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Regional haemodynamic effects of platelet activating factor in the rat

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Abstract

The effects of platelet activating factor (PAF) on haemodynamics in the absence and presence of the potent PAF receptor antagonist TCV-309 {3-bromo-5-[*N*-phenyl-*N*-[2-[[2-(1,2,3,4-tetrahydro-2-isoquinolyl-carbonyloxy)ethyl]carbamoyl]ethyl]carbamoyl]-1-propylpyridinium nitrate} were studied by the microsphere technique in pentobarbitone-anaesthetized rats. I.v. infusion of the low dose PAF (0.05 μg kg^{-1} min^{-1}) did not significantly alter mean arterial pressure, cardiac output or total peripheral resistance but increased arterial conductances in the stomach, intestine, caecum and colon and reduced conductance in the spleen. I.v. infusion of the high dose of PAF (0.3 μg kg^{-1} min^{-1}) markedly reduced mean arterial pressure (−53 mm Hg) and cardiac output (−62%) and insignificantly increased total peripheral resistance. Arterial conductances in the lungs, stomach, intestine, caecum and colon, kidneys and spleen were reduced and those in the heart and muscle were increased. TCV-309 (10 μg kg^{-1}) abolished all changes in arterial pressure, cardiac output and total peripheral resistance and arterial conductances elicited by either the low or the high dose of PAF. The results show that a non-hypotensive dose of PAF caused vasodilatation of the gastrointestinal organs and vasoconstriction of the spleen. A high dose of PAF which markedly decreased arterial pressure and cardiac output caused vasodilatation of the heart and muscle and vasoconstriction of the lungs (bronchial), gastrointestinal organs, kidneys and spleen. All haemodynamic changes were blocked by TCV-309 indicating the involvement of PAF receptors.

Keywords: PAF (platelet activating factor); Hemodynamics; Blood flow, regional; PAF receptor antagonist; Microsphere

1. Introduction

Platelet activating factor (PAF), an ether-linked phospholipid, is released from many cell types in response to immune and non-immune stimuli and is implicated in endotoxic shock and anaphylaxis (see Braquet et al., 1987). In addition to platelet activation, PAF decreases blood pressure, cardiac output and peripheral vascular resistance (Goldstein et al., 1984; Sirén and Feuerstein, 1989; Bellan et al., 1992; Yamanaka et al., 1992). In anaesthetized dogs, a low dose of PAF (0.02 μg kg^{-1}) caused a transient (< 1 min) depressor response accompanied by reduced peripheral vascular resistance and increased cardiac output; however, a high dose (0.5 μg kg^{-1}) of PAF caused sustained hypotension (> 10 min), reduced cardiac output but increased vascular resistance (Yamanaka et al., 1992).

Close arterial administrations of PAF caused renal (Handa et al., 1991; Buckalew et al., 1993) and coronary (Sagach et al., 1992) vasodilatation. In rats, a high dose dilated the renal artery and abdominal aorta and constricted the mesenteric artery but a low dose vasodilated all three beds (Sirén and Feuerstein, 1989). In cats, i.v. bolus PAF increased pulmonary vascular resistance but reduced systemic resistance and resistances in the mesenteric, hindquarter and renal beds (Bellan et al., 1992).

In vitro studies show that PAF vasodilated preconstricted perfused rat mesenteric artery (Kamata et al., 1989; Chiba et al., 1990) and the response was inhibited by the PAF receptor antagonist CV-6209 (Kamata et al., 1989). In the isolated perfused rat heart, PAF produced vasodilatation and vasoconstriction (Man et al., 1990; Hu et al., 1991); the vasodilatation was blocked by CV-6209 and the constriction was blocked

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by the PAF receptor antagonists FR-900452, WEB 2086 and BN-50739 (Hu and Man, 1991) suggesting the existence of subtypes of PAF receptors in the coronary bed.

