Br. J. Pharmac. (1979), **65**, 163–165

CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE AND THE MECHANISM OF ACTION OF THREE COMMON ANTI-INFLAMMATORY DRUGS

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1 The effects of indomethacin, dexamethasone and colchicine on cyclic adenosine 3′,5′-monophosphate (cyclic AMP) concentration in leucocytes during a crystal-induced pleurisy in rats were studied.

2 Each of the drugs significantly increased leucocyte cyclic AMP content within 3 h of the injection of crystals.

3 By 6 h, leucocyte cyclic AMP levels were returning toward control levels and could not be sustained at the higher level by an additional administration of the respective anti-inflammatory drug.

Introduction

Recent experiments have established that cyclic adenosine 3′,5′-monophosphate (cyclic AMP) plays a central role in acute inflammation (Willoughby, Dunn, Yamamoto, Capasso, Deporter & Giroud, 1975; Yamamoto, Dunn, Capasso, Deporter & Willoughby, 1975a; Yamamoto, Dunn, Deporter, Capasso & Willoughby, 1975b; Deporter, Dieppe & Willoughby, 1976a). A common time course pattern for leucocyte cyclic AMP levels was demonstrated in different types of inflammation in the pleural cavities of rats and guinea-pigs. Following an initial and variable rise 1 h after intrapleural injection of an irritant, the concentration of cyclic AMP in pleural exudate leucocytes always fell precipitously below basal levels by 3 h and returned towards basal levels only as the inflammatory reactions subsided. These results suggested that an inflammatory reaction will proceed normally only when the concentration of cyclic AMP in participating leucocytes is lower than in resting leucocytes, and that artificial elevation of cyclic AMP levels in leucocytes might have an anti-inflammatory effect. The effect of the anti-inflammatory drugs, indomethacin, dexamethasone and colchicine on the content of cyclic AMP in inflammatory leucocytes was therefore investigated. Pleurisy induced in rats by calcium pyrophosphate crystals was used. This model has proved useful in the evaluation of drugs for the treatment of human arthritis (Willoughby, 1976).

Methods

Pyrophosphate crystal pleurisy was produced in male Wistar rats (200 to 250 g) as previously described (Deporter *et al.*, 1976b). At 3 h and 6 h after the injection of crystals, the cyclic AMP content of pleural exudate leucocytes was determined by protein-binding radioassay (Deporter, Dieppe, Glatt & Willoughby, 1977).

Indomethacin was administered orally in 0.9% w/v NaCl solution (saline) at a dose of 3 mg/kg body wt. 12 h and 1 h before intrapleural injection of pyrophosphate crystals. Dexamethasone was given orally at 2 mg/kg in saline 1 h before the reaction. Colchicine was injected into the tail vein at a dose of 0.2 mg/kg body wt. 1 h before the intrapleural reaction was elicited.

In one set of experiments the animals were given an additional dose of their respective drugs 3 h after the onset of the reaction, and the content of leucocyte cyclic AMP was determined at 6 h.

Results

The effects of the three drugs studied on leucocyte cyclic AMP levels at 3 and 6 h after intrapleural injection of pyrophosphate crystals are shown in Figure 1. While indomethacin produced a small but significant increase in leucocyte cyclic AMP content, dexamethasone and colchicine increased the content dramatically. By 6 h the effect of indomethacin had completely worn off but dexamethasone and colchicine still maintained a significant, albeit smaller, increase over control levels of cyclic AMP. However, an additional dose of the respective drug given 3 h after the injection of crystals had no additional effect on leucocyte cyclic AMP levels at 6 h (Figure 2).

Discussion

It is not certain how indomethacin, dexamethasone and colchicine might affect leucocyte cyclic AMP con-

0007-1188/79/020163-03 $01.00

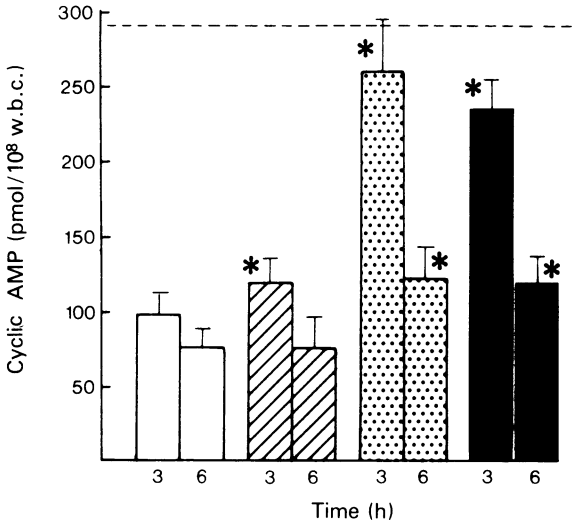

Figure 1 The effects of indomethacin (hatched columns), dexamethasone (stippled column) and colchicine (solid columns) on cyclic adenosine 3',5'-monophosphate (cyclic AMP) content of leucocytes (w.b.c.) from 3 and 6 h pyrophosphate-induced pleural exudates. * Significant difference ($P < 0.05$) from values in control exudates (open columns); vertical lines show s.e. mean. The broken line denotes the concentration of cyclic AMP in 'resting' leucocytes obtained from the pleural cavity of animals not injected with crystals or drugs (s.e. mean $\pm$ 45 pmol).

