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LEUKOTRIENE B_4: A MEDIATOR OF VASCULAR PERMEABILITY

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1 Intradermal injection of leukotriene B_4 (LTB_4) or prostaglandin E_2 (PGE_2), 1 to 100 ng per skin site produced little or no change in plasma exudation in the rabbit, guinea-pig and rat.

2 Intradermal injection of LTB_4 together with PGE_2 produced a significant increase in plasma exudation in the rabbit, guinea-pig and rat.

3 Intradermal injection of LTB_4 or PGE_2 together with bradykinin (500 ng) resulted in a significant potentiation of the plasma exudation produced by bradykinin alone in the rabbit and guinea-pig.

4 LTB_4 (1 to 10 ng) had no effect on blood flow in the rabbit skin, in contrast to PGE_2 which was a potent vasodilator in this species.

5 It is concluded that LTB_4 is a mediator of vascular permeability and that this effect can only be observed in the presence of a vasodilator such as PGE_2.

Introduction

Leukotriene B_4 (LTB_4) is a metabolite of arachidonic acid (AA) formed via a lipoxygenase enzyme (Samuelsson & Hammerström, 1980). LTB_4 is released by polymorphonuclear leucocytes (PMNs) (Borgeat & Samuelsson, 1979a; Stenson & Parker, 1979), eosinophils (Goetzl, Weller & Sun, 1980) and macrophages (Doig & Ford-Hutchinson) when these cells are exposed to the calcium ionophore A23187 and is also present in perfusates obtained from antigen-challenged lung (Morris, Taylor, Piper & Tippins, 1979). LTB_4 is a potent chemokinetic and chemotactic agent for PMNs and macrophages *in vitro* and *in vivo* (Ford-Hutchinson, Bray, Doig, Shipley & Smith, 1980; Smith, Ford-Hutchinson & Bray, 1980) and has been proposed as a potential mediator of the cellular aspects of inflammation (Smith *et al.*, 1980). The present work was designed to investigate a possible role for this compound in the vascular aspects of inflammation.

Methods

Animals used were New Zealand white rabbits (female, 2.5 to 3 kg) (Buxted Rabbits, Buxted, Sussex), Dunkin-Hartley guinea-pigs (female, 250 to 300 g) (Redfern Animal Breeders) and Wistar rats (female, 250 to 300 g) (Olac (1976) Ltd., Oxfordshire).

Preparation of leukotriene B_4

LTB_4, corresponding to the enzymatically produced isomer III (Rådmark, Malmsten, Samuelsson, Clark, Goto, Marfat & Corey, 1980), was prepared from rat peritoneal PMNs exposed to the calcium ionophore A23187 and was purified by ether extraction, silicic acid chromatography and reverse phase high pressure liquid chromatography as previously described (Ford-Hutchinson *et al.*, 1980). The concentration of LTB_4 was determined using an ϵ_{281}^{MeOH} of 39,500 (Borgeat & Samuelsson, 1979b).

Vascular permeability changes

Vascular permeability changes were monitored in the rat, guinea-pig and rabbit by the extravasation of [^{125}I]-human serum albumin (Radiochemical Centre, Amersham) (Williams, 1979). Briefly [^{125}I]-albumin (15 μCi/kg) in Evans Blue solutions (2.5% w/v in saline) was administered intravenously immediately prior to intradermal injections of LTB_4, prostaglandin E (PGE) or bradykinin in 0.1 ml Tyrode solution. Thirty min after the last intradermal injection, blood samples were taken and the animals killed. Skin sites were punched out with a 18 mm diameter punch and were counted in a γ counter. Aliquots of blood were counted in order to estimate ct min^{-1} μl^{-1} in the plasma and results have been expressed as μl of plasma exudate per skin site. In man, 100 ng or 10 ng of LTB_4 in 0.1 ml of saline was injected into the forearm and the skin observed at intervals for erythema and induration.