The vascular effect of PAF has only been studied in selected regional beds. There is as yet no published information on the relative organ or tissue sensitivity to the effects of PAF and the influence of PAF receptor antagonists on haemodynamics. We examined the effects of PAF on regional blood flow in anaesthetized rats and the influence of TCV-309. TCV-309 is long-acting and is 2, 67 and 537 times more potent than CV-6298, WEB2086 and CV-3988 in inhibiting PAF-induced hypotension (Terashita et al., 1992). As well, TCV-309 protected sensitized mice from death induced by PAF and anaphylactic stimulus, and protected rats from death induced by PAF and endotoxin.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (350–400 g) were anaesthetized with pentobarbitone (60 mg kg^{-1} i.p.). Body temperature was maintained at 37°C via a rectal thermometer and a heating pad connected to Thermistemp Temperature Controller (Model 71, Yellow Springs Instrument Co., Ohio, USA). Cannulae filled with heparinized normal saline (0.9% NaCl, 25 I.U. ml^{-1}) were inserted into the left ventricle via the right carotid artery for the injections of radioactively-labelled microspheres, and into the left iliac artery for blood withdrawal, as described in detail elsewhere (Pang, 1983). Cannulae were also inserted into the right iliac artery for the recording of mean arterial pressure by a pressure transducer (PD23DB, Gould, Statham, CA, USA) and into the right femoral vein for the administration of drugs or vehicle. Mean arterial pressure was continuously monitored from a cannula inserted into an iliac artery and recorded by a Grass Polygraph (Model RPS 7C8). Heart rate was determined electronically by a tachograph (7P4G Grass).

2.2. Microspheres studies

A well-stirred suspension (200 μl) containing 30 000–40 000 microspheres (15 μm diameter), labeled with either ^{57}Co or ^{113}Sn (Du Pont Canada, Ontario, Canada), was injected and flushed over 10 s into the left ventricle in the control period and 10 min after the i.v. infusion of a drug or vehicle. Beginning at 10 s before the injection of each set of microspheres, blood was withdrawn (Harvard infusion/withdrawal pump) from the iliac arterial cannula into a heparinized syringe at 0.35 ml/min for 1 min. The order of administration of the microspheres was reversed in half the experiments in each group. At the end of the experiments, blood samples, whole organs of lungs, heart, liver, stomach, intestine, caecum and colon, kidneys, spleen, testes and brain, as well as 40 g each of skeletal muscle (from chest, abdomen, back, diaphragm, forelimbs) and skin (from dorsal and ventral areas) were removed for the counting of radioactivity using a 1185 Series Dual Channel Automatic Gamma Counter (Nuclear-Chicago, Illinois, USA) with a 3 inch NaI crystal at energy settings of 80–160 keV and 330-480 keV for ^{57}Co and ^{113}Sn, respectively. At these energy settings, the 'spill-over' from ^{57}Co to the ^{113}Sn channel was negligible (0.03%), and from ^{113}Sn to the ^{57}Co channel was 28%. A correction was made for the ^{113}Sn 'spill-over'. In rare instances ($< 15\%$) when microspheres trapped in the two kidneys differed by more than 20%, the experiment was rejected, as it was assumed that the mixing of the microspheres was inadequate. Cardiac output, total peripheral resistance, organ blood flow and vascular conductance were calculated as in Pang (1983).

2.3. Experimental protocol

Rats were divided into six groups ($n = 7$ each) and given 1 h to stabilize. A first set of microspheres was injected into three groups to determine baseline flow. This was followed 10 min later by the infusion of the vehicle (0.9% NaCl, 0.07 ml min^{-1}), or a low dose (0.05 μg or 0.1 nmol kg^{-1} min^{-1}) or a high dose (0.3 μg or 0.6 nmol kg^{-1} min^{-1}) of PAF. After another 10 min, a second set of microspheres was injected to determine the effects of the vehicle (time control) and the low or high dose of PAF. In three other groups, TCV-309 (10 μg or 15 nmol kg^{-1}) was i.v. bolus injected into the rats and this was followed 10 min later by the injection of the first set of microspheres to measure flow in the presence of TCV-309. After another 10 min, either the vehicle, the low dose or the high dose of PAF was infused into the rats and another set of microspheres was injected 10 min later to determine the effects of time and the ability of TCV-309 to block the haemodynamic effects of PAF.

2.4. Drugs

PAF was obtained from Calbiochem; the PAF receptor antagonist, TCV-309 {3-bromo-5-[*N*-phenyl-*N*-[2-[[2-(1,2,3,4-tetrahydro-2-isoquinolylcarbonyloxy)ethyl]carbamoyl]ethyl]carbamoyl]-1-propylpyridinium nitrate}, was kindly provided by Takeda Chemical Industries, Osaka, Japan.

