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Release of tissue-type plasminogen activator is induced in rats by leukotrienes C_4 and D_4, but not by prostaglandins E_1, E_2 and I_2

N. Tranquille & [1]J.J. Emeis

Gaubius Institute TNO, Herenstraat 5d, 2313 AD Leiden, The Netherlands

1 Acute release of plasminogen activator (PA) was studied in rat isolated hindleg system perfused with Tyrode solution.

2 Leukotriene C_4 (LTC_4) and LTD_4 dose-dependently induced the release of PA, which plateaued at 160 nmol l^{-1} and 200 nmol l^{-1}, respectively. The amount of PA released was about 1 iu ml^{-1}. The effects of LTC_4 and LTD_4 were not additive.

3 The PA released was identified as tissue-type PA (t-PA) by quenching experiments using anti-human t-PA IgG, by fibrin autography, and by the dependence of its activity on the presence of soluble fibrin.

4 LTE_4 (300 and 450 nmol l^{-1}) and 5-hydroxy-eicosatetraenoic acid (600 nmol l^{-1}) did not induce any t-PA release in the perfusion system used.

5 Release of t-PA induced by LTC_4 and LTD_4 was inhibited by the leukotriene-receptor antagonist FPL 55712 (10 μmol l^{-1}), whereas FPL 55712 did not inhibit t-PA release induced by platelet-activating factor (Paf-acether).

6 *In vivo* LTC_4 and LTD_4 (2 μg kg^{-1} i.v.) also induced an acute increase of t-PA activity in rat blood as evidenced by decreased blood clot lysis times.

7 Prostaglandin E_1 and E_2, prostacyclin and the stable prostacyclin analogue ZK 36374 at concentrations of 0.1–3.0 μmol l^{-1} induced little or no t-PA release.

Introduction

The fibrinolytic and thrombolytic activity of blood is to a large extent determined by its content of plasminogen activators and their inhibitors. Of the various functionally and immunologically distinct types of plasminogen activator present in plasma (Emeis *et al.*, 1985), only tissue-type plasminogen activator (t-PA) can show large and rapid changes in plasma activity (Prowse & Cash, 1984; Emeis, 1987a). A better insight into factors regulating changes in blood t-PA levels might be of importance to develop means of manipulating blood fibrinolytic and thrombolytic activity pharmacologically.

In vivo, t-PA is synthesized and stored by vascular endothelial cells, which are considered to be the major, if not only, source of t-PA present in blood (Emeis, 1987a). From these endothelial cells, t-PA can be released into the blood, resulting in rapid and large increases in blood fibrinolytic activity (Prowse & Cash, 1984). The humoral and cellular mechanisms resulting in this acute release of t-PA are only vaguely understood (Emeis, 1987a). In a previous study we suggested, on the basis of inhibition studies, that products of a lipoxygenase pathway were involved in the acute release of t-PA from vessel walls (Emeis & Kluft, 1985).

In the present paper we will show that leukotriene C_4(LTC_4) and LTD_4, though not other eicosanoids, can induce in rats the acute release of t-PA, both *in vivo* and in a perfused hindleg vascular bed.

[1] Author for correspondence.

Methods

Rat hindleg perfusion

The rat perfused hindleg system was used to study the release of tissue-type plasminogen activator (t-PA) from a perfused vascular bed (Emeis, 1983). Male Wistar rats (Centraal Proefdierbedrijf TNO, Zeist, the Netherlands) weighing 220–300 g were anaesthetized

with pentobarbitone (Nembutal, 60 mg kg^{-1} intraperitoneally). With the animal anaesthetized, the abdominal cavity was opened and the aorta and inferior vena cava were carefully dissected out. Ligatures were applied round the renal vessels and these were tightened to prevent leakage during the perfusion. Double ligatures were loosely applied round the aorta and vena cava separately. The upper ligature round the aorta was tightened and an 18-gauge needle was immediately inserted into the vessel and pushed distally up to the bifurcation. The lower ligature was then tightened securing the needle in place and the perfusion started by means of a constant flow roller pump. Next the upper ligature around the vena cava was tightened and the vessel was severed distal to the tied ligature to allow unimpeded outflow. The animal's thorax was opened and the pulmonary vessels severed to kill the animal. A cannula was inserted distally into the vena cava and tied into place. The rat hindlegs were perfused at a constant flow of 9 to 10 ml min^{-1} using Tyrode solution (composition in mmol l^{-1}: NaCl 146, KCl 6, $CaCl_2$ 3, $MgCl_2$ 0.5, KH_2PO_4 0.3, $NaHCO_3$ 20, glucose 5.6) containing 0.1 mg ml^{-1} bovine serum albumin (BSA), pH 7.4 at 37°C and oxygenated with 95% O_2 and 5% CO_2. Perfusion pressure was measured just proximal to the inflow by means of a mercury manometer.

