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# CHEMOKINESIS OF RAT POLYMORPHONUCLEAR LEUCOCYTES AND THE EFFECT OF CYCLIC ADENOSINE 3',5'-MONOPHOSPHATE

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**1** The chemotactic and chemokinetic properties of various substances were studied using rat polymorphonuclear leucocytes (PMN) in Boyden chambers.

**2** Casein and the exudate from a carrageenan-induced pleurisy possessed both chemotactic and chemokinetic properties, whereas erythrocyte lysates and albumin showed only chemokinetic activity.

**3** Dibutyryl cyclic adenosine 3',5'-monophosphate (db cyclic AMP) had little or no effect on the migration towards casein and the inflammatory exudate, but stimulated the migration towards erythrocyte lysates and albumin.

**4** It appears therefore that db cyclic AMP is able to increase a chemokinetic response initiated by other substances. The lack of effect of this compound on cell migration towards substances possessing both chemotactic and chemokinetic properties probably results from the equilibrating effect of a simultaneous stimulation of chemokinesis and inhibition of chemotaxis.

**5** These results suggest that studies designed to investigate the effect of anti-inflammatory drugs on cell migration should include the separate assessment of their ability to influence both chemotaxis and chemokinesis.

## Introduction

The migration of polymorphonuclear leucocytes (PMN) towards sites of inflammation is probably a function not only of chemotactic effects but also of chemokinetic effects, i.e. effects related to the speed of the cells rather than to their direction of movement (Keller, Wilkinson, Abercrombie Becker, Hirsch, Miller, Ramsey & Zigmond, 1977). The importance of chemokinesis to cell accumulation at inflammatory loci and to the action of anti-inflammatory drugs has yet to be determined but it is likely that changes in cell speed could greatly affect cell accumulation at a specific site even if an increase in cell speed could not totally account for the cell localisation.

We have recently studied the effects of cyclic adenosine 3,'5-monophosphate (cyclic AMP) on the migration of rat PMN observed by both a microscopic technique and the Boyden chamber filter technique (Bradshaw, Roch-Arveiller & Giroud, 1978; 1979), and shown that there were discrepancies between the two methods, which could possibly be accounted for by differences in the stimulus to which the cells were responding. Thus in the microscopic technique, in which the cells are responding to substances released from an erythrocyte after laser-lysis, cyclic AMP caused an inhibition of chemotaxis. With the filter technique, in which it is possible to use various stimulatory substances, we found that dibutyryl cyclic AMP (db cyclic AMP) had little or no effect on cell migration towards casein or exudates from pyrophosphate or carrageenan-induced pleurisies but stimulated the migration of cells towards supernatants derived from erythrocytes lysed with hypotonic NaCl solutions. These results suggested that the various stimulatory substances might be acting via different mechanisms with different susceptibilities to cyclic AMP. We have thus examined this question further and the results suggest that two different mechanisms, represented by the processes of chemotaxis and chemokinesis respectively, could well underlie the varying effects of cyclic AMP described above.

## Methods

### *Rat polymorphonuclear leucocytes*

Rats were injected intrapleurally with 1 ml isologous serum. Four hours later the fluid was withdrawn from the pleural cavity and contained cells which consisted of approximately 95% PMN (Giroud, Roch-Arveiller & Muntaner, 1979). After centrifugation, the cells were washed three times with Hanks' solution and resuspended so as to give a final concentration in the Boyden chambers of $5 \times 10^6$ cells/ml.

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*Casein*

Casein (Merck Ltd.) was dissolved at 10 mg/ml in dilute NaOH (pH 11.5). After adjustment of the pH to 7.2 with $NaH_2PO_4$ the solution was diluted to 5 mg/ml with Hanks' solution.

*Inflammatory exudates*

Four hours after the injection of 0.1 ml of 1% carrageenan into the rat pleural cavity, the exudate was collected and centrifuged to remove cells. The supernatant was used as stimulant in Boyden chambers (see below).

*Erythrocyte lysates*

Heparinised whole blood (100 µl) was added to 1 ml of hypotonic NaCl solution (0.6%). After centrifugation the supernatant was used as the stimulant in Boyden chambers.

*Assessment of cell migration*

Cell migration was assessed with modified Boyden chambers similar to those described by Keller, Gerber, Hess & Cottier (1975). The filters used were made of a mixture of cellulose esters and had a pore diameter of 3 µm (Millipore).

After incubation of the chambers in air at 37°C for 90 min, ethanol was added for fixation and the filters were then stained with haematoxylin and mounted on glass slides. Cell migration was assessed under the light microscope by the leading front technique (Zigmond & Hirsch, 1973), in which the distance into the filter travelled by the leading front of cells (two or more cells per high-power field) is measured with the micrometer fine adjustment on the microscope.

*Assessment of chemotactic and chemokinetic properties of various substances*

To assess chemotactic and chemokinetic properties, cell migration in either the presence or absence of a concentration gradient of the substance was examined. To obtain a positive gradient the stimulatory substance was placed solely in the lower compartment of the chamber and filters were moistened with Hanks' solution. Cells were suspended in Hanks' solution. For experiments performed in the absence of a gradient, filters were moistened with, and cells were suspended in, the same substance as was placed in the lower compartment of the chambers. In chemokinetic studies with bovine serum albumin (Sigma Co.), various concentrations were added to the lower compartment of the chambers and filters were moistened with the same concentrations. Cells were suspended in the same concentrations of albumin either in the absence or presence of dibutyryl cyclic AMP (1 mM), incubated for 15 min at 37°C and then added to the chambers.