2.5. Statistics

All data are shown as mean ± S.E.M. The results were analyzed by the analysis of variance followed by Duncan's multiple range test, with $P < 0.05$ as the level of statistical significance.

3. Results

3.1. Effects of PAF on mean arterial pressure, heart rate, cardiac output and total peripheral resistance in the absence or presence of TCV-309

I.v. infusion of the vehicle did not affect mean arterial pressure, heart rate, cardiac output or total peripheral resistance either in the absence or presence of the PAF receptor antagonist TCV-309 (Fig. 1).

I.v. infusion of the low dose of PAF caused a small, but insignificant reduction in mean arterial pressure and a small increase in heart rate, but did not affect cardiac output or total peripheral resistance when the results were compared with predrug controls (baseline readings) within the same group (Fig. 1). Both changes in mean arterial pressure and heart rate were abolished by TCV-309. Compared with predrug controls, the high dose of PAF markedly reduced mean arterial pressure accompanied by a drastic reduction in cardiac output, an insignificant increase in total peripheral resistance but no change in heart rate. All changes in mean arterial pressure, cardiac output and total peripheral resistance elicited by the high dose of PAF were abolished by pretreatment with TCV-309.

3.2. Effects of PAF on haemodynamics in the absence or presence of TCV-309

The vehicle did not affect blood flows in any organs or tissues, either in the absence or presence of TCV-309 (Table 1). The vehicle also did not affect vascular conductances (results not shown) indicating the reproducibility of the dual-microsphere technique.

The low dose of PAF increased blood flows to the stomach, intestine and caecum and colon, decreased flows to the spleen and brain and did not significantly affect flows in other organs or tissues, when the read-

