Role of R-type calcium channels in the response of the perfused arterial and venous mesenteric vasculature of the rat to platelet-activating factor

Audrey Claing, *Ghassan Bkaily, Nathalie Berthiaume, Pierre Sirois, †Marek Rola-Pleszczynski, & †Pedro D'Orléans-Juste

Departments of Pharmacology, *Physiology and Biophysics and †Pediatrics (Immunology Division), MRCC team in Immuno-Cardiovascular Interactions, Faculty of Medicine, Université de Sherbrooke, Sherbrooke (Québec) J1H 5N4, Canada

1 The vasoactive properties of platelet-activating factor (PAF) were studied in the arterial and venous vasculature of the rat double-perfused mesenteric bed. Although PAF (0.01–0.3 pmol) induced a dose-dependent vasodilatation of the arterial mesenteric vasculature, it triggered only vasoconstrictions on the venous side, with an intact endothelium as bradykinin induced a significant venodilatation.

2 N^G-nitro-L-arginine methyl ester (L-NAME, 100 µM), a nitric oxide synthase inhibitor, markedly reduced the vasodilatation induced by PAF in the arterial mesenteric vasculature and potentiated the contractile responses of the venous side to the same agent.

3 The PAF antagonist, WEB-2170, markedly reduced the response to PAF on both sides of the mesenteric vasculature. However, the IC₅₀ of WEB-2170 against PAF was reached at a much higher concentration (1×10^{-8} M) on the arterial side than on the venous side (5.3×10^{-11} M). Furthermore, a second antagonist of PAF receptors, SRI-63441, although being less potent on the venous vasculature than WEB-2170, was equipotent in antagonizing the venoconstriction and the arterial dilatation induced by PAF (IC₅₀ of SRI-63441, arterial side: 2.9×10^{-9} M; venous side: 3.1×10^{-9} M).

4 The dual L- and R-calcium channel blocker, isradipine (PN 200-110), but not the L-type calcium channel blocker, nifedipine, markedly reduced the PAF-induced vasoactive properties on both sides of the mesenteric vasculature.

5 Our results illustrate the differential vasoactive properties of PAF in the mesenteric vasculature of the rat. These vasoactive responses occur following activation of specific receptors for PAF or, alternatively, through activation of R-type calcium channels.

Keywords: Platelet-activating factor; WEB-2170; nitric oxide; N^G-nitro-L-arginine methyl ester (L-NAME); receptors, mesenteric artery and vein; R-type calcium channels

Introduction

Intravascular administration of platelet-activating factor (PAF) triggers a marked hypotensive response in the rat (Braquet *et al.*, 1987). However, besides its potent hypotensive properties, PAF is also a potent enhancer of plasma extravasation which does not appear to be platelet-mediated in the same animal model (Sanchez-Crespo *et al.*, 1982; Pirotzky *et al.*, 1984; Filep *et al.*, 1991). This latter property of PAF may be greatly influenced by regional differences in the responses of various vasculatures to the phospholipid (Benveniste *et al.*, 1983; Pirotzky *et al.*, 1985; Martins *et al.*, 1987; Chu *et al.*, 1988; Lagente *et al.*, 1988). Indeed, opposite effects of PAF in the pre- and post-capillary circuits in various organs may favour or alternatively counteract plasma leakage induced by PAF.

In the present study, we were interested to assess the effect of PAF in the endothelium-intact, perfused arterial and venous mesenteric vasculature of the rat. An endothelium-dependent relaxation induced by PAF in the perfused mesenteric arterial vasculature of the rat has been previously shown (Chiba *et al.*, 1990). Furthermore, other studies have reported that the rat portal vein contracted to PAF in a concentration-dependent fashion (Hellegouarch *et al.*, 1988). However, the contribution of the venous mesenteric endothelium in the response to PAF had yet to be investigated.