Each experiment was started with a 30 min perfusion of the Tyrode/BSA solution through the hindleg region to clear the vessels from residual blood. Then compounds to be tested (see Results section) were added to the Tyrode/BSA and immediately perfused through the hindlegs. Sample collections were taken every 30 s for 30 s from the vena cava cannula and placed on ice. Subsequently, a 5 min wash with Tyrode/BSA solution was carried out before another compound, generally platelet-activating factor (Paf-acether), was perfused to test the responsiveness of the vascular bed. On completion of the experiment the samples were centrifuged (3000 *g* for 10 min), mixed at a 10:1 ratio with a solution containing 0.5 M Tris HCl (pH = 7.5) and 1% Triton X-100, and stored at − 20°C. This procedure stabilized the activity of the samples for at least four months (data not shown). The samples in 30 s blocks were usually analysed for t-PA activity immediately after experimentation.

To ensure that the hindleg region was totally perfused and no blockages had occurred, Evans Blue dye was injected into the inflow tube: the passage of the dye through all vessels indicated a complete perfusion.

Compounds and solvents

Leukotrienes were obtained in a solvent composed of methanol, water, acetic acid and ammonium hydroxide (65:35:0.03:0.04) and added as such to the Tyrode/BSA. Prostaglandin E_1 (PGE_1) and PGE_2 were dissolved in ethanol, and PGI_2 in 0.1 N NaOH, and added to Tyrode/BSA immediately before perfusion. FPL 55712 and ZK 36374 were dissolved directly in the Tyrode/BSA. Paf-acether was prepared as described by Emeis & Kluft (1985).

Spectrophotometric plasminogen activator assay

The PA activity of the sample was determined by the indirect spectrophotometric rate assay described by Verheijen *et al.* (1982). In brief: to wells of a 96-well microtiter plate were added: 75 µl buffer (0.1 mol l^{-1} Tris HCl, pH 7.65 containing 0.1% Tween 80), 20 µl soluble fibrin digest (1 mg ml^{-1}), 30 µl sample, 100 µl S-2251 (0.66 mmol l^{-1}) and 25 µl human plasminogen (1.11 µmol l^{-1}). The microtiter plate was incubated at 37°C and after 45, 65, 85, 105 and 125 min; absorption was measured at 405 nm in a Titertek multiscan spectrophotometer (Flow Laboratories, Irvine, Scotland). PA activity was then calculated according to Drapier *et al.* (1979). The detection limit for the assay was 0.09 iu ml^{-1}.

Dilutions of human melanoma t-PA (Kluft *et al.*, 1983) were run in each plate for calibration. The PA activity of samples will be expressed in International Units (iu), as defined by the International Standard of t-PA (Gaffney & Curtis, 1985).

In some experiments either the soluble fibrin digest or the human plasminogen was omitted from the incubation mixture.

Quenching experiments

The quenching experiments used the same spectrophotometric t-PA assay as mentioned above with the following addition: to the buffer, fibrin digest, and sample, 0–40 µl of a rabbit anti-human t-PA IgG solution was added. The plate was incubated for 10 min at 37°C and then the substrate and plasminogen were added and the incubation proceeded as described above.

In vivo *release of plasminogen activator*

Rats were injected intravenously with LTC_4 or LTD_4 at a dose of 2 µg kg^{-1} body weight. Leukotrienes (0.1 ml in solvent) were diluted with 0.9 ml of saline to a concentration of 1 µg ml^{-1}. Controls received only the solvent (2 ml kg^{-1}). Blood samples were obtained from a cannula in the carotid artery before and at 1, 2, 3, 5, 7 and 10 min after injection.

Blood (0.2 ml) was diluted to 10% in 1.7 ml of 0.12 mol l^{-1} sodium acetate (pH 7.4), clotted with 0.1 ml thrombin (20 u ml^{-1}) and incubated at 37°C. Lysis times were read in minutes. When indicated,

antibodies were added to the diluted blood 5 min before the addition of thrombin.