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Blood flow changes

The effects of LTB_4 on blood flow were measured in separate experiments in rabbits using the ^{133}Xe clearance technique described by Williams (1979). For comparison PGE_2 was used as an example of a known vasodilator and noradrenaline as an example of a vasoconstrictor.

Drugs

The following were used: calcium ionophore A23187 (Calbiochem); PGE_1, PGE_2 (Dr J.E. Pike, Upjohn); bradykinin triacetate (Sigma).

Results

The effects of LTB_4 and PGE_2 on vascular permeability changes in rabbit, guinea-pig and rat skin are shown in Table 1. Neither LTB_4 nor PGE_2 alone produced any significant change in vascular permeability, except at the highest dose of each compound in rabbit skin where a small, statistically significant effect was observed. In all three species, plasma exudation was observed when LTB_4 was administered together with 10 ng of PGE_2, statistically significant effects being observed with doses of LTB_4 of 1, 10 and 100 ng in the rabbit, 10 and 100 ng in the guinea-pig and 100 ng in the rat. No further effects were seen in the rabbit when the dose of PGE_2 was raised to 100 ng as used by other workers (Wedmore & Williams, 1980). PGE_1 has been reported (Williams & Morley, 1973) to be a more effective potentiator than PGE_2 in the guinea-pig. No difference was observed between the effects of LTB_4 on vascular permeability in the presence of 100 ng of PGE_1 rather than 10 ng of PGE_2.

Both LTB_4 and PGE_2 potentiated the vascular permeability changes produced by bradykinin in the rabbit and guinea-pig (Table 2). In contrast, in the rat, no potentiation of the response to 500 ng of bradykinin was observed with either agent. LTB_4 produced no changes in blood flow in the rabbit skin over the dose range 1 to 100 ng per skin site (Figure 1). In contrast PGE_2, (0.1 to 10 ng per skin site) produced a dose-related increase in blood flow and 1 ng of noradrenaline produced a significant decrease in blood flow. When injected into the human forearm, LTB_4 (10 or 100 ng) produced no visible erythema or induration and no pain responses were observed (M.A. Bray, personal experience).

Discussion

The results of the present work show that either LTB_4

Table 1 Effects of leukotriene B_4 (LTB_4) and the E type prostaglandins (PGE) on plasma exudation in the rabbit, guinea-pig and rat skin

LTB_4 concentration (ng/0.1 ml)	Rabbit			Guinea-pig			Rat		
	Tyrode alone	PGE_2 (10 ng/0.1 ml)	PGE_2 (100 ng/0.1 ml)	*Tyrode alone*	PGE_2 (10 ng/0.1 ml)	PGE_1 (100 ng/0.1 ml)	*Tyrode alone*	PGE_2 (10 ng/0.1 ml)
0	4.6 ± 2.4	9.2 ± 2.8	20.5 ± 6.0*	12.1 ± 2.4	10.5 ± 2.9	7.9 ± 2.0	10.7 ± 2.4	25.5 ± 6.5
1	26.0 ± 10.0	19.9 ± 4.9**	21.1 ± 1.9	12.6 ± 1.9	16.3 ± 5.7	7.1 ± 3.4	10.1 ± 3.3	35.9 ± 2.2
10	7.4 ± 3.6	77.8 ± 13.0**	78.2 ± 12.4**	13.1 ± 5.8	19.1 ± 5.3**	13.2 ± 2.6	17.1 ± 2.0	35.1 ± 8.6
100	29.2 ± 7.8*	166.0 ± 13.0**	110.3 ± 15.0**	21.0 ± 6.1	38.8 ± 2.2**	31.0 ± 4.3**	25.5 ± 6.5	88.6 ± 7.8**

Results are expressed as µl of plasma exudate and are shown as means ± s.e. mean. * $P < 0.05$ when compared to treatment with Tyrode alone; ** $P < 0.05$ when compared with corresponding treatment in the absence of LTB_4 (Student's t test), $n = 5$–10.

