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CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE AND LEUCOCYTE CHEMOTAXIS *in vivo*

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1 The effect of local elevation of leucocyte cyclic adenosine 3′,5′-monophosphate (cyclic AMP) content on the continued migration of leucocytes to a site of acute inflammation was studied in the pleural cavity of rats.

2 Leucocyte cyclic AMP levels were elevated by injecting with the irritant into the pleural cavity dibutyryl cyclic AMP alone or with theophylline.

3 The treatments both produced a marked reduction in leucocyte migration into a pleural reaction induced by immediate hypersensitivity, but had no effect on cell numbers in pleurisy induced either by pyrophosphate or urate crystals.

Introduction

The pleural cavity of rats has been shown to be a useful site for studying the effect of elevating endogenous leucocyte cyclic adenosine 3′,5′-monophosphate (cyclic AMP) levels on the progression of acute inflammation *in vivo* (Deporter, Capasso & Willoughby, 1976; Deporter, 1977; Deporter, Dieppe, Glatt & Willoughby, 1977). The inflammatory reactions studied included an immediate hypersensitivity (Arthus) reaction and inflammation induced by calcium pyrophosphate crystals, both of which have relevance to human arthritis (Willoughby, 1976). In the present study the same two models have been used to study the effect of local elevation of leucocyte cyclic AMP concentration on the continued migration of leucocytes to a site of acute inflammation.

Methods

Male Wistar rats weighing 200–250 g were used. Pleurisy was induced by a mixture of pyrogen-free monoclinic and triclinic calcium pyrophosphate crystals as previously described (Deporter, Dieppe & Willoughby, 1976) or by immediate-hypersensitivity (reverse passive Arthus reaction) as described by Yamamoto, Dunn, Deporter, Capasso, Willoughby & Huskisson (1975). Briefly, bovine serum albumin was injected intravenously and 20–30 min later the animals were challenged intrapleurally with a purified rabbit antibody to bovine serum albumin. Pleurisy was also produced by injecting 1 ml of a solution of sodium biurate (10 mg/ml of saline) prepared according to the technique of Seegmiller, Howell & Malawista (1962).

To evaluate the effect of elevated leucocyte cyclic AMP content on the migration of more leucocytes into the pleural cavity in the three different pleural reactions, 2.45 mg dibutyryl cyclic AMP (Sigma) with and without 0.99 mg theophylline (BDH) was injected into the pleural cavity with the irritant. Both these drug treatments produce marked increases in the cyclic AMP content of leucocytes present in the pleural reactions under study (Deporter *et al.*, 1977; Deporter, 1977). Three hours after the onset of each reaction the animals were anaesthetized with ether and exanguinated via the carotid artery. Pleural exudates were withdrawn, blood-free, in siliconized Pasteur pipettes. After noting the volume of actual exudate in each animal, the pleural cavity was washed quickly with 2 ml of phosphate-buffered saline pH 7.4. Following brief agitation, the combined exudate and wash-out for each animal was sampled with a WBC pipette, diluted 1:20 with WBC diluent and counted within 30 minutes. Care was taken to count only samples free of WBC clumping within the WBC pipettes. WBC were counted 3 h after injection of the irritants, since by this time the three reactions studied were well advanced. Also, other experiments had shown that an effect on cyclic AMP levels in leucocytes by the drug treatments used could be maintained for 3 h but not longer (Deporter, unpublished results).

Results

The results are shown in Table 1. Dibutyryl cyclic AMP alone or in combination with the cyclic AMP

Table 1 Effect of local administration of dibutyryl cyclic AMP (2.45 mg) with or without theophylline (0.99 mg) on the migration of leucocytes into the pleural cavity of rats during three types of acute pleurisy

Type of pleurisy		*Total WBC ($\times 10^6$)*
Arthus-induced pleurisy	Control	60.4 ± 3.7
	Dibutyryl cyclic AMP	39.2 ± 6.0*
	Dibutyryl cyclic AMP and theophylline	36.4 ± 8.4*
Pyrophosphate pleurisy	Control	33.8 ± 5.2
	Dibutyryl cyclic AMP	34.0 ± 5.1
	Dibutyryl cyclic AMP and theophylline	36.5 ± 7.1
Urate pleurisy	Control	28.2 ± 3.7
	Dibutyryl cyclic AMP	30.8 ± 3.1
	Dibutyryl cyclic AMP and theophylline	26.3 ± 4.9

* $P < 0.05$.

phosphodiesterase inhibitor theophylline significantly decreased leucocyte counts in intrapleural Arthus reactions. In contrast, in pleurisy produced by pyrophosphate or urate crystals, (Table 1) neither drug treatment produced a significant change in the numbers of migrating leucocytes. Moreover, neither drug treatment produced significant changes in exudate volume over control values in any of the three models studied (results not shown).

Discussion

The mechanism by which a local elevation of leucocyte cyclic AMP content could reduce further leucocyte migration to a site of inflammation induced by immediate hypersensitivity is not known. However, since polymorphonuclear leucocytes (PMNs) engaged in phagocytosis of a variety of particles including antigen-antibody complexes release chemotatic factors for other PMNs (Tse & Phelps, 1970; Keller & Borel, 1971; Henson, 1972), it is conceivable that the elevated cyclic AMP levels inhibited the release of chemotactic factors into the Arthus-induced pleural effusions. The different effect of elevated cyclic AMP on cell migration in the different types of acute inflammation studied is difficult to explain. It is not due to an inhibition of particle ingestion since other experiments using the same models have shown that dibutyryl cyclic AMP and theophylline have no effect on leucocyte phagocytosis *in vivo* (Deporter *et al.*, 1977). However, the difference may be related to the fact that only the pleurisy induced by immediate hypersensitivity is complement-dependent (Yamamoto *et al.*, 1975). The two types of crystal-induced inflammation are not complement-dependent (Willoughby, Dunn, Yamamoto, Capasso, Deporter & Giroud, 1975; McCarty & Kozin, 1975). Complement generates factors that are chemotactic for PMNs (Ward, 1974) but the effect of cyclic AMP on the generation of these factors is not yet known and requires *in vitro* investigation.

The present results with urate crystals are in disagreement with those of Tse & Andrews (1973) who showed that the release of a chemotactic factor from PMNs following the intra-articular injection of urate crystals in dogs could be inhibited by injecting cyclic AMP with the crystals. The conflicting results could be due to differences in crystal preparation if, for example, Tse & Andrews did not include heat sterilization at 180°C. Non-heated urate crystals are known to activate the complement system (McCarty & Kozin, 1975). Also, since cyclic AMP levels in the dog leucocytes were not monitored, the inhibition of chemotaxis observed may not have been associated with elevated leucocyte cyclic AMP content. Cyclic AMP penetrates cell membranes less effectively than its dibutyryl derivative and is rapidly degraded by cyclic AMP phosphodiesterase (Robison, Butcher & Sutherland, 1971). Furthermore, *in vitro* butyrylated cyclic AMP derivatives and theophylline but not exogenous cyclic AMP mimic the effects of increased intracellular cyclic AMP (Drezner, Neelson & Lebovitz, 1976).

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