In addition, we have recently reported that PAF induces a

concentration-dependent and sustained increase of intracellular calcium in human and canine cultured endothelial cells (Bkaily *et al.*, 1993) via the specific activation of the recently reported R-type calcium channel (Bkaily *et al.*, 1992). Indeed, we have shown that PAF increases calcium via an isradipine-sensitive calcium channel whose activation was unaffected by nifedipine, an L-type calcium channel blocker. As suggested by Bkaily *et al.* (1992, 1993), R-type calcium channels may have an important role in sustained contraction of vascular smooth muscle and in excitation-secretion coupling mechanisms in vascular endothelial cells and subsequent release of vasoactive factors, such as endothelium-derived relaxing factor (EDRF). Hence, in the present study, the dual L- and R-type calcium channel blocker, isradipine (Bkaily *et al.*, 1992), as well as the L-type calcium channel blocker, nifedipine, were compared for their blocking properties on the response induced by PAF on both sides of the mesenteric vasculature of the rat.

Finally, the inhibitory effects of the PAF receptor antagonists, WEB-2170 (Casals-Stenzel, 1987) and SRI-63441 (Handley *et al.*, 1987), were assessed against the PAF-induced vasoactive responses on both sides of the mesenteric vasculature. Our results suggest that PAF induces an endothelium-dependent arterial dilatation and an endothelium-independent venoconstriction of the mesenteric vasculature. Moreover, R-type calcium channel activation importantly contributes to the vasoactive properties of PAF in the mesenteric vasculature of the rat.

¹ Author for correspondence; (Fax: 819-564-5400)

Methods

The simultaneously-perfused superior mesenteric arterial and venous vascular bed of the rat

The simultaneously-perfused arterial and venous vessels of the superior mesenteric vascular bed of the rat were prepared as described previously (Warner, 1990). Male albino Wistar rats (250–350 g) were killed by stunning and exsanguinated. The abdomen was opened and the ileocolic and colic branches of the superior mesenteric artery were tied. The portal and superior mesenteric veins were separated from the connecting tissue in the region of the portal vein. A plastic cannula (Portex, size 3FG, external diameter 1.02 mm, internal diameter 0.61 mm) was inserted retrogradely into the portal vein and passed into the superior mesenteric vein 1–1.3 cm distal to the portal-mesenteric junction. The superior mesenteric artery was cannulated, as described previously (McGregor, 1965), with an identical cannula. The superior mesenteric vascular bed was perfused via the artery for 5 min at 2 ml min^{-1} with Krebs solution (at room temperature) containing heparin (100 u ml^{-1}). During this period, the effluent from the bed passed out from the venous cannula. At the end of this period, the intestine was separated from the mesentery by cutting close to the intestinal border and the preparation supported on a Petri dish while the arteries and veins were perfused independently at a constant flow of 2 ml min^{-1} with warm (37°C), oxygenated (95% O_2 ; 5% CO_2) Krebs solution containing indomethacin ($5 \mu\text{M}$). When the arterial side was precontracted with methoxamine ($100 \mu\text{M}$), the flow was increased to 4 ml min^{-1} to reduce the marked spontaneous activity of the preparation (Warner, 1990). Changes in perfusion pressures were measured with a Statham pressure transducer (Model P-23A) and recorded on a Grass physiograph (Model 7-D).

Vasodilatation responses of the arterial and venous mesenteric vasculature

Following an equilibration period of 45 min, the vasoactive effect of PAF, acetylcholine (ACh) or bradykinin (BK) was evaluated. The perfusion pressure on both sides of the mesenteric circulation was increased by infusing either a sympathomimetic, methoxamine ($100 \mu\text{M}$), on the arterial side or a thromboxomimetic, U46619 ($0.5 \mu\text{M}$), on the venous side, as previously described (Warner, 1990; Claing *et al.*, 1992; D'Orléans-Juste *et al.*, 1993). When a plateau was reached, the agents were administered by bolus injections (1 to $5 \mu\text{l}$). Vasodilator responses were expressed as % reduction of induced tone on both sides of the mesenteric vasculature, as previously reported (Warner, 1990). In some experiments, the effects of inhibitors and antagonists were evaluated. They were all infused 15 min before the administration of PAF, bradykinin, or acetylcholine, except for N^G -nitro-L-arginine methyl ester (L-NAME) which was infused 30 min before. The apparent affinities of WEB-2170 and SRI-63441 were obtained by monitoring the vasoactive responses of the mesenteric vasculature to PAF (arterial: 0.1 pmol ; venous: 5 pmol) in the presence of increasing concentrations of the antagonists and their IC_{50} , calculated from a linear regression analysis.