Fibrin autography

Sodium dodecyl sulphate/8% polyacrylamide slab gels were prepared according to Laemmli (1970). Fibrin autography was performed according to Loskutoff & Mussoni (1983).

Materials

All the chemicals used were of analytical grade. The products necessary for the spectrophotometric assay, such as the fibrin digest, substrate S-2251 and plasminogen were described previously (Verheijen *et al.*, 1982). Conditioned medium from rat L_2 cells (Wewer *et al.*, 1981) was used as a source of rat t-PA. Rabbit anti-human t-PA IgG (100 $\mu g\,ml^{-1}$) was prepared in our institute (Rijken *et al.*, 1984).

The compounds used were obtained from the following sources: LTC_4, LTD_4 and LTE_4 as free acids from Paesel GmbH & Co, Frankfurt, West Germany; PGE_1, PGE_2, PGI_2 and bovine albumin (fraction 5) from Sigma, St Louis, U.S.A.; ZK 36374 (5-(E)-(1S,5S,6R,7R)-7-Hydroxy-6-[(E)-(3S,4RS)-3-hydroxy-4-methyl-1-octane-6-inyl]bicyclo[3.3.0] octane-3-ylidenepentanoic acid) from Schering AG, Berlin, West Germany; FPL 55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propyl-phenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate) from Fisons Pharmaceuticals, Loughborough, U.K.; bovine thrombin from Leo Pharmaceuticals, Ballerup, Denmark; Nembutal from Sanofi, Paris, France; Paf-acether from Bachem, Bubendorf, Switzerland; 5-hydroxy-eicosatetraenoic acid (5-HETE) from Unilever Research Laboratories, Vlaardingen, the Netherlands.

Results

The release of PA by LTC_4 and LTD_4

Perfusion using only Tyrode/BSA solution did not result in PA release. When LTC_4 (8–320 nmol l^{-1}) was added to the Tyrode/BSA solution and perfused through the rat hindleg region, analysis of the samples collected showed the presence of plasminogen activator (PA) in the perfusate. The amount of PA released was found to be dose-dependent, increasing and reaching a maximum at a dose of 160 nmol l^{-1} (Figure 1). LTD_4 (10–400 nmol l^{-1}) induced PA release in a very similar fashion to LTC_4. The release of PA was also dose-dependent, in this case reaching a maximum at a dose of 200 nmol l^{-1}. The maximal amount of PA released was similar for LTC_4 and LTD_4, and amounted to about 1 iu ml^{-1} (Figure 1). Both LTC_4 and LTD_4 followed a similar time course of PA release with peak values being always present in the 60–90 s sample block and decreasing gradually over the next few minutes (Figure 2).

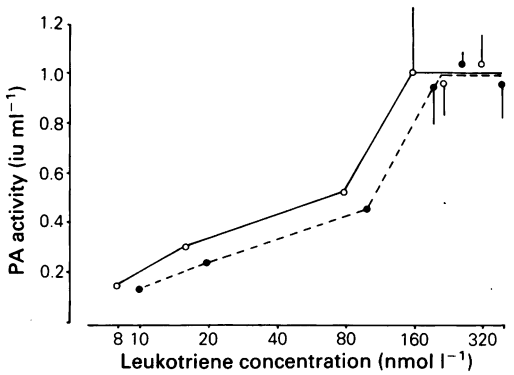

Figure 1 Dose-dependent release of tissue-type plasminogen activator (t-PA) induced by leukotriene C_4 (LTC_4 ○) and LTD_4 (●) in perfused hindlegs of the rat. Data are shown as means with vertical lines indicating s.d. ($n = 4$), or as mean ($n = 2$) values, of t-PA concentrations in the 60–90 s sample blocks.

In two experiments, LTC_4 (160 nmol l^{-1}) and LTD_4 (200 nmol l^{-1}) were added together in the same perfusion buffer. The PA activity released by the com-