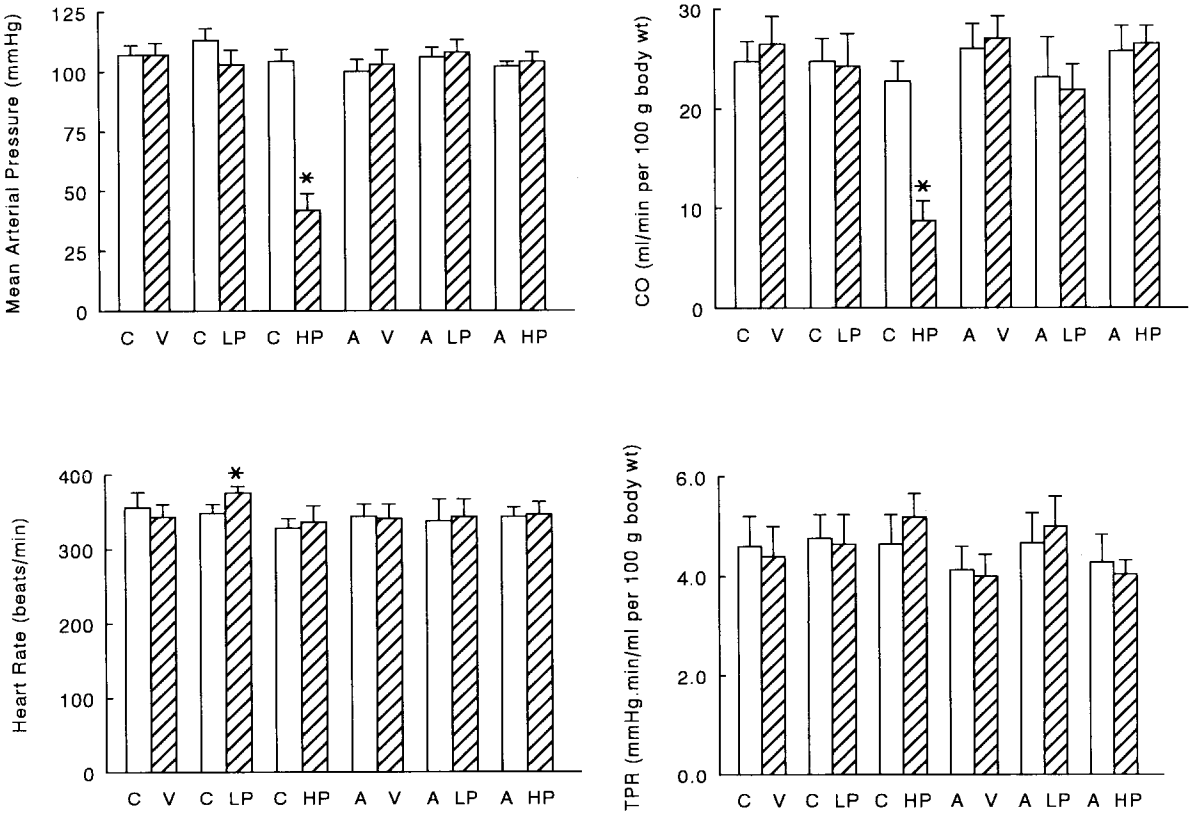

Fig. 1. Effects (mean ± S.E.M.) of vehicle (V, 0.9% NaCl), low dose PAF (LP, 0.05 $\mu g\ kg^{-1}\ min^{-1}$), high dose PAF (HP, 0.3 $\mu g\ kg^{-1}\ min^{-1}$), in the absence (first 6 columns) and presence (last 6 columns) of 10 $\mu g\ kg^{-1}$ of TCV-309 on mean arterial pressure, heart rate, cardiac output (CO) and total peripheral resistance (TPR) in six groups of pentobarbitone-anaesthetized rats. C = pretreatment control; A = TCV-309-pretreated control. $n = 7$ in each group. * Significant difference from control values ($P < 0.05$).

Table 1
Effects (mean ± S.E.M.) of vehicle (0.9% NaCl) on blood flow in two groups ($n = 7$ each) of pentobarbitone-anaesthetized rats in the absence and presence of TCV-309 (10 $\mu g\ kg^{-1}$)

Organ	Blood flow (ml min^{-1} per 100 g)			
	Control		TCV-309	
	Baseline	Vehicle	Baseline	Vehicle
Lung	77.8 ± 11.1	88.9 ± 11.1	55.6 ± 11.1	66.7 ± 16.7
Heart	269 ± 29	291 ± 29	342 ± 51	364 ± 44
Liver	7.7 ± 1.7	10.5 ± 2.8	13.2 ± 5.5	14.3 ± 2.8
Stomach	81.9 ± 14.5	86.7 ± 14.5	48.2 ± 4.8	62.7 ± 4.8
Intestine	207 ± 16	234 ± 19	207 ± 16	252 ± 27
Caecum/colon	148 ± 16	164 ± 16	151 ± 20	174 ± 23
Kidney	533 ± 49	583 ± 63	542 ± 30	569 ± 49
Spleen	132 ± 20	132 ± 20	172 ± 20	192 ± 20
Muscle	4.3 ± 0.3	4.5 ± 0.5	4.75 ± 0.5	5 ± 0.5
Skin	13.3 ± 1.8	14.5 ± 1.8	13.0 ± 1.3	15.5 ± 2.0
Testes	15.6 ± 2	19.5 ± 2	15.6 ± 0.0	19.5 ± 2.0
Brain	70.8 ± 10.9	70.8 ± 10.9	70.8 ± 16.3	76.2 ± 10.9

ings were compared with the predrug controls within the same group (Fig. 2). Vascular conductances were calculated for individual beds to reflect active changes in vasomotor tone, i.e., responses independent of changes in mean arterial pressure. Vascular conductances of the stomach (200% of control), intestine

