Interaction of vasoactive substances released by platelet-activating factor in the rat perfused heart

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1 The coronary vascular effects of platelet-activating factor (PAF) have been intensively studied and it has been proposed that they are mediated by the release of vasoactive substances. In this study, a cascade perfusion model using two rat perfused hearts was developed to investigate the properties of PAF-released vasoactive substances and the interplay of these substances. The properties of the vasoactive substances after an injection of PAF (100 pmol) in the rat perfused heart were examined by collecting the effluent from the first heart for the perfusion of a second (recipient) heart. The presence of vasoconstrictor substances in the effluent was characterized by an increase in the perfusion pressure of the recipient heart.

2 Previous exposure of the recipient heart of PAF (100 pmol) abolished the response of the heart to subsequent administration of PAF, but did not affect the response of the recipient heart to the effluent. This suggested that the coronary vasoconstrictor response of the recipient heart was not due to the presence of PAF in the effluent but to other vasoactive substances.

3 Pretreatment of the recipient heart with the leukotriene receptor antagonist, L-649,923 (5 μM), partially reduced the vasoconstrictor effect of the effluent. Pretreatment of the first heart with indomethacin (2.8 μM) also partially reduced the vasoconstrictor effect of the effluent. The combination of indomethacin pretreatment of the first heart and L-649,923 pretreatment of the recipient heart completely abolished the vasoconstrictor effect of the effluent suggesting that both prostaglandins and leukotrienes are involved in the vasoconstrictor effect of the effluent.

4 Pretreatment of both hearts with L-649,923 or the first heart with the leukotriene synthesis inhibitor (MK-886, 10 μM) completely abolished the vasoconstrictor effect of the effluent. This suggested that the indomethacin sensitive vasoconstrictor component of the effluent might be regulated by leukotrienes in the first heart. However, infusion of leukotrienes (LTB$_4$, LTC$_4$ and LTD$_4$) to the first heart did not reproduce this vasoconstrictor component of the effluent in the recipient heart.

5 In conclusion, our study demonstrated through the use of a leukotriene receptor antagonist, a leukotriene synthesis inhibitor and a cyclo-oxygenase inhibitor that the vasoconstrictor effect of the effluent of the rat perfused heart after an injection of PAF is mediated by leukotrienes and prostaglandins. The ability of leukotriene receptor blockade and inhibition of leukotriene synthesis to mimic the effect of indomethacin indicates that the production and/or release of cyclo-oxygenase products in the effluent by PAF can be modulated by leukotrienes. The inability of exogenously applied leukotrienes to modulate the production and/or the release of cyclo-oxygenase products in the effluent suggests that the PAF-induced production of prostaglandins may be mediated by intracellular leukotrienes or at sites not accessible to exogenously applied leukotrienes.

Keywords: Platelet-activating factor; coronary vascular effects; leukotrienes; prostaglandins; rat perfused heart; cascade perfusion model

Introduction

Platelet-activating factor (PAF), a new class of phospholipid mediator, has been identified as a very potent vasoactive compound (Braquet *et al.*, 1987). PAF elicits a variety of striking cardiovascular changes including hypotension, selective vasodilatation, vasoconstriction, and cardiac depression (Braquet *et al.*, 1987). PAF has been detected in the effluent blood from the coronary sinus in patients with coronary artery disease undergoing atrial pacing (Montrucchio *et al.*, 1986) and release of PAF from ischaemic-reperfused rabbit heart has been demonstrated (Montrucchio *et al.*, 1989). The effects of PAF in the coronary circulation have been shown in a number of *in vivo* (Levi *et al.*, 1984; Piper & Stewart, 1986; 1987; Man *et al.*, 1990) and *in vitro* studies (Feuerstein *et al.*, 1984; Jackson *et al.*, 1986; Mehta *et al.*, 1986; Ezra *et al.*, 1987; Fiedler *et al.*, 1987).