Vasoconstrictor responses on the venous mesenteric vasculature

The vasoconstrictor properties of PAF and angiotensin II were evaluated on the venous mesenteric vasculature perfused at basal pressure. Bolus doses of PAF (0.1 – 5 pmol) were administered in the absence and presence of the various inhibitors or antagonists. To avoid tachyphylaxis, the consecutive doses were administered at 1 h time intervals or when the perfusion pressure was returned to basal value.

Vasoconstrictor responses were expressed as the increase of the perfusion pressure.

All studies using nifedipine, isradipine, SRI-63441 and WEB-2170 were carried out in the dark. The effects of isradipine were studied in mesenteric vasculatures pretreated with nifedipine in order to discount any influence at L-type calcium channels.

Materials

The Krebs solution had the following composition (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.2, $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ 2.5, NaHCO_3 25 and glucose 5.5. Indomethacin, methoxamine, heparin, sodium nitroprusside, N^G -nitro-L-arginine methyl ester (L-NAME), nifedipine and acetylcholine were purchased from Sigma Chemical Co. (St-Louis, U.S.A.). Angiotensin II and bradykinin were synthesized in the laboratory of Dr D. Regoli (Université de Sherbrooke, Sherbrooke, Québec, Canada). U46619 (11 α , 9 α -epoxymethane PGH_2) was purchased from Cayman Chemical Company (Ann Arbor, U.S.A.). C_{16} -PAF was obtained from Bachem (U.S.A.) and WEB-2170 (3-[4-(2-chlorophenyl)-9-methyl-6H-thieno[3,2-F] [1,2,4]triazolo-4[3-a] [1,4]-diazepin-2-Y1)-1-(4-morpholinyl)-1-propanone) was a generous gift from Dr H. Heuer (Boehringer-Ingelheim, Germany). Isradipine (PN 200-110) was obtained from Sandoz Canada Inc. and SRI-63441 (cis($\pm$)-1-[2-[hydroxy[tetrahydro-5-[(octa-decylaminocarbonyl)oxy]-methyl]furan-2-yl]-methoxy-phosphinyl-oxy]ethyl]-quinolinium hydroxide) was a generous gift from Dr D. Handley (Sandoz, East Hanover, NJ).

All drugs were dissolved in phosphate buffered saline (PBS; pH: 7.4), except for nifedipine which was dissolved in 10% dimethylsulphoxide (DMSO), isradipine in 60% ethanol and indomethacin in Trizma base (pH 7.4; 0.2 M, Sigma).

Statistics

Results are shown as mean values $\pm$ s.e.mean for n experiments. All data are subjected to comparison and analysis by a one-way analysis of variance (ANOVA), followed by a Mann-Whitney U-test to assess the statistical significance of the results, except for the experiment with L-NAME. In this case, Student's t test was used. A P value less than 0.05 was considered statistically significant.

Results

Effect of PAF on arterial and venous mesenteric vasculature of the rat perfused at basal pressure or precontracted

The basal perfusion pressures of arterial and venous sides of the mesenteric vasculature were $5.1 \pm 0.3 \text{ mmHg}$ ($n = 40$) and $1.7 \pm 0.2 \text{ mmHg}$ ($n = 35$), respectively, when perfused at 2 ml min^{-1} . An increase in perfusion flow to 4 ml min^{-1} also increased the perfusion pressure of the arterial vasculature to $12.1 \pm 0.9 \text{ mmHg}$ ($n = 25$). The infusion of methoxamine ($100 \mu\text{M}$, arterial side) and U46619 ($0.5 \mu\text{M}$, venous side) increased the perfusion pressures by $39.1 \pm 3.6 \text{ mmHg}$ ($n = 27$) and $7.1 \pm 1.2 \text{ mmHg}$ ($n = 8$), respectively.

