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Cyclic AMP production during adjuvant-induced arthritis in rats

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Following the observation that prostaglandin E_1 (PGE_1) and theophylline act synergistically in alleviating adjuvant arthritis in rats (Bonta, Parnham, van Vliet & Vincent, 1977), we have investigated cyclic AMP production during perfusion of arthritic paws.

Male Lewis rats received 0.1 ml complete or incomplete Freund's adjuvant in the left hind paw. After 10, 14, 18 or 22 days a stainless steel coaxial catheter was inserted, sub-cutaneously, during urethane anaesthesia, into the thigh of the right (chronic) hind leg until the tip covered the tibiotarsal joint. Left (acute, 6 h) metatarsal joints were perfused (s.c.) from 2 opposing needles. The catheter was tied in place and the joint perfused for 2 h with 6% dextran-saline at 0.2 ml/min, the perfusate being collected over ice. Two groups of arthritic rats were treated with either saline (1 ml kg^{-1} day^{-1} s.c.) or PGE_1 (0.5 mg kg^{-1} day^{-1} s.c.) on days 16 to 22 before perfusion on day 22. Total lipids were extracted from 4 ml of each perfusate with 10 ml chloroform: methanol (2 : 1) and the dextran emulsified with 2×10 ml ethanol. Following centrifugation at 2500 rev/min for 5 min, the supernatant was evaporated to dryness and resuspended in 350 µl water for duplicate assay of cyclic AMP by the method of Gilman (1970).

In the acute phase of adjuvant arthritis, despite a significant increase in paw volume, cyclic AMP levels were unaltered (Table 1). During the chronic phase

Table 1 Cyclic AMP levels in perfusates of inflamed joints of rats with adjuvant-induced arthritis

Treatment	*Acute inflammation†*		*Chronic inflammation*		
	cAMP (pmoles/h)††	*% increase in paw volume††*	*days after adjuvant injection*	*cAMP (pmoles/h)††*	*paw volume (ml)††*
Complete adjuvant (0.1 ml)	59.4 ± 2.8 (3)	94.3 ± 10.4*(4)	10	83.1(80.0–85.9) (2)	—
			14	41.4 ± 8.9**(5)	—
			18	30.1 ± 8.8**(4)	—
			22	86.2 ± 31.0 (5)	—
Incomplete adjuvant (0.1 ml)	53.0 ± 11.8 (4)	63.4 ± 11.6 (4)	22	111.9 ± 25.9 (3)	—
Complete adjuvant + saline (1 ml/kg s.c.)§	—	—	22	152.2 ± 32.0 (5)	1.060 ± 0.069 (5)
Complete adjuvant + PGE_1 (0.5 mg/kg s.c.)§	—	—	22	212.2 ± 74.0 (5)	1.198 ± 0.015***(5)

All values are means ± s.e. mean except the cAMP value for day 10 where the range of values is given. The numbers of observations are given in brackets. † 6 h. †† corrected for 100 g body weight. § daily dose, days 16–22. Significance of differences in paw volume and of differences in cAMP values from day 22 incomplete adjuvant controls was calculated by Student's *t*-test.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

cyclic AMP levels decreased until day 18 but rose again between days 18 and 22, when paw volume was still increasing (Bonta *et al.*, this volume). PGE_1 (0.5 mg kg^{-1} day^{-1}) did not significantly alter the late increase in cyclic AMP, though paw volume was increased.

These results suggest that, in adjuvant arthritis, gross changes in cyclic AMP levels at the inflammatory site cannot be correlated with the inflammatory response. They may reflect different changes in individual cell populations, as observed with lymphocyte subpopulations *in vitro* (Bach, 1975).

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Actions of phospholipase-A on mast-cell histamine release and paw oedema in the rat

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Local administration of E-prostaglandins potentiates carrageenin-induced oedema formation in the rat paw (Moncada, Ferreira & Vane, 1973). A prostaglandin precursor, arachidonic acid, also potentiates carrageenin paw oedema and this action is abolished by administration of a non-steroid anti-inflammatory drug, indomethacin, known to inhibit prostaglandin synthesis (Lewis, Nelson & Sugrue, 1975). In the present work, we have investigated the effects on paw oedema of phospholipase-A (PL-A), an enzyme involved with the liberation of endogenous prostaglandin precursors, and have studied the actions of anti-inflammatory agents. However, since crude PL-A is known to release inflammatory mediators from rat mast cells (Thomas & Whittle, 1976) which therefore could lead to oedema formation, we have first compared the ability of PL-A obtained from various sources to liberate histamine from rat mast cells with their effects on rat paw oedema.

Mast cells were obtained by lavage of the rat peritoneal cavity with a modified buffer solution (pH7) and the histamine release following incubation (20 min at 37°C) was determined by fluorometric assay. Crude phospholipase-A from *vipera russellii* venom caused a dose-dependent release of histamine; a concentration of 20 μg/ml (0.1 unit/ml enzyme activity) gave a $75 \pm 7\%$ (mean ± s.e. mean, $n=5$) release of the total histamine content of the mast cells. However, the PL-As from other sources gave only a low histamine release; in five experiments, PL-A from *crotalus terrificus* venom (20 μg/ml; 6 units/ml) gave $9 \pm 2\%$ release, that from bee venom (20 μg/ml; 31 units/ml) gave $8 \pm 2\%$ release and the PL-A_2 from pig pancreas (20 μg/ml; 16 units/ml) $13 \pm 1\%$ release. Thus, the histamine-releasing activity was not correlated with PL-A enzyme activity, and may be due to presence of a lytic factor in the crude PL-A.

Changes in rat paw volume following subplantar injections were determined with a mercury-displacement plethysmograph (Van Arman, Begany, Miller & Pless, 1965). PL-A from *vipera russellii* (0.5–5 μg in 0.1 ml) gave a rapid marked rise in paw volume reaching a maximum after 30 min, as was found with the histamine liberator, compound 48/80 (1–10 μg in 0.1 ml). The PL-A from *crotalus* and bee venoms (5–20 μg) also caused an increase in paw volume following local injection, but were less effective. In contrast, pig pancreas PL-A_2 (5–20 μg) had no consistent effect on paw volume. However, this PL-A_2 (10 μg) significantly potentiated the increase in paw volume (by 212 ± 28 μl, $n=20$; after 1.5 h, $P<0.001$) following simultaneous subplantar administration of carrageenin (0.1 ml, 2% suspension). Pretreatment with indomethacin (15 mg/kg, s.c.; 1 h prior to carrageenin) in a dose causing 75% inhibition of the carrageenin-induced paw oedema, reduced this potentiation with PL-A_2 (to 92 ± 10 μl, $n=14$; $P<0.01$), whereas pretreatment with an equi-active anti-inflammatory dose of dexamethasone (100 μg/kg, s.c.) did not significantly alter this response (177 ± 33 μl, $n=14$).

These results show that local administration of PL-A_2, like exogenous prostaglandins, can potentiate carrageenin-induced rat paw oedema. The ability of a prostaglandin synthetase inhibitor to reduce this response may suggest that endogenous prostaglandin formation is involved in the potentiation. The failure of dexamethasone to inhibit this PL-A_2 response could indicate that the anti-inflammatory steroids exert their effects at a stage prior to, or independent of, the involvement of PL-A_2 (see Gryglewski, 1976), although the present findings do not preclude the possibility of actions on the activation or release of endogenous PL-A_2.

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