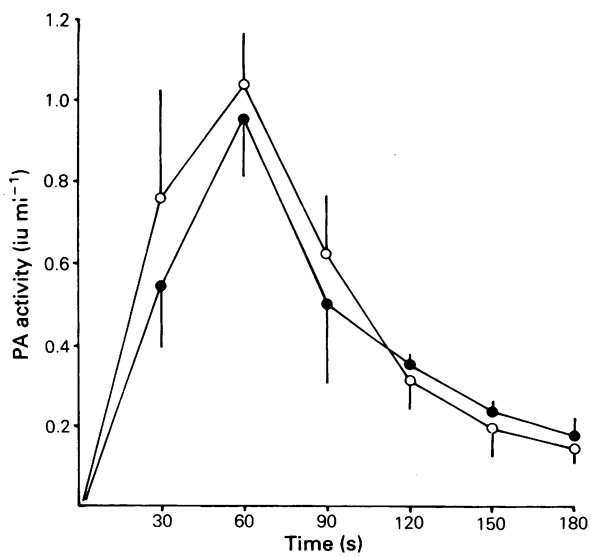

Figure 2 Time course of tissue-type plasminogen activator (t-PA) release induced by leukotriene C_4 (○; 320 nmol l^{-1}) and leukotriene D_4 (●; 400 nmol l^{-1}) in perfused hindlegs of the rat. Each point represents the mean ($n = 4$) and vertical lines indicate s.d.

Table 1 Induction of tissue-type plasminogen activator (t-PA) release in the rat perfused hindleg system

Compound	*Concentration* (nmol l^{-1})	n	*t-PA release* (iu ml^{-1})
LTC_4	320	4	1.04 ± 0.11*
LTD_4	400	4	0.94 ± 0.14
LTE_4	300	3	ND
	450	2	ND
5-HETE	600	5	ND
Solvent controls	—	5	ND
PGE_1	600	2	ND
	3000	2	<0.10
PGE_2	3000	4	<0.15
PGI_2	3000	3	<0.10
ZK 36374	100	3	ND
	1000	3	ND
Paf-acether	20	4	2.79 ± 0.81

*Mean ± s.d. of maximal t-PA concentration obtained during perfusion of rat hindlegs in the presence of the indicated concentration of release-inducing compound. Maximal concentrations were always found in the 60–90 s sample block. ND = none detected.

Table 2 Effect of FPL 55712 on tissue-type plasminogen activator (t-PA) release in rat perfused hindlegs

Compound	*Concentration* (nmol l^{-1})	n	*t-PA release* (iu ml^{-1})
LTC_4	160	4	1.00 ± 0.32*
LTC_4 and FPL 55712	160 / 10^4	4	ND
LTD_4	200	3	0.94 ± 0.11
LTD_4 and FPL 55712	200 / 10^4	3	ND
Paf	20	4	2.79 ± 0.81
Paf and FPL 55712	20 / 10^4	6	3.01 ± 0.79

*Mean ± s.d. of maximal t-PA concentration obtained (in the 60–90 s blocks) by perfusing rat hindlegs with the indicated concentration(s) of compound(s). ND = none detected.

bined leukotrienes (0.99, 0.90 iu ml^{-1}) was equivalent to the amount of PA activity released by each of them when given alone, and not equivalent to the sum of these amounts. In the perfusions using the leukotrienes no pressure changes or variations in the flow were noted. No oedema was seen in any of the animals following these perfusions. In contrast to LTC_4 and LTD_4, LTE_4 in concentrations of 300 and 450 nmol l^{-1} did not induce any PA release in the hindleg system (Table 1).

Perfusions were also done using 5-HETE, but no PA release was induced using this compound at a concentration of 600 nmol l^{-1} (Table 1). Paf-acether is known to induce the release of large amounts of t-PA in the hindleg model (Emeis & Kluft, 1985). To test each individual experiment and ensure that the animal was responsive, Paf-acether (20 nmol l^{-1}) was perfused for several minutes after each of the compounds investigated. In all the experiments, Paf-acether did induce t-PA release, whether the compound perfused previously to it had induced t-PA release or not. Following the perfusion with Paf-acether, some oedema was seen in the animal's hind quarter; a slight increase in pressure and decrease in the flow was also noted. In solvent control experiments no PA release was detected.

The effect of FPL 55712 on PA release

To investigate the mode of release of PA by LTC_4 and LTD_4, FPL 55712, a leukotriene receptor antagonist (Augstein *et al.*, 1973; Musser *et al.*, 1986), was used (Table 2). FPL 55712 (10 μmol l^{-1}) was added to the Tyrode/BSA buffer and perfused through the hindleg region for 20 min before the addition of either LTC_4 (160 nmol l^{-1}) or LTD_4 (200 nmol l^{-1}). FPL 55712 totally blocked the release of PA by both LTC_4 and LTD_4. However, it did not affect the release of PA induced by Paf-acether and so was not an inhibitor of the release reaction.