Typical traces of Figure 1 illustrate the vasoactive properties of PAF on the arterial and venous vasculatures, precontracted respectively with methoxamine ($100 \mu\text{M}$) and U46619 ($0.5 \mu\text{M}$) or on the venous vasculature perfused at basal pressure. Acetylcholine (100 pmol) induced a transient vasodilatation on the arterial side and bradykinin (1000 pmol) induced a vasodilatation of the venous vasculature. PAF (0.1 pmol) induced only a vasodilatation of the arterial mesenteric vasculature. In venous preparations perfused at basal pressure, angiotensin II (100 pmol) and PAF (5 pmol) induced an increase of the perfusion pressure (Figure 1c).

Figure 2 illustrates the dose-dependent vasodilatation by

PAF (0.01–0.3 pmol) on the arterial side precontracted with the methoxamine (Figure 2a), the effect of PAF (0.1 and 1 pmol) on the venous side in the precontracted vasculature (U46619, 0.5 μ M) (Figure 2b) and the effect of PAF (1 and 5 pmol) on the venous vasculature perfused at basal pressure (Figure 2c).

Effect of L-NAME on the vasodilator and vasoconstrictive properties of PAF

Acetylcholine and PAF induced a vasodilatation in the arterial mesenteric vasculature precontracted with methoxamine. In Figure 3a, a 30 min preinfusion of L-NAME (100 μ M) reduced the vasodilatation induced by acetylcholine (100 pmol) by 82% ($n = 7$, $P < 0.01$) and PAF (0.1 pmol) by 99% ($n = 8$, $P < 0.01$) when compared to the responses to these vasoactive agents in the absence of L-NAME, without affecting the vasodilatation induced by sodium nitroprusside (1000 pmol) ($n = 8$). Furthermore, as illustrated in Figure 3b, L-NAME significantly potentiated the constrictive properties of PAF (5 pmol) ($n = 6$, $P < 0.05$) and angiotensin II (100 pmol) ($n = 6$, $P < 0.05$) when compared to the effect of these two constrictor agents in the absence of L-NAME in the venous mesenteric vasculature perfused at basal pressure. Infusion of L-NAME increased the perfusion pressure of the precontracted arterial side and non-precontracted venous vasculature by 42.0 ± 5.8 ($n = 8$) and 3.6 ± 1.2 mmHg ($n = 6$), respectively.

Figure 1 Typical trace illustrating the effect of acetylcholine (ACh) (100 pmol), bradykinin (BK) (1000 pmol) and PAF (0.1–1 pmol) on the arterial (a) and venous (b) vasculature precontracted respectively with methoxamine (100 μ M) and U46619 (0.5 μ M). This trace illustrates also the effect of angiotensin II (AII 100 pmol) and PAF (5 pmol) on the venous vasculature perfused at basal pressure (c). This trace is representative of five other experiments.

Effect of WEB-2170 and SRI-63441 on the vasoactive properties of PAF in the arterial and venous mesenteric vasculatures

Figures 4 and 5 illustrate, respectively, the effects of WEB-2170 and SRI-63441, on the vasodilator and venoconstrictor responses to PAF of the mesenteric vasculature. The PAF antagonists significantly reduced the vasodilator properties of PAF in the arterial mesenteric vasculature. WEB-2170 was inactive at 10^{-10} M (not shown) and a concentration ten times higher was necessary to reduce significantly the vasodilator properties of PAF (0.1 pmol); the IC_{50} of the antagonist was estimated at 1×10^{-8} M. The IC_{50} value of the second antagonist, SRI-63441, against PAF (0.1 pmol)-induced vasodilatation was 2.9×10^{-5} M. In contrast, a concentration of 1×10^{-7} M of WEB-2170, which was sufficient to block the vasodilator properties of PAF by 88%, did not affect the endothelium-dependent vasodilatation induced by acetylcholine (100 pmol) (Figure 4a).