Regarding the mechanism of the coronary vascular actions of PAF, it has been suggested that the effects of PAF are largely mediated by the release of other vasoactive substances. The importance of lipoxygenase products, leukotriene B$_4$ (LTB$_4$), LTC$_4$, and LTD$_4$ and cyclo-oxygenase products,

prostaglandins and thromboxane A$_2$ in the coronary vascular effects of PAF has been demonstrated (Piper & Stewart, 1986; 1987). More recently, we showed that LTC$_4$ and LTD$_4$ may be responsible for the vasoconstrictor effect while LTB$_4$ may be responsible for the vasodilator effect of PAF in the rat perfused heart (Hu *et al.*, 1991). Inasmuch as the precise mechanisms of actions of PAF on circulatory function have not been fully elucidated, the complex interactions between PAF and archidonic acid metabolities in the coronary vascular effects of PAF are also not well defined. In the present study, a cascade perfusion model of rat isolated heart was developed to investigate the properties of vasoactive substances released by PAF. The objectives of this study were (a) to clarify the role of arachidonic acid metabolites in the coronary vascular effects of PAF and (b) to gain additional insight into the characteristics of the vasoactive mediators released by PAF.

Methods

Rat heart perfusion

Following cervical dislocation, hearts from Sprague-Dawley rats (250–350 g) were rapidly excised and placed in cool

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Krebs-Henseleit solution oxygenated with 95% O_2 : 5% CO_2. The solution had the following composition (mM) : NaCl 120, NaH_2PO_4 1.18, $MgSO_4$ 1.18, KCl 4.76, $CaCl_2$ 1.25, $NaHCO_3$ 25.0 and glucose 11. The aorta was cannulated for coronary perfusion. The heart was allowed to beat spontaneously. The temperature of the perfusate was maintained at 37 ± 0.5°C and the coronary flow was controlled by a roller pump. The perfusion pressure was measured by a pressure transducer attached to a side arm of the aortic cannula. The perfusion pressure was recorded on a Gould chart recorder and monitored with a digital display. The detailed methodology of the isolated heart perfusion system had been reported previously (Hu *et al.*, 1991).

The cascade perfusion model

In the cascade perfusion model, two hearts were first separately perfused with oxygenated Krebs-Henseleit solution. Each heart was equilibrated with Krebs-Henseleit solution for 20 min. During this period, the flow rate was adjusted to obtain a control perfusion pressure of 65–75 mmHg and the flow rate was maintained constant for the rest of the experiment. Coronary vascular effects were measured as changes in the perfusion pressure as described previously (Man *et al.*, 1990). The perfusate for each heart could be controlled by a multiple way selector to enable drug pretreatments. During cascade perfusion, the effluent from the first heart was collected by a funnel and immediately pumped to a second (recipient) heart. The effluent was oxygenated with 95% O_2 : 5% CO_2 to maintain the Po_2, and Pco_2 at the same level as the perfusate for the first heart. The pH, Po_2 and Pco_2 of the solutions were measured by a pH/blood gas analyzer. Na^+ and K^+ contents were measured by flame photometry. The coronary flow rates of both hearts were controlled by the same roller pump motor so that the flow rate was kept identical. Solution accumulation in the funnel was kept to a minimum in order to reduce the dilution of vasoactive substances released from the first heart after challenge with PAF. One min before the injection of PAF to the first heart, the perfusion of the recipient heart was switched to the effluent from the first heart and continued to the end of experiment. Heart rates were monitored from the electrocardiographic recordings.

Drug preparations and administration

In the experiments which required a drug pretreatment, 10 min of equilibration with Krebs-Henseleit solution was followed by 10 min of pretreatment with a Krebs-Henseleit solution containing a leukotriene antagonist (L-649,923, Jones *et al.*, 1986) or the leukotriene synthesis inhibitor (MK-886, Gillard *et al.*, 1989), and continued throughout the administration of PAF. The cyclo-oxygenase inhibitor, indomethacin, was added to the perfusion solution at the start of the stabili-