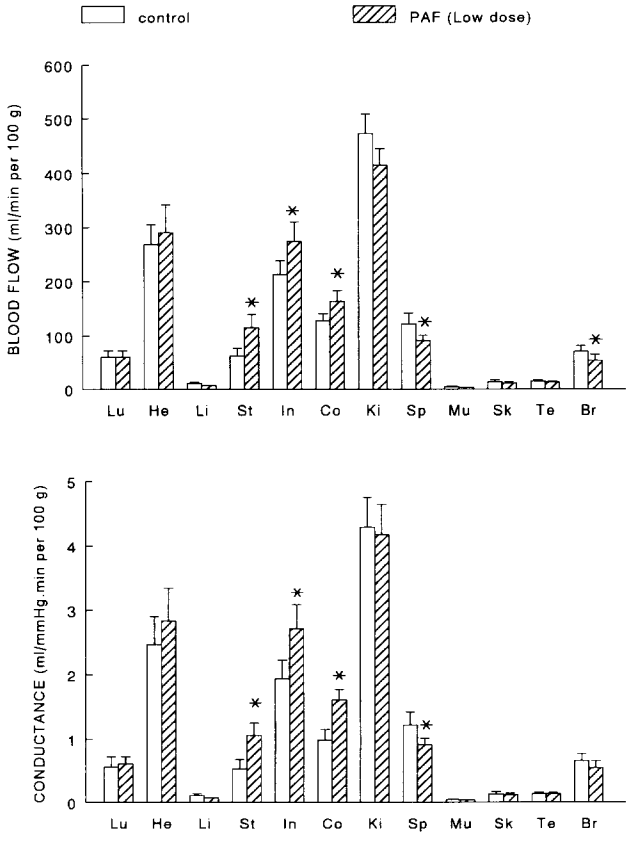

Fig. 2. Effects (mean ± S.E.M.) of the low dose PAF (0.05 $\mu g\ kg^{-1}\ min^{-1}$) on blood flow and conductance in pentobarbitone-anaesthetized rats ($n = 7$). * Significant difference from pretreatment values ($P < 0.05$). Tissue samples were lung (Lu), heart (He), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), skeletal muscle (Mu), skin (Sk), testes (Te) and brain (Br).

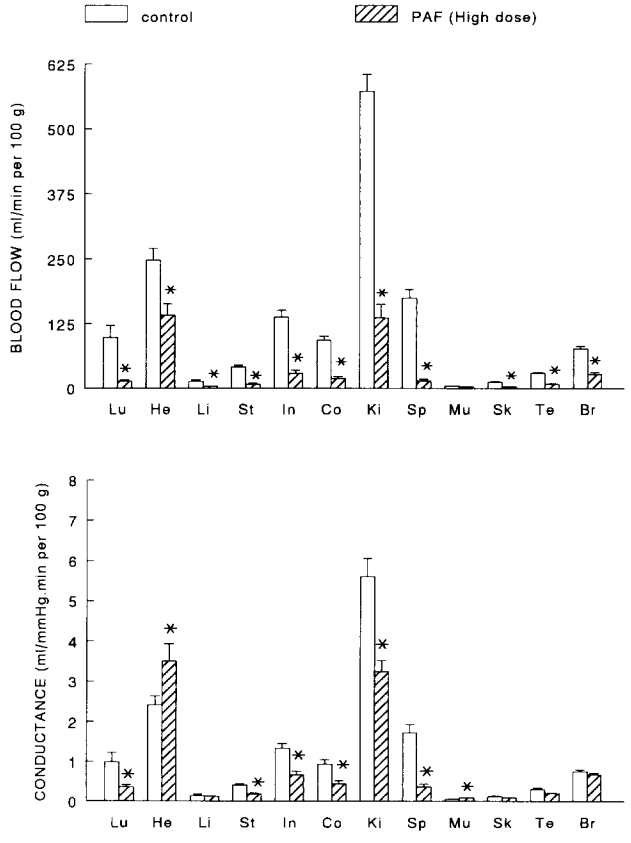

Fig. 3. Effects (mean ± S.E.M.) of the high dose PAF (0.3 $\mu g\ kg^{-1}\ min^{-1}$) on blood flow and conductance in pentobarbitone-anaesthetized rats ($n = 7$). Values are mean ± S.E.M. * Significant difference from pretreatment value ($P < 0.05$). Tissue samples were lung (Lu), heart (He), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), skeletal muscle (Mu), skin (Sk), testes (Te) and brain (Br).

