4-8$ ) of alterations relative to baseline values. Start of the bolus injections was indicated by an arrow. Injection of BSA, SPP, SPPC, sphingosine (SPH) or glucopsychosine (GLU) did not detectably alter MAP (A) or HR (B) up to  $100 \mu\text{g kg}^{-1}$ .

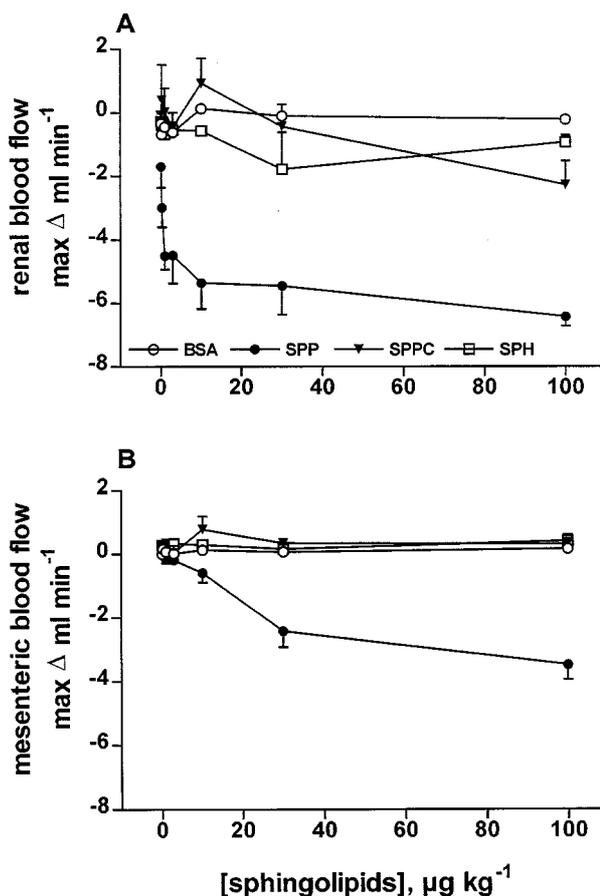


**Figure 4** Effects of  $100 \mu\text{g kg}^{-1}$  intrarenally injected sphingolipids on RBF and MBF in anaesthetized rats. Data are mean  $\pm$  s.e. mean ( $n=5-7$ ) of alterations relative to baseline values. Start of the bolus injections was indicated by an arrow. RBF (A) and MBF (B) were rapidly and transiently reduced by injection of SPP (\*\* $P < 0.01$  in a one-way analysis of variance); the blood flow reduction was more pronounced than upon intravenous injection. SPPC also reduced RBF but not MBF to a lower extent compared to SPP (\*\* $P < 0.01$  in a one-way analysis of variance). BSA and sphingosine (SPH) had no effects.

thereafter angiotensin II ( $1 \mu\text{g kg}^{-1}$ ). Methoxamine and angiotensin II-induced elevations of MAP ( $15 \pm 4$  vs  $18 \pm 2$  mmHg and  $48 \pm 8$  vs  $35 \pm 6$  mmHg, respectively) and reductions of RBF ( $1.0 \pm 0.1$  vs  $1.0 \pm 0.1$  ml min $^{-1}$  and  $3.6 \pm 0.5$  vs  $3.6 \pm 0.6$  ml min $^{-1}$ , respectively) were not significantly altered by PTX treatment.