zation period and the effect of PAF injection was tested after 20 min of perfusion in the presence of indomethacin. All stock solutions were made fresh daily and kept at 4°C between experiments. The leukotriene antagonist, L-649,923 (sodium (*βS**, *R**)-4-(3-4(-acetyl-4-hydroxy-2-propylphenoxy)- propyl-thio)-γ-hydroxy-β-methylbenzenebutanoate, Merck Frosst Canada Inc.) and the leukotriene synthesis inhibitor, MK-886 (3-[1-(4-chrofobenzyl)-3-*t*-butyl-thio-5-iospropylindol - 2 - yl] - 2,2-dimethylpropanoic acid, Merck Frosst Canada Inc.) were dissolved in distilled water and then diluted in Krebs-Henseleit solution to a concentration of 5 μM and 10 μM respectively. Indomethacin (Sigma Chemical Co.) was dissolved in 0.1 M Na_2CO_3 solution and diluted in Krebs-Henseleit solution to a final concentration of 2.8 μM. PAF, (1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, prepared from bovine heart, Sigma Chemical Co.) was prepared fresh daily in saline (0.9% NaCl) containing 0.25% bovine serum albumin (Sigma Chemical Co.). Bolus injections of PAF were given in a volume of 0.1 ml and over a 1 s period, into the perfusion line, 5–6 cm proximal to the aortic cannula. LTB_4, LTC_4 and LTD_4 (Merck Frosst Canada Inc.) were prepared fresh daily in saline (0.9% NaCl). The appropriate amount of LTB_4, LTC_4 or LTD_4 was continuously infused to the perfusion line 5–6 cm proximal to the aortic cannula for 5 min. Changes in perfusion pressure and the time at which maximum changes occurred were recorded.

Statistical analyses

Data were analyzed by Students *t* test and analysis of variance (ANOVA) followed by Duncan's test where appropriate. Values are expressed as means ± standard deviations (s.d.) and $P < 0.05$ was considered statistically significant.

Results

The effect of the effluent from the first heart on the recipient heart in the cascade perfusion model

After perfusion through the first heart, the Po_2 of the effluent was reduced. In order to maintain the pH, Po_2, and Pco_2 constant, the effluent from the first heart was oxygenated with 95% O_2 : 5% CO_2 before being perfused into the recipient heart. The pH, Po_2, and Pco_2 of the effluent were in the same range as normal oxygenated Krebs-Henseleit solution (Table 1). The Na^+ and K^+ concentrations of the effluent and normal Krebs-Henseleit solution were identical. Heart rate, contractility and the electrocardiogram were not altered by perfusion for up to 60 min with the effluent from the first heart (data not shown). There was also no significant alteration in the perfusion pressure of the recipient heart by perfusion with effluent (66.5 ± 1.3 and 68.8 ± 1.0 mmHg at time 0 and after 30 min of perfusion with the effluent respectively, $n = 4$).

Table 1 Po_2, Pco_2, pH, Na^+ and K^+ concentrations of the solutions used in the cascade perfusion model: (A) solution before perfusion into the first heart, (B) effluent collected from the first heart, (C) solution before perfusion into the second heart, and (D) effluent collected from the second heart

	A	*B*	*C*	*D*	n
Po_2 (mmHg)	462.4 ± 34.0	199.3 ± 32.3	434.0 ± 14.0	196.2 ± 20.9	7
Pco_2 (mmHg)	43.6 ± 9.3	40.4 ± 5.7	42.6 ± 8.7	40.7 ± 4.9	7
pH	7.37 ± 0.07	7.37 ± 0.05	7.36 ± 0.07	7.36 ± 0.04	6
Na^+ (mEq l^{-1})	149 ± 2	150 ± 2	150 ± 2	151 ± 2	6
K^+ (mEq l^{-1})	5.5 ± 0.5	5.5 ± 0.5	5.5 ± 0.5	5.5 ± 0.5	6

Values represent mean ± s.d., n = number of experiments. Na^+ concentration was determined to 1 mEql^{-1} accuracy and K^+ concentration to 0.1 mEql^{-1} accuracy.

