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Comparative effects of leukotriene B_4, prostaglandins I_2 and E_2, 6-keto-$PGF_{1\alpha}$, thromboxane B_2 and histamine on selected smooth muscle preparations

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A number of oxidative metabolites of arachidonic acid are synthesized and released during various inflammatory and hypersensitivity reactions. Among them, there are the prostaglandin endoperoxides which are unstable and rapidly transformed to the various prostaglandins and to the thromboxanes. Other compounds are formed through the action of the platelet lipoxygenase. Recently, the leukotrienes (LTs), a newly discovered group of metabolites (Borgeat & Samuelsson, 1979a, 1979b) formed in polymorphonuclear leukocytes by a lipoxygenase, have been identified as the major chemical bioactive components of Slow Reacting Substance of Anaphylaxis (SRS-A) (Murphy et al 1979; Hammarstrom et al 1979; Samuelsson et al 1980). The LTs are characterized by a conjugated triene structure and are produced through series of enzymatic reactions involving a 5-hydroperoxyeicosatetraenoic acid and an unstable intermediate named LTA_4. LTA_4 could be transformed into LTB_4 or LTC_4 which could undergo another transformation into LTD_4 or SRS-A (for a review, see Sirois & Borgeat 1980). As part of a project designed to evaluate the biological significance of the leukotrienes in hypersensitivity reactions, these experiments were done to compare the activity of selected metabolites of arachidonic acid and of histamine in various smooth muscle preparations.

Smooth muscle preparations: Rat stomachs were obtained from albino animals, prepared according to Vane (1957) and cut in 3–4 cm strips; the guinea-pig and rat ileum and the guinea-pig duodenum were prepared according to the technique published by the Department of Pharmacology of the University of Edinburgh; the rat and guinea-pig ascending and descending colon were trimmed free from mesentery and prepared according to Gagnon & Sirois (1972); the rat and guinea-pig oesophagus were removed together with the lungs and trachea, dissected free from fat and cut to 2–3 cm; the guinea-pig and rabbit aorta and trachea were cut spirally so as to produce strips of 3–4 × 30 mm; the guinea-pig pulmonary vein was cut open longitudinally; the guinea-pig and rabbit lung parenchymal strips (3 × 3 × 30 mm) were prepared from the edges of the lobes; the chick rectum was trimmed free from fat and cut in 2 cm pieces. Tyrode solution bubbled with air was used with intestinal smooth muscles and Krebs solution bubbled with 5% CO_2 in oxygen with other preparations.

Superfusion technique: After dissection, three smooth muscles were fixed to the bottom of three baths in cascade and to Grass FTO3C isometric transducers and were superfused with either Tyrode or Krebs solution at 37 ° (5 ml min^{-1}). The agonists were injected as a bolus (approx. 50 μl) in the superfusing fluid and the responses of the tissues were recorded on a Grass polygraph.

Drugs used: Histamine dihydrochloride was purchased from Sigma Chem. Co. Prostaglandins I_2 and E_2, 6-keto-$PGF_{1\alpha}$ and thromboxane B_2 were supplied as a generous gift by Dr J. E. Pike, Upjohn Co., Kalamazoo. Leukotriene B_4 was prepared from human polymorphonuclear leukocytes as described by Sirois et al (1980). In brief, the cells were incubated for 4 min in the presence of arachidonic acid (50 μg ml^{-1}) and ionophore A23187 (10 μg ml^{-1}). The lipids were extracted with ether at pH 3 and fractionated by silicic acid

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Table 1. Comparative effects of histamine and selected metabolites of arachidonic acid on smooth muscles.

Tissues		Hist.	PGE_2	PGI_2	TxB_2	6-Keto $PGF_{1\alpha}$	LTB_4	n
Rat	Oesophagus	+	0	0	0	0	0	4
	Stomach	0	++++	+++	0	+++	+	11
	Jejunum	0	++	ND	0	++++	ND	4
	Ileum	0	+	0	0	0	+	8
	Caecum	0	0	0	0	ND	ND	4
	Ascending colon	0	++++	0	0	+	0	10
	Descending colon	+++	+	+++	++	++	0	6
	Aorta	ND	+	++	+++	++	ND	7
Guinea-pig	Oesophagus	++	+	+	0	0	0	4
	Duodenum	++++	++	++	0	+	+++	9
	Jejunum	+++	++	+	0	0	ND	4
	Ileum	++++	++	++	+	+	++	9
	Aorta	++	0	0	0	0	+	3
	Pulmonary vein	+	0	0	0	0	0	3
	Trachea	+	0	0	0	0	+	9
	Parenchyma	++	—	+	+	+	++	6
Rabbit	Aorta	++	0	0	0	0	0	6
	Trachea	0	0	0	0	0	0	2
	Parenchyma	+	0	0	0	0	0	2
Chick	Rectum	+++	++	0	0	0	0	6

++++ Very strong contraction
+++ Strong contraction
++ Contraction
+ Slight contraction
0 No contraction
— Relaxation
ND Not determined
n Number of tissues

chromatography. The fraction containing LTB_4 was purified twice by h.p.l.c. and the purity was verified by gas chromatography and mass spectrometry. LTB_4 concentration was established by ultraviolet spectrophotometry ($\epsilon \simeq 51000$ at 270 nm) and the purified substance kept in methanol–water (1 : 1 v/v).