Figure 2 (a) Dose-dependent vasodilatation induced by PAF (0.01–0.3 pmol) on the arterial side precontracted with methoxamine (100 μ M), (b) constriction of the venous side induced by PAF (0.1 and 1 pmol) in precontracted (U46619, 0.5 μ M) mesenteric vasculature or (c) constriction by PAF (1 and 5 pmol) of the venous vasculature perfused at basal pressure. The vasodilator effect of acetylcholine (ACh) (100 pmol) and sodium nitroprusside (NaNP) (1000 pmol) is also determined on the arterial side (a) and that of bradykinin (BK) (1000 pmol) on the venous side (b). The vasoconstrictor effect of angiotensin II (AII) (100 pmol) is determined on the venous side (c). Each column represents the mean $\pm$ s.e. mean of four to 10 determinations.

Figure 3 Effect of N^G -nitro-L-arginine methyl ester (L-NAME) (100 μ M, 30 min) (solid columns) on the response of the arterial (a) mesenteric vasculature (precontracted with methoxamine) to acetylcholine (ACh) (100 pmol), PAF (0.1 pmol) and sodium nitroprusside (NaNP) (1000 pmol) and on the venous side perfused at basal pressure (b) to angiotensin II (AII) (100 pmol) and PAF (5 pmol). Each column represents the mean $\pm$ s.e. mean of 6 to 8 determinations. * $P < 0.05$, ** $P < 0.01$, when compared to control values (open columns).

WEB-2170 and SRI-63441 also markedly reduced the vasoconstrictive properties of PAF (5 pmol) in the mesenteric vasculature at threshold concentrations of 1×10^{-11} M and 1×10^{-9} M, respectively (Figure 4b and 5b). In terms of antagonism of PAF-induced vasoconstriction, the IC_{50} value of WEB-2170 was lower (5.3×10^{-11} M) than that of SRI-63441 (3.1×10^{-9} M).

Effect of isradipine on the vasoactive properties of PAF

The effects of a L-type calcium channel blocker, nifedipine, and a dual L- and R-type calcium channel blocker, isradipine, were tested for their inhibitory properties on the response of PAF and other vasoactive agents in the mesenteric vasculature. Figures 6 and 7 illustrate that the L-type calcium channel blocker, nifedipine (0.1 μ M), did not affect the vasodilator properties of PAF on the arterial side nor the vasoconstrictor properties of the same agent. In contrast, in the presence of nifedipine (0.1 μ M), treatment with isradipine ((+) or (-) enantiomer) significantly reduced the vasoactive effects of PAF on the arterial and venous mesenteric vasculature, without affecting the vasodilator properties of acetylcholine on the arterial side and the vasoconstrictor properties of angiotensin II (Figures 6 and 7). Finally, the co-infusion of isradipine enantiomers and WEB-2170 did not further reduce the vasoactive effect of PAF when compared to mesenteric vasculatures treated only with isradipine (Figures 6 and 7).

Discussion

The present study illustrates the heterogeneity of the vasoactive properties of PAF in the mesenteric vasculature of the

Figure 4 Effect of PAF (0.1 pmol) and acetylcholine (ACh) (100 pmol) in the absence of ((1), open columns) and presence of different concentrations of WEB-2170 ((2): 0.001 μ M; (3): 0.01 μ M; (4): 0.1 μ M; (5): 1 μ M) on the precontracted arterial (a) mesenteric vasculature. The vasoconstrictor effect of PAF (5 pmol) and angiotensin II (AII) (100 pmol) was also assessed on the venous (b) vasculature perfused at basal pressure in absence (open columns) and in presence of various concentrations of WEB-2170 ((2): 0.001 nM; (3): 0.01 nM; (4): 0.1 nM; (5): 1 nM). Each column represents the mean $\pm$ s.e. mean of 5 to 6 determinations. * $P < 0.05$; ** $P < 0.01$ when compared to control values ($\square$).