Determining the type of PA released

The activator released by LTC_4 and LTD_4 was identified as tissue-type PA (t-PA) by the following observations.

Firstly, a series of quenching experiments were done using the IgG fraction of a rabit anti-human t-PA antiserum. The specific anti-human t-PA IgGs quenched human t-PA, rat t-PA and the PA present in the perfusate samples dose-dependently (see Figure 3). As expected, heterologous rat t-PA was quenched less efficiently than the homologous human t-PA.

Secondly, the activation of plasminogen by t-PA is enhanced by soluble fibrin (ogen) fragments whereas plasminogen activation by urokinase-type plasminogen activators is not (Verheijen *et al.*, 1982). Several spectrophotometric assays were done omitting the soluble fibrin digest; plasminogen activation by the perfusate samples was only detectable in the presence of soluble fibrin fragments.

Thirdly, in fibrin autography (Figure 4) both rat t-PA from L_2 cells and perfusate samples of LTC_4 and

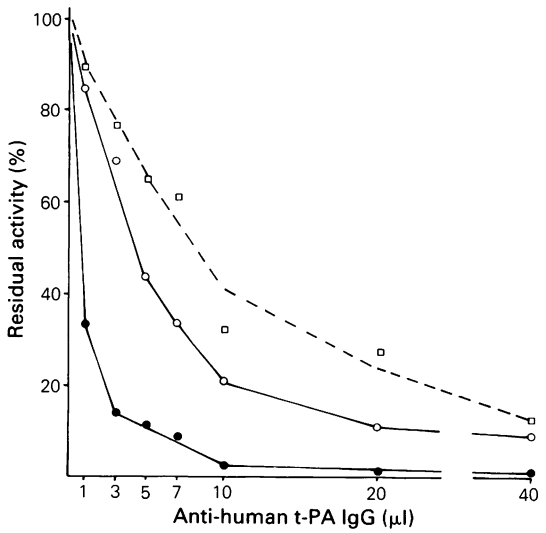

Figure 3 Quenching of plasminogen activator (PA) activity in perfusate samples by anti-human PA tissue-type (t-PA) antibodies. Perfusate samples (30 min from leukotriene C_4 (LTC_4)- or LTD_4-stimulated rat hindlegs were incubated with increasing amounts of rabbit anti-human t-PA IgG (100 $\mu g\,ml^{-1}$) for 10 min at 37°C, and subsequently the residual PA activity was determined spectrophotometrically. Data shown are means of duplicate determinations; the percentages of residual activity for LTC_4- and LTD_4- induced perfusates are averaged (○), as these percentages did not differ by more than 4%. Also shown for comparison is the quenching by anti-human t-PA Ig of human t-PA (●; 30 miu) and of rat L_2 t-PA (□; 60 miu).

LTD_4 showed a single lysis zone at the molecular weight of approx. 70,000, identical to the M_r of human t-PA, but different from that of urokinase-type PA (molecular weights 55,000 and 33,000).

In vivo *experiments*

To see whether LTC_4 and LTD_4 would also induce t-PA release *in vivo*, each compound was injected intravenously into rats. Both LTC_4 and LTD_4 (2 $\mu g\,kg^{-1}$) were found to increase blood fibrinolytic activity, as evidenced by decreased blood clot lysis times (Figure 5). At one min after injection, dilute blood clot lysis times were decreased by 86 ± 14 min in the leukotriene-treated animals, as compared to 7 ± 13 min ($n = 5$) in the control, solvent-treated, rats. No decreased blood clot lysis times were seen with the solvent controls. The increase in blood fibrinolytic activity could be quenched by pre-incubation of the diluted blood with antibodies against human t-PA (Figure 5).

Investigation of other eicosanoids

Several cyclo-oxygenase products were also tested in the hindleg perfusion system (see Table 1). PGE_1 was perfused at concentrations of 600 $nmol\,l^{-1}$ and 3 $\mu mol\,l^{-1}$. Whereas no PA was detected at the lower dose, at the higher dose a very small amount of t-PA release was induced. PGE_2 (3 $\mu mol\,l^{-1}$) and PGI_2 (3 $\mu mol\,l^{-1}$) also induced the release of small amounts of t-PA, which were significantly less than those released by LTC_4 and LTD_4.

ZK 36374, a stable prostacyclin analogue (Schrör *et al.*, 1981), was also perfused through the hindleg system (at 100 $nmol\,l^{-1}$ and 1 $\mu mol\,l^{-1}$) but no PA release was detected.