Assessment of PAF-released vasoactive substances in the effluent using the recipient heart of the cascade perfusion model

When 100 pmol PAF was administered to the first heart, a brief vasodilator response followed by a vasoconstrictor response were observed as described previously. The effluent from this heart caused an increase in the perfusion pressure of the recipient heart (30.8 ± 8.7 mmHg, $n = 6$) at about 1.5 min after the injection of PAF to the first heart. However, no vasodilator response was observed in the recipient heart. The results of the effects of PAF on the perfusion pressure of the first and recipient hearts are summarized in Figure 1. To determine whether the coronary vascular effect of the effluent was due to the presence of PAF per se or of released vasoactive substances, the recipient heart was pretreated with PAF (100 pmol) in order to eliminate the vasoconstrictor response to subsequent exposure to PAF (Piper & Stewart, 1986; Man et al., 1990). The vasoconstrictor response of the recipient heart to the effluent was not affected by such a pretreatment (Figure 1). When both hearts were pretreated with PAF, no vasoconstrictor response to the effluent was observed in the recipient heart (Figure 1). The above results demonstrated that the vasoconstrictor response of the recipient heart to the effluent from the first heart was not caused by PAF itself, but by the presence of other PAF-released vasoactive substances. To eliminate the possible interference of the presence of PAF in the effluent in the determination of the properties of the PAF-released vasoactive substances in the effluent, 100 pmol PAF was administered to the recipient hearts in all subsequent experiments.

The effects of L-649,923, indomethacin and MK-886 on the response to PAF in the cascade perfusion model

To gain further insight into the characteristics of PAF-released vasoactive substances, pretreatments with a leukotriene receptor antagonist (L-649,923, 5 μM), a cyclo-oxygenase inhibitor (indomethacin, 2.8 μM) and a leukotriene synthesis inhibitor (MK-886, 10 μM) were used. The effects of pretreatment of the first heart with L-649,923, indomethacin and MK-886 on the coronary responses of these hearts to a bolus injection of 100 pmol PAF are shown in Figure 2. Pretreatment with indomethacin did not significantly affect the vasodilator or the vasoconstrictor responses to PAF while hearts

Figure 2 The effects of 100 pmol PAF on the perfusion pressure of the first heart after various pretreatments: (a) shows the initial vasodilator response to PAF and (b) shows the subsequent vasoconstrictor response to PAF. The concentrations of L-649,923, indomethacin and MK-886 were 5.0, 2.8 and 10 μM, respectively. Pretreatments: none (9), open columns; indomethacin (7) hatched columns; L-649,923 (6) cross-hatched columns; MK-886 (6) solid columns. Numbers in parentheses indicate the number of experiments. Columns represent mean with s.d. shown by vertical bars. $**P < 0.01$ when compared to the response of hearts with no pretreatment.

pretreated with L-649,923 and MK-886 abolished both the vasodilator and vasoconstrictor responses to PAF.

The responses of the recipient hearts following the various pretreatments are summarized in Figure 3. Pretreating only the recipient heart with L-649,923 significantly attenuated ($P < 0.01$) but did not completely abolish the vasoconstrictor response of the recipient heart to the effluent from the first heart after an administration of 100 pmol PAF. The remaining vasoconstrictor component was abolished by pretreating the first heart with indomethacin and the recipient heart with L-649,923 ($P < 0.05$ when compared to the group pretreated with L-649,923 alone). Pretreatment of both hearts with L-649,923 or the first heart with MK-886 also abolished the vasoconstrictor effect of the effluent from the first heart on the recipient heart (Figure 3).

The coronary vascular effects of the effluent from hearts given leukotriene infusions