Table 2 Effects of leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2) on the plasma exudation response to intradermal bradykinin (500 ng/0.1 ml) in the rabbit and guinea-pig

LTB_4 or PGE_2 (ng/0.1 ml)	*Rabbit*		*Guinea-pig*	
	$+LTB_4$	$+PGE_2$	$+LTB_4$	$+PGE_2$
0	77.2 ± 9.4		196.2 ± 13.3	
1	104.8 ± 53.7*	95.4 ± 5.5*	219.3 ± 22.5	212.1 ± 13.7
10	128.6 ± 24.1*	178.8 ± 13.0*	228.1 ± 11.7	245.1 ± 17.6*
100	185.8 ± 13.0*	334.2 ± 36.8*	257.3 ± 17.7*	298.5 ± 20.1*

Results are expressed as µl of plasma exudate and are shown as means ± s.e. mean. * $P < 0.05$ when compared with treatment with bradykinin alone (500 ng 0.1 ml^{-1}) (Student's *t*-test). $n = 5$ for rabbit and 10 for guinea-pig.

or PGE_2, when injected into rabbit, guinea-pig or rat skin, cause little or no change in vascular permeability (Table 1). When the compounds are administered together a significant increase in plasma exudation occurs (Table 1) and in the rabbit and guinea-pig, both LTB_4 and PGE_2 potentiate the vascular permeability response to bradykinin (Table 2). LTB_4 has no effect on blood flow in the rabbit (Figure 1) in contrast to PGE_2, a potent vasodilator in this species (Williams, 1979). These results support the general hypothesis, advanced by Wedmore & Williams, that chemotactic agents, including the complement-derived peptide C5a and the synthetic peptide f-met-leu-phe, are mediators of vascular permeability (Wedmore & Williams, 1980). The increase in plasma exudation, due to LTB_4 or other chemotactic mediators, can only be observed in the presence of a vasodilator such as PGE_2 and we suggest that this increase may result from PMN accumulation at the site of injection. Potentiation of the bradykinin response by PGE_2 and LTB_4, therefore, occurs through different mechanisms. PGE_2 potentiation of this response is a result of a vasodilator action of PGE_2 potentiating the vascular permeability response of bradykinin. The effects of LTB_4 on the bradykinin response, however, is a result of the vasodilator actions of bradykinin (Williams & Peck, 1977) potentiating the vascular permeability response to LTB_4. Finally, increased plasma exudation induced by a combination of PGE_2 and LTB_4 is caused by potentiation of the vascular permeability effects of LTB_4 by the vasodilator actions of PGE_2. The lack of vasodilator activity shown by LTB_4 may explain the failure of this compound to potentiate carrageenan-induced paw oedema in the rat (Smith, Ford-Hutchinson & Bray, 1980). In contrast, substances with known vasodilator actions such as PGI_2, PGE_1 and PGE_2 cause a considerable enhancement of this reaction (Ford-Hutchinson, Walker, Davidson & Smith, 1978).

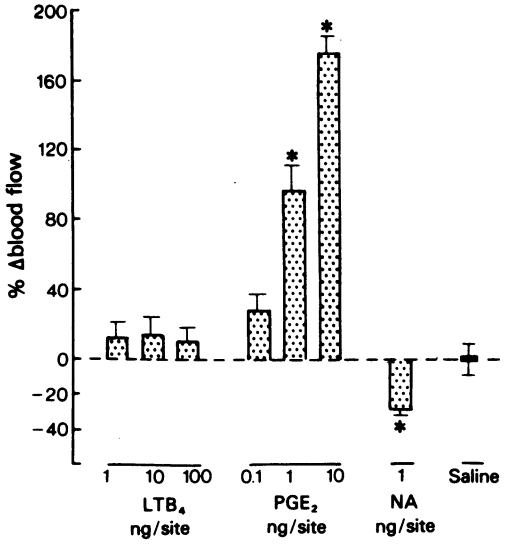

Figure 1 The effects of leukotriene B_4 (LTB_4), prostaglandin E_2 (PGE_2) and noradrenaline (NA) on normal blood flow in rabbit skin measured over a 20 min period. Columns represent means ($n = 6$); vertical lines show s.e. mean. * $P < 0.05$ compared to treatment with saline alone (Student's *t* test).