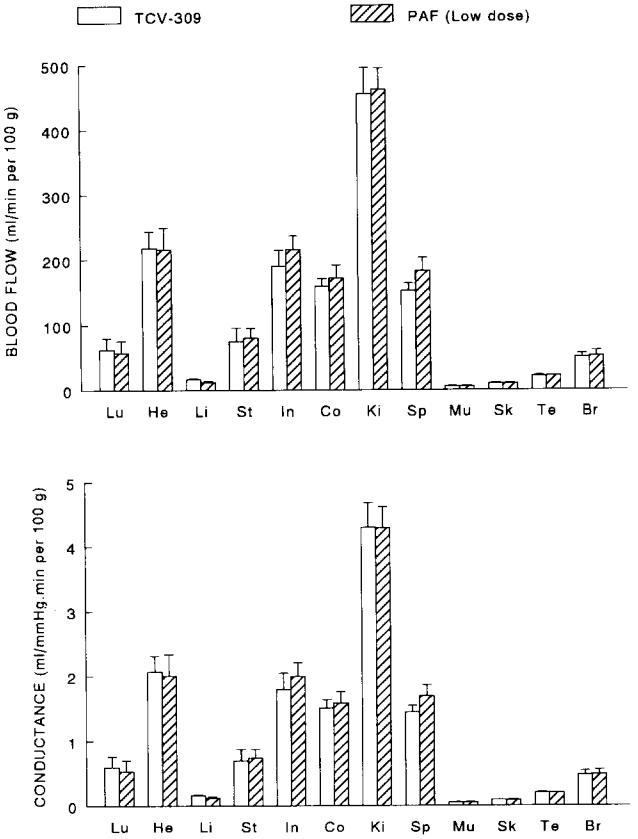

Fig. 4. Effects (mean ± S.E.M.) of the low dose PAF (0.05 μg kg^{-1} min^{-1}) after pretreatment with TCV-309 (10 μg kg^{-1}) on blood flow and conductance in pentobarbitone-anaesthetized rats ($n = 7$). Tissue samples were lung (Lu), heart (He), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), skeletal muscle (Mu), skin (Sk), testes (Te) and brain (Br).

(140%) and caecum and colon (160%) were increased whereas conductance of the spleen (70% of control) was reduced (Fig. 2). Conductances in other organs and beds were not affected. All changes in vascular conductances were abolished by pretreatment with TCV-309 (Fig. 3).

The high dose of PAF, on the other hand, reduced blood flows in all beds; significant reductions were obtained in all organs except the muscle (Fig. 4). Vascular conductances in the lungs (36% of control), stomach (46%), intestine (50%), caecum and colon (47%), kidneys (58%) and spleen (21%) were significantly reduced but conductances in the heart (145% of control) and muscle (169%) were increased. In rats pretreated with TCV-309, the high dose of PAF did not alter flow or conductance in any organs or tissues (Fig. 5).

4. Discussion

Our results showed that even a low dose of PAF which insignificantly decreased mean arterial pressure produced significant vasodilatation of the gut (stomach, intestine, colon and caecum) vasculature and vasoconstriction of the spleen. These results suggest that the gut vasculature is most sensitive to the dilator effects of low doses of PAF. In contrast to the results of Sirén and Feuerstein (1989), there was no renal vasodilatation in response to a low dose of PAF and this was likely due to differences in the experimental conditions in the Sirén and Feuerstein vs. our study, namely, the use of conscious vs. anaesthetized rats, bolus injection vs. infusion, different doses of PAF (0.3 vs. 1 nmol kg^{-1}), and the use of a hypotensive vs. a non-hypotensive dose of PAF, respectively. Since no sympathetic blockers were given, it is unclear if the splenic vasoconstrictor response to PAF in our study was direct or mediated by baroreflex. All changes in haemodynamics were abolished by TCV-309 suggesting the involvement of PAF receptors.

A high dose of PAF caused a depressor response which was mediated via a marked reduction (−40%) in cardiac output as total peripheral resistance was insignificantly increased. Due to the drastic reduction in