Discussion

Peptidoleukotrienes affect the vascular system in various ways. Injection of leukotrienes C_4 or D_4 into rats results in a short-lived increase in blood pressure, followed by hypotension (Piper, 1983, Feuerstein, 1984). In cultured vascular endothelial cells LTC_4 shows high-affinity plasma membrane binding, which can be inhibited by FPL 55712 (Chau *et al.*, 1986). Moreover, LTC_4 and LTD_4, but not LTB_4 or LTE_4, stimulate endothelial cell prostacyclin synthesis (Benjamin *et al.*, 1983; Cramer *et al.*, 1983; Pologe *et al.*, 1984; Clark *et at.*, 1986a), presumably because leukotrienes activate phospholipase A_2 in endothelial cells (Clark *et al.*, 1986b, c). Not only prostacyclin synthesis, but synthesis of Paf-acether as well, is enhanced by LTC_4 and LTD_4 though again not by LTE_4 or LTB_4 (McIntyre *et al.*, 1986). Endothelial cells *in vitro* can also degrade LTC_4 into LTD_4 and LTE_4 (Pologe *et al.*, 1984). Whether endothelial cells can synthesize leukotrienes from arachidonic acid is, however, doubtful (Feinmark & Cannon, 1986). Together these observations show that endothelial cells respond to LTC_4 and LTD_4, both *in vivo* and *in vitro*, and can convert LTC_4 into LTD_4 and LTE_4. In our constant flow-perfused hindleg system of the rat, LTC_4 LTD_4 and LTE_4 did not change perfusion pressure and did not cause oedema formation within the experimental time period, in agreement with the relatively small effects on hindleg blood flow and vascular resistance described by Eimerl *et al.* (1986) after *in vivo* injection of graded doses of leukotrienes.

LTC_4 and LTD_4 were approximately equi-effective in inducing the release of t-PA, while LTE_4 had no effect at all. The combined application of both LTC_4 and LTD_4 did not result in an enhanced release of t-PA compared to the release induced by LTC_4 or LTD_4 separately. In combination with the complete suppression of t-PA release by FPL 55712, the data suggest that both LTC_4 and LTD_4 interact with a single