These results clearly demonstrate that LTB_4 affects vascular aspects of inflammation as well as cellular aspects. The mechanism of action of LTB_4 is different from that of the prostaglandins and the combined effects of vasodilators such as prostaglandins with LTB_4 may be an important aspect in the initiation and development of acute inflammatory reactions.

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References

BORGEAT, P. & SAMUELSSON, B. (1979a). Arachidonic acid metabolism in polymorphonuclear leucocytes: effects of ionophore A23187. *Proc. natn. Acad. Sci. U.S.A.* **76**, 2148–2152.

BORGEAT, P. & SAMUELSSON, B. (1979b). Metabolism of arachidonic acid in polymorphonuclear leucocytes. Structural analysis of novel hydroxylated compounds. *J. biol. Chem.*, **254**, 7865–7869.

DOIG, M.V. & FORD-HUTCHINSON, A.W. (1980). The production and characterisation of products of lipoxygenase enzyme systems released by rat peritoneal macrophages. *Prostaglandins*, (in press).

FORD-HUTCHINSON, A.W., BRAY, M.A., DOIG, M.V., SHIPLEY, M.E. & SMITH, M.J.H. (1980) Leukotriene B: a potent chemokinetic and aggregating substance released from polymorphonuclear leucocytes. *Nature*, (in press).

FORD-HUTCHINSON, A.W., WALKER, J.R., DAVIDSON, E.M. & SMITH, M.J.H. (1978). PGI_2: a potential mediator of inflammation. *Prostaglandins*, **16**, 253–258.

GOETZL, E.J., WELLER, P.F. & SUN, F.F. (1980). The regulation of human eosinophil function by endogenous mono-hydroxy-eicosatetraenoic acids (HETEs). *J. Immunol.*, **124**, 926–933.

MORRIS, H.R., TAYLOR, G.W., PIPER, P.J. and TIPPINS, J.R. (1979) Slow reacting substance of anaphylaxis: studies on purification and characterisation. In *Prostaglandins and Inflammation*. ed. Rainsford, K.D. & Ford-Hutchinson, A.W. pp. 27–36. Basel: Birkhäuser Verlag.

RÅDMARK, O., MALMSTEN, L., SAMUELSSON, B., CLARK, D.A., GOTO, G., MARFAT, A. & COREY, E.J. (1980). Leukotriene A: stereochemistry and enzymatic conversion to leukotriene B. *Biochem. biophys. Res. Commun.*, **92**, 954–961.

SAMUELSSON, B. & HAMMARSTRÖM, S. (1980). Nomenclature for leukotrienes. *Prostaglandins*, **5**, 645–648.

STENSON, W.F. & PARKER, C.W. (1979). Metabolism of arachidonic acid in ionophore-stimulated neutrophils. *J. clin. Invest.* **42**, 293–304.

SMITH, M.J.H., FORD-HUTCHINSON, A.W. & BRAY, M.A. (1980). Leukotriene B: a potential mediator of inflammation. *J. Pharm. Pharmac.*, (in press).

WEDMORE, C.V. & WILLIAMS, T.J. (1980). The control of vascular permeability by polymorphonuclear leucocytes in inflammation. *Nature*, (in press).

WILLIAMS, T.J. (1979). Prostaglandin E_2, prostaglandin I_2 and the vascular changes in inflammation. *Br. J. Pharmac.*, **65**, 517–524.

WILLIAMS, T.J. & MORLEY, J. (1973). Prostaglandins as potentiators of increased vascular permeability in inflammation. *Nature*, **246**, 215–217.

WILLIAMS, T.J. & PECK, M.J. (1977). Role of prostaglandin-mediated vasodilatation in inflammation. *Nature*, **270**, 530–532.

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