rat. Our results support the report by Chiba *et al.* (1990) who have suggested that PAF induced an endothelium-dependent relaxation in the rat mesenteric arterial vasculature. In contrast, PAF was found to be a potent vasoconstrictor of the venous mesenteric vasculature in which the functional endothelium was monitored through the vasodilator properties of bradykinin (Warner, 1990; Claug *et al.*, 1992). This heterogeneity of the response of the mesenteric vasculature to the potent vasoactive factor, PAF, is of interest considering that this compound is a potent enhancer of vascular permeability changes in conscious rats (Sirois *et al.*, 1988). Indeed, assuming that PAF induces a marked endothelium-dependent vasodilator effect in the precapillary vasculature as well as a marked endothelium-independent constriction of the post-capillary circulation, one can suggest that hydrostatic pressure changes play an important role in the plasma leakage induced by PAF in the mesenteric vasculature. The contribution of vasoconstriction in the plasma leakage induced by PAF has been previously discussed by Heffner *et al.* (1989) and Sirois *et al.* (1990) who suggested that the complex phenomenon involved in capillary extravasation was com-

Figure 5 Effect of PAF (0.1–5 pmol) in the absence ((1), open column) and presence of different concentrations of SRI-63441 ((2): 0.0001 μ M; (3): 0.001 μ M; (4): 0.01 μ M; (5): 0.1 μ M) on the precontracted arterial (a) and venous (perfused at basal pressure) (b) mesenteric vasculature. Each column represents the mean $\pm$ s.e.mean of 5 to 6 determinations. * P < 0.05; ** P < 0.01 when compared to control values (open columns).

posed, among other mechanisms, of both post-capillary disjunction of endothelial cells and constriction.

EDRF, as previously suggested (Chiba *et al.*, 1990), greatly contributes to the vasodilator properties of PAF in the arterial mesenteric vasculature. Indeed, these authors have shown that methylene blue, an inhibitor of guanylate cyclase, markedly blunted the response of the arterial mesenteric vasculature to PAF and acetylcholine. We have confirmed these data by the use of a specific nitric oxide synthase inhibitor, L-NAME (Moore *et al.*, 1990).

On the venous side, although PAF did not induce an endothelium-dependent vasodilatation, L-NAME still markedly potentiated the increase of perfusion pressure triggered by PAF, suggesting that basally-released EDRF may contribute as a physiological antagonist of the vasoconstrictor properties of PAF as well as those of other agonists, such as angiotensin II, neurokinins and endothelin-1 in the rat mesenteric vasculature (D'Orléans-Juste *et al.*, 1991, 1993; Claing *et al.*, 1992).

The PAF antagonist, WEB-2170, markedly blocked the response of both sides of the mesenteric vasculature to PAF, suggesting that this mediator may very well activate specific receptors to induce its vasodilator effects as well as its vasoconstrictive properties. The IC_{50} of WEB-2170 as an antagonist of the arterial vasodilator properties of PAF was

Figure 6 Effect of PAF (0.1 and 5 pmol) on the arterial and venous sides, respectively) in the absence ((1) open column) or presence of nifedipine (0.1 μ M) (2) or nifedipine (0.1 μ M) and (+)-isradipine (1 μ M) (3) or nifedipine (0.1 μ M), (+)-isradipine (1 μ M) and WEB-2170 (arterial: (a) 0.1 μ M; (4): venous; (b): 0.1 nM; (4)). The same mixture of antagonists was tested against acetylcholine (ACh) (100 pmol) on the arterial and angiotensin II (AII) (100 pmol) on the venous mesenteric vasculature. Each column represents the mean $\pm$ s.e.mean of 5 to 8 determinations. NS: not significant. ** P < 0.01 when compared to control values (open columns).

lower (2.5 log units) when compared to the IC_{50} of the same antagonist to block PAF-induced venoconstriction. The discrepancy in concentrations found with this antagonist was not seen with another PAF receptor antagonist (SRI-63441; Handley *et al.*, 1987). Indeed, the latter antagonist markedly reduced the arterial vasodilator properties as well as the venoconstrictor properties of PAF at similar concentrations (IC_{50} values of 2.9×10^{-9} M and 3.0×10^{-9} M, respectively). The fact that WEB-2170 was more sensitive as a blocker of the venoconstriction than of the arterial vasodilatation may explain the heterogeneity of the antagonist properties of that compound in PAF-induced plasma extravasation (Sirois *et al.*, 1988; Henriques *et al.*, 1990), as pre- and post-capillary vasculatures may be affected differently by the antagonist. The discrepancy may be further explained by the presence of high affinity receptors for PAF in the venous smooth muscle as opposed to lower affinity receptors in the arterial endothelium. It is of interest that WEB-2170 has been used to discriminate between high and low affinity receptors for PAF in human HL-60 myeloid leukaemia cells (Rola-Pleszczynski & Stankova, 1992). By the use of another PAF antagonist BN52021, those authors were unable to distinguish between the two receptor populations. We suggest that SRI-63441, as