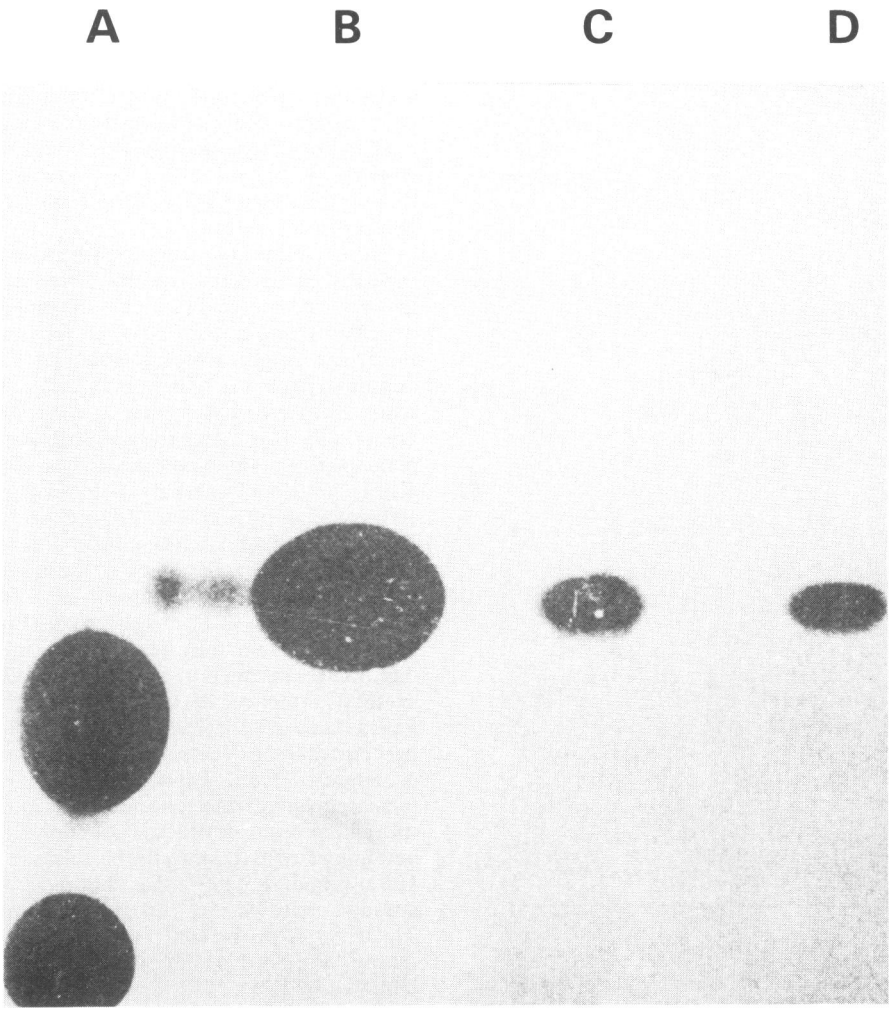

Figure 4 Fibrin autography of perfusate samples from leukotriene-stimulated rat hindlegs. Human urokinase (lane A), rat L_2 tissue-type plasminogen activator (t-PA, lane B) and perfusate samples after stimulation with 160 nmol l^{-1} of leukotriene C_4, (LTC_4, lane C) or 200 nmol l^{-1} of LTD_4 (lane D) were electrophoresed in 8% polyacrylamide slab gels (Laemmli system). The gel was then soaked for 2 h in 2.5% Triton X-100 (with one change), washed, and placed on top of a plasminogen-rich fibrin-agarose gel. The dark areas indicate lysis of the fibrin gel, caused by PA diffused from the polyacrylamide gel into the fibrin gel. The mobility of the PA-activity in the perfusate samples is identical to that of rat t-PA (M_r = 70,000), but different from that of urokinase (M_r = 55,000 and 33,000).

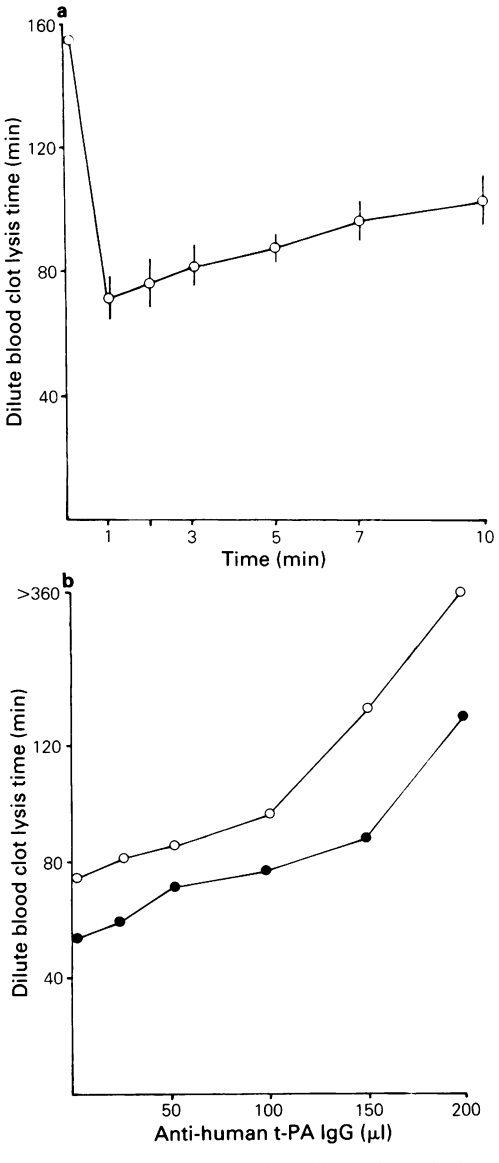

Figure 5 (a) Time course of dilute blood clot lysis times after injection of leukotrienes. Data are shown as mean lysis times from four rats (two injected with leukotriene C_4 (LTC_4), 2 $\mu g\,kg^{-1}$; two with LTD_4, 2 $\mu g\,kg^{-1}$); vertical lines indicate s.d. (b) Incubation of blood from a LTC_4- and a LTD_4-injected rat with anti-human tissue-type plasminogen activator (t-PA) IgG normalizes the decreased dilute blood clot lysis times. Blood was obtained from rats one minute after the i.v. injection of leukotriene C_4 (○) or D_4 (●), both at a dose of 2 $\mu g\,kg^{-1}$.