The effect of a 5 min infussion of LTC_4 or LTD_4 (300 pmol min^{-1}) on the perfusion pressure of the first and
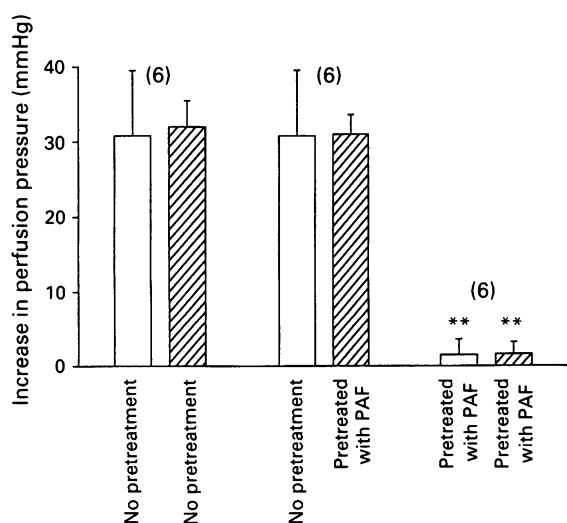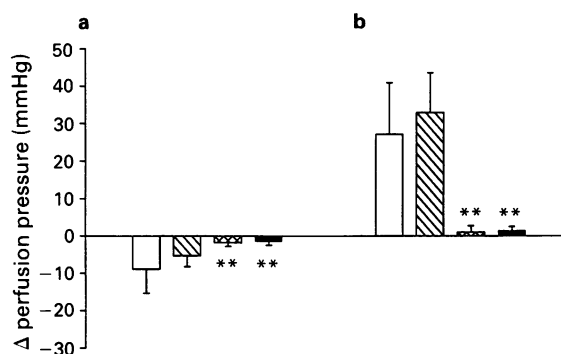
Figure 1 The effects of 100 pmol PAF on the perfusion pressure of the first (open column) and recipient (hatched column) hearts with and without pretreatment with PAF. Columns represent mean with s.d. shown by vertical bars. Numbers in parentheses indicate the number of experiments. $**P < 0.01$ when compared to the response of the corresponding group with no pretreatment.
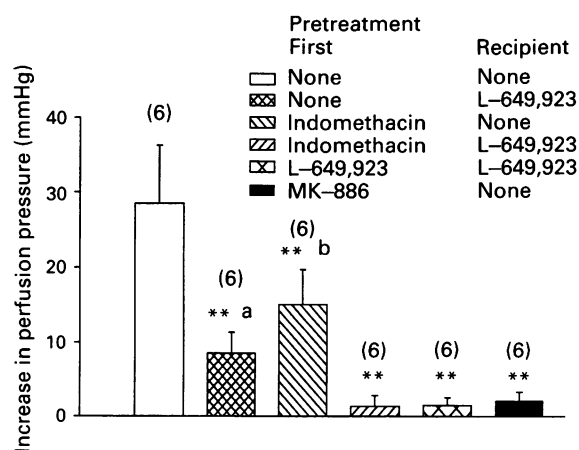
Figure 3 The response of the recipient heart to the effluent from the first heart: the effect of various pretreatments to the first and recipient hearts. The concentrations of L-649,923, indomethacin and MK-886 were 5.0, 2.8 and 10 μM. Columns represent mean with s.d. shown by vertical bars. Numbers in parentheses indicate the number of experiments. $**P < 0.01$ when compared to the response of recipient hearts with no pretreatment. $^aP < 0.05$ when compared to the responses of the last four groups. $^bP < 0.01$ when compared to the responses of the last three groups.
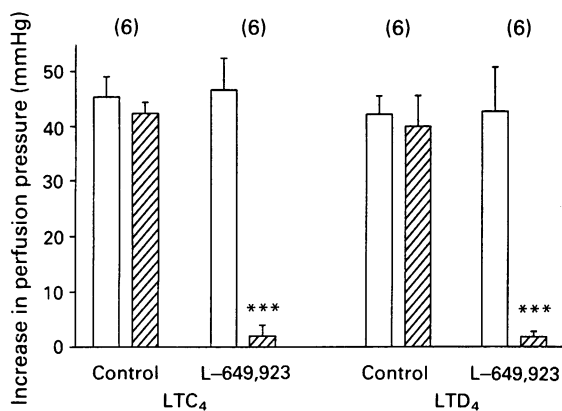
Figure 4 The effects of leukotriene C_4 (LTC_4) and LTD_4 infusion on the perfusion pressure of the first (open columns) and recipient (hatched columns) hearts. LTC_4 and LTD_4 infusion rates were 300 pmol min^{-1} and the amount of L-649,923 used for pretreatment of the recipient heart was 5.0 μM. Columns represent mean with s.d. shown by vertical bars. Numbers in parentheses indicate the number of experiments. ***$P < 0.001$ when compared to the response of the corresponding values with no L-649,923 pretreatment.

