# Mutual enhancement of the effects of prostaglandin $E_1$ and theophylline on the Freund adjuvant-induced arthritis syndrome of rats

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Prostaglandin $E_1$ ($PGE_1$) counteracts the adjuvant-induced arthritis of rats (Zurier, Hoffstein & Weissmann, 1973). *In vitro*, $PGE_1$ suppresses lymphocyte function (Morley, 1974) and increases leucocytic cyclic AMP levels, the latter effect being enhanced by theophylline (Bourne, Lichtenstein & Melmon, 1972). The mutual effect of these two drugs has now been examined on the adjuvant arthritis of rats.

Male Lewis rats received 0.1 ml adjuvant (5 mg/ml *Mycobacterium butyricum* in liquid paraffin) in the left hind paw and acute inflammation was evaluated after 6 h by the increase in volume of the treated paw. Chronic inflammation (day 28) was measured by the increase in volume of the untreated paw. Malfunction in the latter condition was evaluated by a gait test and X-ray photographs of the tibiotarsal joint of the untreated paws were made on day 35. The latter two evaluations were carried out by observers who were unaware of the treatments. The rats were killed on day 35 and the adrenals and spleens removed and weighed. $PGE_1$ (0.75 mg/kg), theophylline (75 mg/kg) or both, were injected s.c. daily from the day of adjuvant administration until autopsy.

The results (Table 1) show that neither drug alone affected acute inflammation, but together they caused inhibition. Chronic inflammation was inhibited by each drug and combined treatment enhanced the effects. Theophylline failed to influence gait function or joint destruction, but $PGE_1$ maximally restored the former and partially counteracted the latter, which was further improved by theophylline. $PGE_1$ did not increase adrenals weight but markedly amplified the effect of theophylline. The effects of the drugs on spleen weight paralleled their inhibition of the chronic inflammation.

We propose that, *in vivo*, $PGE_1$ and theophylline each amplify the effect of the other by increasing cyclic AMP levels in splenic lymphocytes, resulting in enhanced suppression of adjuvant arthritis.

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**Table 1** Effects of $PGE_1$ and theophylline on several parameters of the adjuvant arthritis of the rat.

| | *Acute inflammation† (treated paw % increase)††* | *Chronic inflammation§ (untreated paw % increase)††* | *Spleen weight (mg)††* | *Adrenal weight (mg)††* | *Tibiotarsal joint destruction in untreated paw (%)* | *Malfunction of untreated paw (%)* |
|---|---|---|---|---|---|---|
| Adjuvant control | 109 ± 4 (12) | 63 ± 10 (12) | 247 ± 16 (5) | 20 ± 4 (5) | 83 (6) | 50 (6) |
| Theophylline 75 mg $kg^{-1}$ $day^{-1}$ s.c. | 110 ± 10 (12) | 42 ± 5* (11) | 189 ± 9** (7) | 31 ± 4* (7) | 67 (6) | 43 (7) |
| Prostaglandin $E_1$ 0.75 mg $kg^{-1}$ $day^{-1}$ s.c. | 117 ± 5 (12) | 40 ± 7* (12) | 188 ± 9** (6) | 21 ± 5 (6) | 17* (6) | 0**(6) |
| Theophylline plus Prostaglandin $E_1$ | 70 ± 7***(12) | 30 ± 7**(12) | 155 ± 9***(6) | 57 ± 8**(6) | 0***(5) | 15* (7) |

All values are means ± s.e.mean except the last two columns in which the values were obtained from quantal (all or none) tests. The number of observations are given in brackets. The acute and chronic inflammation values were derived from two separate experiments, other parameters were obtained from one experiment. † 6 h. †† corrected for 100 g body weight.

§ Day 28. Adjuvant controls received saline (1 ml $kg^{-1}$ $day^{-1}$) s.c. Significance versus control in the first 4 columns was calculated with the one tailed Student's *t*-test. In the last 2 columns significance versus control was derived from a $\chi^2$ test.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

**References**

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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 $2 \times 10$ 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.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.