## Discussion

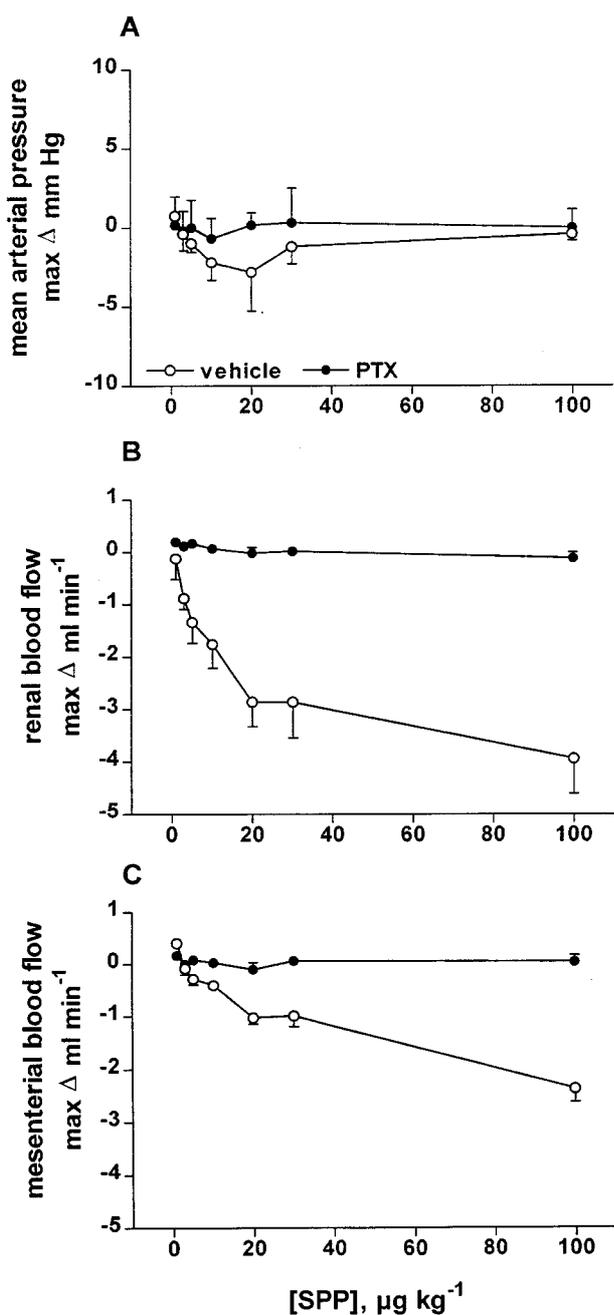
The sphingolipid SPP is stored in and released from human platelets (Yatomi *et al.*, 1995; 1997a), suggesting that it may be a biologically active agent in the cardiovascular system. Sphingolipid effects have been studied in various types of cultured cells including those related to the cardiovascular system. In cultured rat cardiomyocytes, for example, sphingolipids such as sphingosine modulate  $\text{Na}^+$  and  $\text{Ca}^{2+}$  currents (Yasui & Palade, 1996) and tumour necrosis factor-induced negative inotropic effects (Oral *et al.*, 1997). Furthermore, Bünemann *et al.* (1995) observed a SPP-induced activation of muscarinic  $\text{K}^+$  current in guinea-pig atrial myocytes. However, very little is known about the effects of sphingolipids on the cardiovascular system *in vivo*. It has also been reported that sphingolipids, including SPP and SPPC, cause vasoconstriction in rat isolated mesenteric and



**Figure 5** Dose-response curves of intrarenally injected sphingolipids on RBF and MBF in anaesthetized rats. Data are mean  $\pm$  s.e. mean ( $n=5-7$ ) of alterations relative to baseline values. RBF (A) and MBF (B) were dose-dependently reduced by SPP ( $P < 0.0001$  in a two-way analysis of variance) but not by BSA or sphingosine (SPH). SPPC had a small inhibitory effect at  $100 \mu\text{g kg}^{-1}$ .

intrarenal microvessels; these *in vitro* effects were dose-dependent, stereospecific and PTX-sensitive, suggesting a mediation *via* receptors coupled to  $\text{G}_i$ -type G-proteins (Bischoff *et al.*, 2000).

In the present study, systemic and intrarenal bolus injections of SPP dose-dependently reduced RBF and, to a smaller extent, MBF in anaesthetized rats. On the other hand, MAP and HR remained unaffected by any tested sphingolipid, indicating a preferential sphingolipid effect on the vasculature rather than the heart. The lack of MAP and HR alterations despite RBF and MBF reductions could be explained haemodynamically by a reduced resistance in other vascular beds and/or lowered cardiac output upon SPP administration; the latter could result from a reduced HR and/or a reduced stroke volume. A reduced HR cannot explain the lack of MAP change, since HR was unaltered in the present study and was even reported to be increased in a recent study with an isolated canine heart preparation. A reduced stroke volume, and hence a reduced cardiac output, should be considered, since SPP was a reported cause of coronary vasoconstriction and mild negative inotropic effects in an isolated canine heart preparation (Sugiyama *et al.*, 2000). SPP was also reported to activate the muscarinic  $\text{K}^+$  current in guinea-pig atrial cardiomyocytes (Bünemann *et al.*, 1995). Moreover, SPPC and sphingosine were reported to inhibit  $\text{Na}^+$  and  $\text{Ca}^{2+}$  channels in cardiomyocytes isolated from adult rats (Yasui & Palade, 1996), and sphingosine was reported to cause negative



**Figure 6** Dose-response curves of systemically injected SPP on MAP, RBF and MBF in PTX- and vehicle-treated anaesthetized rats. Data are mean  $\pm$  s.e. mean ( $n = 5-6$ ) of alterations relative to baseline values. SPP did not alter MAP (A), but dose-dependently reduced RBF (B) and MBF (C) in vehicle- but not in PTX-treated rats ( $P < 0.0001$  in a two-way analysis of variance).

inotropy in isolated feline cardiomyocytes (Oral *et al.*, 1997). Taken together, these data make a reduced cardiac output even with unchanged HR a likely cause of unaltered MAP, despite lowered RBF and MBF. However, the existence of SPP-induced vasodilatation in some vascular beds cannot be excluded, based on the present data, as an additional and/or alternative cause for unchanged MAP values upon SPP infusion. Moreover, differences between *in vitro* and *in vivo* studies should be considered when taking the above *in vitro* data on cardiac function into consideration as an explanation for the present *in vivo* findings.