recipient hearts is summarized in Figure 4. With no pretreatment, LTC_4 and LTD_4 infusion resulted in a prominent increase in perfusion pressure in the first and recipient hearts and the increases were similar (Figure 4). With pretreatment of only the recipient heart with L-649,923 (5 μM), the effect of the effluent from hearts given LTC_4 or LTD_4 infusion on the perfusion pressure of the recipient heart was completely blocked ($P < 0.001$). Infusion of LTB_4 (300 or 600 pmol min^{-1}) resulted in vasodilatation in the first heart (-5.7 ± 1.2, $n = 12$ and -10.7 ± 1.9 mmHg, $n = 12$ respectively). However, only a small vasodilatation was detected in the recipient heart when perfused with the effluent from hearts with these LTB_4 infusions (-2.2 ± 0.9, $n = 6$ and -4.3 ± 2.4 mmHg, $n = 6$ for 300 and 600 pmol min^{-1} LTB_4 infusions, respectively). Pretreatment of the recipient heart with L-649,923 also did not result in any significant change in the response to effluent from hearts given LTB_4 in infusions (-1.5 ± 0.8, $n = 6$ and -1.2 ± 1.5 mmHg, $n = 6$ for 300 and 600 pmol LTB_4 infusions, respectively).

Discussion

In the cascade perfusion model, the recipient heart was pretreated with a bolus injection of PAF (100 pmol). After this treatment, the recipient heart loses the ability to respond to the direct effects of a further exposure to PAF (Piper & Stewart, 1986; 1987; Man *et al.*, 1990). This enabled us to determine the properties of PAF-released vasoactive substances in the effluent of the first heart without the possible interference of the effect of PAF being presence in the effluent.

Our results confirmed the existence of vasoactive substances in the effluent of the rat perfused heart after an administration of PAF. This is consistent with the concept that PAF itself does not directly initiate the coronary vascular effects but mediates its effects through the release of other vasoactive substances (Piper & Stewart, 1986; 1987; Hu *et al.*, 1991). Our results showed that one PAF-induced vasoconstrictor component in the effluent was mediated by leukotrienes (blocked by the leukotriene receptor antagonist in the recipient heart or the leukotriene synthesis inhibitor in the first heart) while another component was mediated by cyclo-oxygenase products (blocked by the cyclo-oxygenase inhibitor pretreatment in the first heart). Use of the cascade model enabled us to study the interplay of the various vasoactive substances in mediating the coronary vascular effects of PAF.

There was an interaction between the two major arachidonic acid products, prostaglandins and leukotrienes in the perfused heart. This conclusion is based on the observation that

the indomethacin-sensitive vasoactive component could also be blocked by pretreatment of the first heart with a leukotriene receptor antagonist or leukotriene synthesis inhibitor. These results demonstrate for the first time that cyclo-oxygenase products may be regulated by leukotrienes in the rat perfused heart and that this effect is receptor-mediated. Although it is more common to observe regulation of 5-lipoxygenase products by cyclo-oxygenase products, there have been a number of studies that demonstrate leukotrienes directly regulating the synthesis and/or release of cyclo-oxygenase products in other systems. Busija & Leffler (1986) found that leukotrienes were able to increase the levels of prostaglandins ($PGF_{2\alpha}$ and PGE_2) in the cerebral cortex of new born pigs. A dose-dependent increased production of PGI_2 from human lymphatics by LTC_4 and LTD_4 has also been described and each type of leukotriene tested (LTC_4, LTD_4 and LTE_4) has a similar effect on prostaglandin synthesis (Sinzinger *et al.*, 1986). In addition it was suggested that the synthesis and release of 6-keto-$PGF_{1\alpha}$ and thromboxane A_2 in guinea-pig lung by LTD_4 and LTE_4 was receptor-mediated (Mong *et al.*, 1986).