receptor on endothelial cells, binding to this receptor being sensitive to inhibition by FPL 55712. In cultured bovine endothelial cells, an LTC_4 receptor with K_D = 6.8 nM has been described (Chau *et al.*, 1986). The dose-response curve obtained in our system is compatible with the presence of a similar receptor for LTC_4 and LTD_4 on rat vascular endothelial cells *in vivo*. Our data, however, cannot exclude the (quantitative) conversion of LTC_4 into LTD_4 during passage through the hindleg vascular bed, the t-PA release response being then due to activation of an LTD_4-receptor. Rats are indeed able to metabolize LTC_4 into LTD_4 efficiently (Denzlinger *et al.*, 1985). To decide if a similar conversion is of importance in our experimental system, we will have to await the availability of compounds interfering with the said conversion, or of receptor antagonists specific for LTD_4, rather than LTC_4 (Cheng, 1986; Lee *et al.*, 1984; Musser *et al.*, 1986).

The PA released by leukotrienes proved to be tissue-type PA, as demonstrated by the dependence of its activity on soluble fibrin, by the quenching of its activity by anti-human t-PA antibodies, and by its molecular weight, which was similar to that of cell culture-derived rat and human t-PA, but different from urokinase. Paf-acether and a variety of other compounds also induce the release exclusively of t-PA from perfused rat hindlegs (Emeis, 1983; Emeis & Kluft, 1985) and pig ears (Klöcking *et al.*, 1984). Release of another type of PA, e.g. urokinase, has so far not been observed. As endothelial cells are the only cells in hindlegs to contain t-PA, the induced release of t-PA must be due to release of t-PA from vascular endothelial cells. In a previous paper (Emeis & Kluft, 1985) we showed that lipoxygenase inhibitors (and diethylcarbamazine, a putative peptidoleukotriene synthesis inhibitor, Bach & Brashler, 1986) inhibited Paf-induced t-PA release. As shown by others, in perfused rat lung (Voelkel *et al.*, 1982) and heart (Piper & Stewart, 1986) Paf-acether does indeed cause increased leukotriene concentrations in the perfusate, the cellular origin of which is not known. The maximal amount of t-PA that could be released by LTC_4 or LTD_4 (about 1 iu ml^{-1}) was, however, less than the amount released by 20 nM Paf-acether (about 3 iu ml^{-1}). Also, in contrast to leukotriene-induced release, Paf-acether-induced t-PA release was not inhibited by FPL 55712. Together these two observations suggest that Paf-acether does not induce t-PA release exclusively by inducing the vascular synthesis and release of leukotrienes, which then cause endothelial cells to release t-PA.

The present study suggests that the effects on t-PA release of the lipoxygenase inhibitors and of diethylcarbamazine cannot be explained by decreased peptidoleukotriene release from the vessel wall. Whether other products of a lipoxygenase pathway (e.g. hydroxy-eicosatetraenoic acids or hydroxy-linoleic acid) or a mono-oxygenase pathway are involved in t-PA release, remains to be determined, although the

data make a major role of 5-HETE unlikely. Neither can we exclude that leukotrienes synthesized intracellularly behave differently from exogenously supplied leukotrienes. However, a recent study on the effects of lipoxygenase inhibitors cautions against aspecific inhibitory effects of these compounds on secretion (Razin *et al.*, 1984).

The observation that intravenous injection of LTC_4 or LTD_4 also induced acute release of t-PA, as evidenced by decreased dilute blood clot lysis times, shows that the induction of t-PA release *ex vivo* in a perfused vascular bed is not an artifact induced by the experimental procedure, but that leukotriene-induced t-PA release may be of physiological significance.

In perfused hindlegs, prostaglandins E_1 and E_2 and prostacyclin were unable to induce t-PA release, in agreement with previous negative results on the induction of PA-release by prostacyclin (Nakajima, 1983) and prostaglandins (Markwardt & Klöcking, 1978) in perfused ears of the pig. However, Hussaini & Moore (1985) demonstrated that in rats *in vivo* prostacyclin (and its metabolite 6-keto-PGE_1) induced increased fibrinolytic activity. Maximally increased activity was found at 30–60 min after injection, suggesting that the observed induction of increased fibrinolytic activity by cyclo-oxygenase products may proceed by a pathway different from acute t-PA release (as induced by e.g. leukotrienes) which generally peaks at one min after injection.

Other mechanisms that might be involved in prostacyclin-induced increased fibrinolytic activity (for discussion, see Emeis, 1987b) are now under investigation. Whether the peptidoleukotriene-induced release of t-PA from endothelial cells is of (patho) physiological significance in processes involving increased leukotriene production also remains to be determined.

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