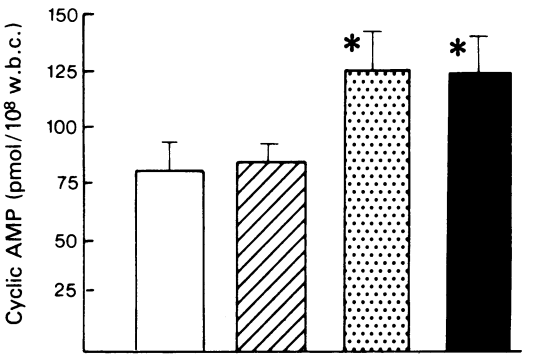

Figure 2 The effects of an additional dose of indomethacin (hatched column), dexamethasone (stippled column) or colchicine (solid column) on cyclic adenosine 3',5'-monophosphate (cyclic AMP) content of leucocytes from 6 h exudates. * Significant difference ($P < 0.05$) from values in control exudates (open column). Vertical lines show s.e. mean.

tent. Studies on chicken epiphyseal and articular cartilage (Newcombe, Thanassi & Ciosek, 1974), human synoviocytes (Ciosek, Ortel, Thanassi & Newcombe, 1974), toad bladder (Flores & Sharp, 1972), frog skin (Hall & O'Reagan, 1975) and purified beef heart phosphodiesterase (Stefanovich, 1974) have shown that indomethacin inhibits not only prostaglandin synthesis but also cyclic AMP phosphodiesterase activity and thereby raises cellular cyclic AMP content. Dexamethasone and other corticosteroids inhibit phosphodiesterase activities and increase cellular cyclic AMP in *in vitro* preparations such as rat hepatoma cells (Manganiello & Vaughan, 1972), rat testis (Schmidtke, Wienker, Flugel & Engel, 1976) and purified beef heart phosphodiesterase (Stefanovich, 1974).

The effect of colchicine on cyclic AMP phosphodiesterase activity is not known. Colchicine increases leucocyte cyclic AMP content *in vitro* and enhances the increase produced by adenylate cyclase stimulators such as isoprenaline and prostaglandin E_1 (Rudolph, Greengard & Malawista, 1977). However, colchicine may produce these effects by stimulating prostaglandin synthesis by leucocytes (Glatt, Graf & Brune, 1975). This would also help to explain why higher levels of cyclic AMP occur in animals treated with colchicine than in those treated with indomethacin where prostaglandin synthesis is inhibited.

We suggest that one or more of the anti-inflammatory effects of indomethacin, dexamethasone and colchicine is mediated through an elevation of leucocyte cyclic AMP content. This rise could then affect the inflammatory reaction by inhibiting the release of inflammatory mediators such as histamine. This hypothesis is corroborated by the finding of Thomas & Whittle (1976) that indomethacin inhibited histamine release from isolated challenged mast cells of the rat, probably through inhibition of mast cell phosphodiesterase activity. A similar inhibition of histamine release *in vitro* (Orange, Kaliner, Laraia & Austen, 1971) and *in vivo* (Deporter, Capasso & Willoughby, 1976a) can be produced by elevation of leucocyte cyclic AMP levels with dibutyryl cyclic AMP and another phosphodiesterase inhibitor, theophylline.

Long-term alteration of leucocyte cyclic AMP levels by anti-inflammatory drugs may not be feasible since at 6 h the effect of indomethacin on cyclic AMP content was negligible, and of dexamethasone and colchicine greatly reduced. A second dose of the drugs did not affect the leucocyte cyclic AMP content. The apparent refractoriness could be due to a feedback regulator for cyclic AMP phosphodiesterase, similar to the one postulated to regulate adenylate cyclase activity (Ho & Sutherland, 1975; Ho, Russell & Asakawa, 1975).

The authors wish to thank the Discretionary fund of St. Bartholomew's Hospital and the European Biological Research Association for financial support. D.A.D. was a postdoctoral fellow of the Medical Research Council of Canada at the time this study was performed. Correspondence to D.A.D., please.

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(Received January 11, 1978. Revised August 22, 1978.)