It has been reported in several studies that MK-886 is a potent inhibitor of leukotriene biosynthesis *in vivo* (Gillard *et al.*, 1989; Miller *et al.*, 1990; Rouzer *et al.*, 1990) and MK-886 has no direct effect on 5-lipoxygenase activity (Rouzer *et al.*, 1990) or cyclo-oxygenase activity (Gillard *et al.*, 1989). The ability of MK-886 to inhibit leukotriene biosynthesis is mediated via binding to the 5-lipoxygenase activating protein resulting in the prevention of translocation of 5-lipoxygenase from the cytosol to the active membrane location (Ford-Hutchinson, 1991). Our results have shown that pretreatment of the first heart with MK-886 completely abolished the PAF-induced vasoconstrictor effect of the effluent in the recipient heart. In contrast, L-649,923 pretreatment of the first heart would still require pretreatment of the recipient heart with L-649,923 since PAF-induced leukotriene production would not be affected. Although a direct inhibition of cyclo-oxygenase by L-649,923 cannot be ruled out in the present study, the evidence from using both L-649,923 and MK-886 strongly supports the concept that the synthesis and/or release of cyclo-oxygenase products are leukotriene receptor-mediated in the coronary circulation of the rat perfused heart as concluded in the previous section.

Piper & Stewart (1986, 1987) showed that indomethacin attenuated the increase in perfusion pressure in rat and guinea-pig isolated hearts following bolus injections of PAF. It had also been reported that indomethacin blocked the coronary vasoconstriction produced by intracoronary injection of PAF in the pig heart *in vivo* (Feuerstein *et al.*, 1984). However, our previous study (Hu *et al.*, 1991) and current data showed that pretreatment of the heart with indomethacin did not affect the coronary vascular effect of PAF in the same heart. Thus cyclo-oxygenase products are unlikely to participate in the coronary vascular response under our experimental conditions, although results obtained from the cascade perfusion model indicate the presence of cyclo-oxygenase products based on the response of recipient hearts to effluents from hearts with or without pretreatment with indomethacin. One possible explanation is that the cyclo-oxygenase products are released from the venous side of the coronary circulation and therefore cause vasoconstriction only in the recipient heart.

Leukotrienes and prostaglandins are extensively metabolized. However, it is clear that a sufficient amount of these vasoactive arachidonic acid products was present in the coronary effluent after an administration of PAF to initiate a vasoconstrictor response in the recipient heart comparable to that in the first heart. In contrast, in spite of the presence of a vasodilator response in the first heart, no noticeable vasodilatation was observed in the recipient heart. This suggests that LTB_4, proposed as the mediator of the vasodilator response in the first heart (Hu *et al.*, 1991), is unstable in coronary effluent and was therefore present in insufficient quantity to produce vasodilatation in the recipient heart.

In summary, the present study used a cascade perfusion model to investigate the characteristics and the interaction of PAF-released vasoactive substances. Our results suggest the following. Upon the administration of PAF in the rat perfused heart, leukotrienes (LTB_4, LTC_4 and LTD_4) are released. While LTB_4 may be responsible for the observed vasodilator effect of PAF, LTC_4 and LTD_4 are responsible for the vasoconstrictor effect of PAF (Hu et al., 1991). In addition to directly mediating part of the coronary vascular effects of PAF, leukotrienes also modulate the synthesis and release of cyclo-oxygenase products via a receptor-mediated mechanism. Both lipoxygenase and cyclo-oxygenase products are found in the effluent after the administration of PAF in the rat perfused heart and contribute to the coronary vascular effects of the effluent observed in the recipient heart. However, exogenously applied leukotrienes cannot mimic the endogenously produced leukotrienes released by PAF in regulating the synthesis of cyclo-oxygenase products. This suggests that the PAF-induced production of prostaglandins may be mediated by intracellular leukotrienes or at sites not accessible to exogenous leukotrienes. Indeed it has been demonstrated that leukotrienes, in particular LTC_4, may serve as intracellular mediator of somatostatin-induced increase of neuronal M-current (Schweitzer et al., 1990) and regulation of prostaglandin synthesis by leukotrienes. Hence, further work regarding the cellular mechanism(s) for the interaction between these PAF-released vasoactive substances and the elucidation of the site where this interaction occurs in the coronary vasculature seems warranted.

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