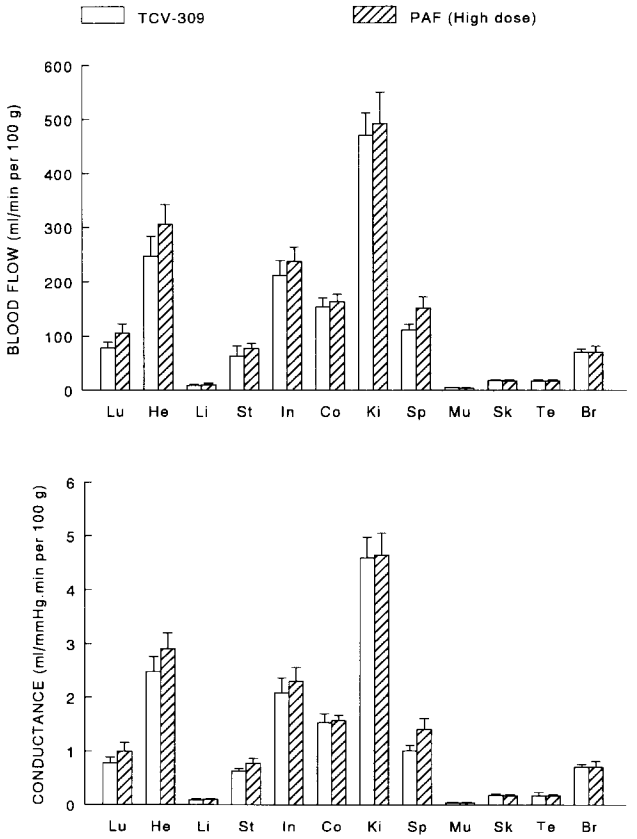

Fig. 5. Effects (mean ± S.E.M.) of the high dose PAF (0.3 μg kg^{-1} min^{-1}) after pretreatment with TCV-309 (10 μg kg^{-1}) on blood flow and conductance in pentobarbitone-anaesthetized rats ($n = 7$). Tissue samples were lung (Lu), heart (He), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), skeletal muscle (Mu), skin (Sk), testes (Te) and brain (Br).

cardiac output, flows to all organs and tissues were reduced; significant reductions were present in all organs except the skeletal muscle bed. The PAF-induced changes in mean arterial pressure, cardiac output and total peripheral resistance were completely abolished by pretreatment with TCV-309, again indicating the involvement of PAF receptors. Important factors which affect cardiac output include heart rate, cardiac contractility, arterial resistance, venous resistance, unstressed blood volume and venous compliance (Greenway, 1982; Pang, 1994). The latter three are parameters which define the venous system. A small (though insignificant) increase in total peripheral resistance by PAF might also have led to a small decrease in cardiac output. Heart rate was unchanged. Assuming that cardiac contractility, similar to heart rate, was also unaffected, the decrease in cardiac output by PAF could be due to an increase in venous resistance, unstressed blood volume and/or venous compliance. More studies are needed to find out if PAF reduced cardiac output by altering venous function. Our results are in agreement with those of Bellan et al. (1992) and Yamanaka et al. (1992) in anaesthetized cats and dogs, respectively, which showed that hypotension occurring at 1–10 min following the i.v. bolus injection of PAF was associated with reduced cardiac output as well as decreased right and left atrial pressures suggesting increased hindrance to venous return. Since aspirin and ibuprofen attenuated PAF-evoked reductions in mean arterial pressure and cardiac output, it is possible that reduced cardiac output was partially due to increased prostanoid synthesis (Yamanaka et al., 1992).

Vascular conductances were also calculated to normalize flow. It was found that a high dose of PAF caused significant vasoconstrictions in the lungs (bronchial flow), gastrointestinal organs (stomach, intestine, colon and caecum), kidneys and spleen. Significant vasodilatations were present in the heart and skeletal muscle bed. The vasodilator responses are undoubtedly mediated via direct actions of PAF. Since mean arterial pressure was markedly reduced, it is unclear if the vasoconstrictor responses to the high dose of PAF were direct or mediated via reflex. Direct vasoconstrictor response to a high dose of PAF has been reported in the isolated perfused heart (Man et al., 1990; Hu et al., 1991). Our results of the effects of PAF in the mesenteric bed are similar to those of Sirén and Feuerstein (1989) who found that PAF caused mesenteric vasodilatation at a low dose and vasoconstriction at a high dose. All changes in conductances were due to the activation of PAF receptors as they were abolished by TCV-309. It has been reported that the PAF receptor antagonist BN 52021 partially blocked the vasodilatation responses to PAF in the renal and hindquarter beds (Sirén and Feuerstein, 1989). Hu and Man (1991) observed that PAF produced a biphasic response: the initial vasodilatation phase was selectively blocked by CV-6209 and the more sustained vasoconstriction was blocked by WEB 2086. In our study, both vasoconstriction and vasodilatation responses to PAF were blocked by TCV-309.

To summarize, the haemodynamic effects of PAF are critically dependent on the dose. A non-hypotensive dose of PAF caused vasodilatation of gastrointestinal organs and vasoconstriction of the spleen. A high dose of PAF caused a marked reduction in cardiac output and mean arterial pressure. Total peripheral resistance was not appreciably altered by the high dose of PAF as vasodilatations occurred in the heart and skeletal muscle and vasoconstrictions occurred in the lungs, gastrointestinal organs, kidneys and spleen. All haemodynamic changes elicited by PAF were abolished by TCV-309 indicating the involvement of PAF receptors.

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