The metabolites of arachidonic acid were generally used at the following doses: PGE_2 (20 ng), PGI_2 (100 ng), thromboxane B_2 (100 ng), 6-keto-$PGF_{1\alpha}$ (1 μg) and LTB_4 (1 μg); in particular cases such as with the guinea-pig lung parenchymal strips, the doses were increased. Histamine was used at doses from 25 ng to 10 μg. Histamine is active on most smooth muscles (Table 1) and was used to ascertain the quality of each muscular preparation; tissues which did not respond to histamine or PGE_2 were discarded.

Results

Unlike histamine, prostaglandins appear more active on rat than on guinea-pig tissues in which contractile activity is restricted to intestinal smooth muscle. At the doses used, arachidonic acid metabolites were inactive on the rabbit aorta, trachea and parenchymal strip. 6-Keto-$PGF_{1\alpha}$, the metabolite of PGI_2, appeared to be nearly as active a myotopic substance as its parent molecule. Thromboxane B_2, the metabolite of thromboxane A_2 slightly contracted the guinea-pig ileum and parenchymal strip and strongly contracted the rat descending colon and aorta.

The newly discovered metabolite of the lipoxygenase arachidonic acid pathway, LTB_4 is also a relatively potent myotropic agent. It induced contractions of most guinea-pig smooth muscles studied (Table 1) whereas it was only active on a few rat tissues and inactive on rabbit tissues. The guinea-pig duodenum appeared as the most sensitive preparation to LTB_4. However, the stability of the lung parenchymal strip preparation allowed the sensitivity of the physiograph to be increased to record contractions at a dose of LTB_4 as low as 2 ng.

Discussion

We have confirmed that selected oxidative metabolic products of both cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism have significant myotropic effects on various smooth muscle preparations (Bergstrom et al 1968; Moncada et al 1977; Sirois et al 1980). Furthermore, as shown in Table 1, histamine and prostaglandins E_2 and I_2 appeared to have most activity on guinea-pig intestinal tissue whereas in the rat the various metabolites of arachidonic acid were not active on every intestinal tissue. The results also show thromboxane B_2 the stable metabolite of thromboxane A_2, to be a relatively potent myotropic substance on the rat aorta. Although the role of thromboxane A_2 has been extensively studied in platelet aggregation, the significance of the contraction produced by thromboxane A_2 or B_2 on the aorta remains unknown.

Prostaglandin I_2 and its metabolite 6-keto-$PGF_{1\alpha}$ contracted the guinea-pig parenchymal strip, confirming

the results of Schneider & Drazen (1980). Since PGI_2 was described as a pulmonary vasodilator (Kadowitz et al 1978), the contribution of vascular smooth muscles to the myotropic activity of the lung parenchymal strip appears to be minor. Our results also confirm the relaxant activity of PGE_2 on the lung strip (Chand & DeRoth 1979; Schneider & Drazen 1980).

Leukotriene B_4 was very active, especially on guinea-pig smooth muscle. Although the duodenum appeared the most sensitive tissue studied, the stability of the lung parenchymal strip afforded better assessment of the biological activity of this lipoxygenase product. The doses we used were high and the response of the lung strips to histamine and LTB_4 difficult to compare, but experiments not presented in this paper have shown that LTB_4 is 50–100 times more potent than histamine on this preparation.

In conclusion, this report has analysed the comparative myotropic effects of selected mediators on rat, guinea-pig, rabbit and chick smooth muscles. However, tissue concentrations of these substances have to be determined before any conclusions can be drawn on their physiological role in hypersensitivity or inflammatory reactions.

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Vascular reactivity to vasopressin in doca-salt hypertensive rats

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Plasma concentrations of arginine vasopressin are elevated in doca-salt hypertensive rats, and their blood pressure is lowered by antagonists of this peptide (Mohring et al 1977; Crofton et al 1979). These observations have prompted the suggestion that vasopressin plays a role in maintaining peripheral resistance in this form of hypertension (Mohring 1978). However, the plasma concentrations of vasopressin in doca-salt hypertensive rats are apparently insufficient to exert a direct vasoconstrictor effect, unless a large increase in 'vascular reactivity' to this peptide has occurred. In the present study, vasoconstrictor responses to vasopressin, noradrenaline and adrenergic nerve stimulation have been compared in isolated mesenteric arteries from doca-salt hypertensive and normotensive rats, to determine whether reactivity to vasopressin is increased and if this increase is of greater magnitude than that seen for other vasoconstrictor stimuli.

Doca-salt hypertension was induced in Alderley Park Wistar rats (80–100 g, either sex), by a subcutaneous implant of desoxycorticosterone acetate (75 mg) and the substitution of 0·8% NaCl for drinking water. Six to eight weeks after this procedure the systolic blood pressure of these rats (196·2 ± 6·4 mmHg, n = 13) was significantly greater ($P < 0.001$) than the blood pressure of untreated control rats (127·7 ± 5·5 mmHg, n = 13, tail cuff method, Gerold & Tschirky 1968). The weights of doca-salt hypertensive (234·9 ± 5·0 g, n = 13) and of control rats (235·6 ± 5·0 g, n = 13) were not significantly different.

Pairs of normotensive and hypertensive animals were anaesthetized (pentobarbitone sodium, 60 mg kg^{-1} i.p.) and their mesenteric arteries cannulated and perfused at a constant flow of 6·0 ml min^{-1} with Krebs solution (37 °C) bubbled with 95% O_2 5% CO_2 (McGregor 1965). Arginine vasopressin (Sigma) and (−)-noradren-