opposed to WEB-2170, is unable to discriminate between the two receptor populations in the mesenteric vasculature. In addition, Henriques *et al.* (1990), by the use of two het-

Figure 7 Effect of PAF (0.1 and 5 pmol on the arterial and venous sides, respectively) in the absence ((1) open columns) or presence of nifedipine (0.1 μ M) (2) or nifedipine (0.1 μ M) and (-)-isradipine (1 μ M) (3) or nifedipine (0.1 μ M), (-)-isradipine (1 μ M) and WEB-2170 (arterial: (a) 0.1 μ M; (4): venous; (b): 0.1 nM; (4)). The same mixture of antagonists was tested against acetylcholine (ACh) (100 pmol) on the arterial and angiotensin II (AII) (100 pmol) on the venous mesenteric vasculature. Each column represents the mean $\pm$ s.e.mean of 5 to 8 determinations. NS: not significant. ** $P < 0.01$ when compared to control values (open columns).

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razepine PAF antagonists, have shown that distinct receptor populations were involved in paw oedema and pleurisy in the mice.

On the other hand, it is suggested that WEB-2170 interferes with entities other than PAF receptors to counteract the effect of that phospholipid, in accordance with a previous study in which we have shown that PAF induced a significant increase of intracellular calcium in human and canine endothelial cells via the opening of calcium channels (Bkaily *et al.*, 1993). This effect was unaltered by nifedipine, yet reversed by isradipine, suggesting that PAF induced a sustained increase of intracellular calcium via the activation of a recently described calcium channel, the resting potential calcium channel (R-type calcium channel) (Bkaily *et al.*, 1992, 1993). In one of those studies, we had also reported that WEB-2170 also reversed not only the sustained increase of Ca^{2+} induced by PAF, but also by high $[\text{K}]_o$ depolarization in human and canine endothelial cells, suggesting that the PAF antagonist may directly interfere with the opening of R-type Ca^{2+} channels (Bkaily *et al.*, 1993).

Since we have shown in the present study that isradipine, but not nifedipine, markedly reduced both the vasodilator and vasoconstrictor properties of PAF, we suggest that this particular agonist may well increase the probability of opening the R-type calcium channels prior to inducing its vasoactive effects in the mesenteric vasculature. The activation of L-type calcium channels was excluded as nifedipine did not alter the vasoactive properties of PAF. Furthermore, Warner *et al.* (1989) have previously shown that Bay K 8644, a dihydropyridine activator of L-type calcium channels, did not trigger the release of endothelium-derived relaxing factor (EDRF) from the arterial mesenteric vasculature of the rat.

In conclusion, our results illustrate the opposite effect of PAF on the arterial and venous mesenteric vasculature of the rat. The use of such a model as the double-perfused mesenteric bed allows a better understanding of the pre- and post-capillary vasoactive properties of PAF and their contribution in capillary leakage. Furthermore, the use of an R-type calcium channel blocker, such as isradipine, should give us further insight into the contribution of ionic channels in pro-inflammatory conditions.

The authors would like to acknowledge H. Morin for secretarial assistance. A.C. is in receipt of a studentship of the Heart and Stroke Foundation of Canada (HSFC). G.B. is a scholar of the Fonds de la recherche en santé due Québec. P.D.J. is a scholar of the HSFC. This work was financially supported by a programme grant (PG-12350) of the Medical Research Council of Canada in Immuno-Cardiovascular Interactions.

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(Received February 15, 1994)

Revised April 4, 1994

Accepted April 18, 1994)