The detectable SPP-induced alterations of local blood flows indicate that sphingolipids can cause vasoconstriction not only

*in vitro* (Bischoff *et al.*, 2000) but also *in vivo*. The pronounced SPP-induced RBF reduction may be surprising in light of the known autoregulation of RBF, but a similar situation has been described for another receptor coupling to PTX-sensitive G-proteins, the neuropeptide Y receptor (Bischoff *et al.*, 1996). The direct effect of SPP on the renal vasculature was further demonstrated by enhanced RBF lowering upon direct intrarenal SPP administration. Upon intrarenal administration, RBF reduction by SPPC also became detectable, which was not seen upon systemic treatment.

SPP and other sphingolipids are bioactive molecules that can act on signal transduction pathways as second messengers and also as ligands at G-protein-coupled receptors of the Edg-family (Goetzl & An, 1998; Hla *et al.*, 1999; Van Brocklyn *et al.*, 1998). Many of the Edg receptor-mediated sphingolipid actions, in contrast to the intracellular effects of these agents, are dependent on PTX-sensitive G<sub>i</sub>-type G-proteins (An *et al.*, 1997; 1999; Goetzl & An, 1998; Hla *et al.*, 1999; Liu *et al.*, 1999; Meyer zu Heringdorf *et al.*, 1997; Okamoto *et al.*, 1999; Van Brocklyn *et al.*, 1998; Zondag *et al.*, 1998). To determine whether the *in vivo* effects of SPP are receptor-mediated, their sensitivity to PTX have been tested. The experimental conditions of *in vivo* PTX treatment were effective since they abolished the vasoconstriction by neuropeptide Y, which acts *via* receptors coupled to PTX-sensitive G-proteins (Michel *et al.*, 1998), but did not cause obvious toxic effects since they did not affect vascular effects of angiotensin II or the  $\alpha_1$ -adrenoceptor agonist methoxamine. Pretreatment with PTX abolished SPP-induced vasoconstriction in anaesthetized rats. As PTX treatment also markedly attenuated the contractile effects of sphingolipids on microvessels *in vitro* (Bischoff *et al.*, 2000), these data strongly suggest that sphingolipid-induced vasoconstriction both *in vivo* and *in vitro* is apparently mediated by receptors coupled to G<sub>i</sub>-type G-proteins. Possible candidates are receptors belonging to the Edg-receptor family which are activated by SPP and SPPC. While Edg-receptors have been detected on cultured cells of cardiovascular origin and in several rat tissues (Yatomi *et al.*, 1997b), to the best of our knowledge no data exist on Edg-receptor distribution in blood vessels.

Despite the similarities between sphingolipid-induced vasoconstriction *in vitro* (Bischoff *et al.*, 2000) and *in vivo* (present study), several differences have also been noticed. First, sphingolipids had greater effects on RBF than on MBF *in vivo* but caused stronger vasoconstriction in mesenteric than in intrarenal microvessels. However, this difference should not be overinterpreted, since the physiological regulation of RBF is complex and the intralobar arteries used in the *in vitro* experiments represent only a part of the intrarenal resistance vasculature. More important, the order of agonist potency differed between the *in vitro* and the *in vivo* studies. *In vivo*, SPP had the greatest effects, SPPC was less active, and the other tested sphingolipids were inactive. In contrast, *in vitro* SPPC had the greatest effects, SPP was less active, and sphingosine and glucosylsphingosine had small but detectable effects (Bischoff *et al.*, 2000). One possible explanation for these differences might be a rapid degradation of SPPC and sphingosine *in vivo*, e.g. by a bloodborn esterase. On the other hand, it should be noted that the observed *in vivo* effects are in line with findings on sphingolipid responses in various types of cultured cells where SPP usually is the most potent agonist (Meyer zu Heringdorf *et al.*, 1996; van Koppen *et al.*, 1996). The development of selective and chemically stable agonists and/or antagonists for sphingolipid receptors will hopefully allow to resolve these discrepancies.

Taken together, the present data demonstrate that the sphingolipid SPP reduces RBF and, to a smaller extent, MBF in anaesthetized rats. This effect appears to occur *via* receptors coupled to G<sub>i</sub>-type G-proteins. To the best of our knowledge, this is the first report on sphingolipid action *via* G<sub>i</sub>-protein-coupled receptors *in vivo* and identifies sphingolipids as a novel endogenous regulator of cardiovascular function.

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