## Results

*Chemotactic and chemokinetic effects of various chemo-attractants*

Casein, a carrageenan exudate, erythrocyte lysates and albumin were found to be chemokinetic, in that cell migration was stimulated when the substances were placed in both compartments of the chambers i.e. in the absence of a gradient (Table 1). Casein and a carrageenan exudate also induced a chemotactic response since cell migration in the presence of a positive gradient of these substances was greater than when the substances were present in uniform concen-

**Table 1** Chemotactic and chemokinetic effects of various chemo-attractants

| | *Migration of cells into filter (µm)* | | |
|---|---|---|---|
| *Chemo-attractant* | *With gradient* | *Without gradient* | |
| Casein (5 mg/ml) | 67.0 ± 3.6 | 48.6 ± 1.9 | $P < 0.001$ |
| Carrageenan exudate | 120* | 89.9 ± 3.4 | |
| Haemolysate | 45.9 ± 3.1 | 49.2 ± 2.4 | NS |
| Albumin (5 mg/ml) | 53.7 ± 2.9 | 86.1 ± 3.4 | $P < 0.001$ |

The migration of rat PMN was assessed in modified Boyden chambers by the leading front technique. Chemo-attractants were added either solely to the lower compartments of the chambers (with gradient) or to both compartments (without gradient). The results represent the mean ± s.e. mean of 15 determinations from triplicate filters in a typical experiment.

* The cells completely traversed the filter, and a precise leading front determination was not possible.

tration (Table 1). By contrast, cell migration in the presence of a gradient of erythrocyte lysate or albumin was smaller than in the absence of a gradient.

This was probably due to the lower concentration of the substances within the filter and/or the time they took to diffuse through the filter from the lower to the upper compartment.

*Effect of cyclic AMP on chemokinesis*

The effects of db cyclic AMP (1 mM) on the migration or rat PMN in a uniform concentration of albumin (1 to 20 mg/ml) were variable, but consistent stimulation was found in low concentrations (Table 2). The range which produced clear dose-response curves varied between cell populations, although 0 to 1.0 mg/ml was normally satisfactory.

Cells pre-incubated in db cyclic AMP and tested in the presence of a uniform concentration of 1 mM db cyclic AMP, showed no more cell migration than cells incubated in Hanks' solution. Thus, db cyclic AMP had no detectable direct chemokinetic activity.

## Discussion

Our previous results indicated that the effect of cyclic AMP on stimulated PMN migration varied according to the stimulus employed to induce locomotion, and therefore suggested that the various stimulatory substances might act via different mechanisms (Bradshaw *et al.*, 1979).

Our present findings indicate that cyclic AMP enhances chemokinetic, but not chemotactic responses of PMN. In particular, db cyclic AMP increased the chemokinetic response to albumin which was chosen because it does not possess chemotactic activity (Keller, Hess & Cottier, 1977; Wilkinson & Allan, 1978; present results). Wilkinson & Allan (1978) found that cyclic AMP increased PMN migration induced by albumin, and suggested that albumin bound cyclic AMP and presented it to the cell surface and thus allowed it to act as a chemotactic stimulus. This does not agree with our present results which show that migration was increased even when albumin and cyclic AMP were present in uniform concentrations without a gradient between the chambers. We therefore interpret our results as indicating a stimulation, by cyclic AMP, of the chemokinesis produced by other substances.

Using a microscopic technique which appears for the most part to be independent of chemokinetic effects, we have observed an inhibition by db cyclic AMP of chemotaxis (Bradshaw *et al.*, 1978). It is possible therefore that cyclic AMP does not modify PMN migration induced by substances possessing both chemotactic and chemokinetic properties, because the stimulation of chemokinesis by cyclic AMP is balanced by the inhibition of chemotaxis. This may also account for the different data on the effect of cyclic AMP on PMN chemotaxis obtained by Borel (1973) and Rivkin, Rosenblatt & Becker (1975), since they employed different chemo-attractants which probably had chemokinetic and chemotactic activities in different proportions.

The high dose of cyclic AMP required, and the lack of chemokinetic effect when used alone, suggest that cyclic AMP plays only a modulatory role in cell locomotion and is probably not directly involved in chemotaxis and chemokinesis. Rivkin *et al.* (1975) arrived at a similar conclusion.

The results described in this paper indicate a clear distinction between the processes of chemotaxis and chemokinesis and it seems likely that both these processes will be involved in the accumulation of cells at inflammatory sites. The search for drugs which in-

**Table 2** Effect of dibutyryl cyclic AMP on albumin-induced chemokinesis

| *Albumin concentration (mg/ml)* | *Migration of cells into filter (μm)* | | |
|---|---|---|---|
| | *Albumin alone* | *Albumin + db cyclic AMP* | |
| 1.0 | 72.7 ± 4.9 | 89.8 ± 6.4 | $P < 0.001$ |
| 0.8 | 64.2 ± 4.7 | 82.3 ± 5.4 | $P < 0.001$ |
| 0.6 | 60.8 ± 6.5 | 70.8 ± 6.9 | $P < 0.01$ |
| 0.4 | 51.5 ± 6.2 | 55.5 ± 3.9 | NS |
| 0.2 | 29.8 ± 7.1 | 43.0 ± 3.2 | $P < 0.001$ |
| 0.1 | 21.5 ± 6.7 | 30.4 ± 3.6 | $P < 0.001$ |

Cell migration in the presence of uniform concentrations of albumin was assessed using modified Boyden chambers. Cells were incubated for 15 min at 37°C either in the presence or absence of db cyclic AMP (1 mM) before addition to the chambers.
Results represent the means ± s.e. mean of the experiments.

fluence cell accumulation as a basis for anti-inflammatory activity should therefore include a consideration of their ability to influence not only chemotaxis but also chemokinesis.

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