heptadine-like antagonists inhibit only the post-synaptic receptor, thus counteracting the effects of post-synaptic stimulation, but are incapable of inhibition of the pre-synaptic receptor, thus producing negligible changes in the 5-HT synthesis, and not counteracting the inhibitory action of 5-HT-mimetics on 5-HT synthesis rate.

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Effect of cyclic AMP and cholera toxin on the migration of rat polymorphonuclear leucocytes in boyden chambers

D. BRADSHAW*, M. ROCH-ARVEILLER, J. P. GIROUD, *Department of Pharmacology, Hôpital Cochin, 27 rue du Faubourg Saint-Jacques 75014 Paris—France*

The accumulation of polymorphonuclear leucocytes (PMN) at inflammatory loci is generally considered to be the result of the chemotactic attraction of these cells to substances (cytotaxins) produced and/or released at these sites. Since these cells play a major role in inflammation through phagocytosis and release of their lysosomal enzymes, modification of their accumulation via the pharmacological manipulation of chemotaxis is a possible method by which inflammatory processes could be controlled. Therefore PMN migration has been studied. The techniques used in vitro to do so include both the direct microscopic observation of cell migration on glass slides and the measurement of the degree of migration of cells into filters. We have found, as have Allan & Wilkinson (1978), that the parallel use of both types of technique is advantageous in that they furnish some different information. Several workers have studied the effect of cyclic (c) AMP on chemotaxis (see review by Hill 1978) and both an inhibition of migration (Rivkin et al 1975) and a lack of effect (Borel 1973) have been reported. We have recently studied the effect of cAMP and cholera toxin (which raises intracellular cAMP concentrations in rat) (Roch-Arveiller et al 1979) on the chemotaxis of rat PMN towards a laser-lysed erythrocyte as observed under the phase-contrast microscope. Both substances produced a marked inhibition of the chemotactic response (Bradshaw et al 1978). The results now presented are those of a similar study but in which a filter technique instead of a microscopic technique was used for the assessment of cell migration.

The method was a modification of the Boyden chamber technique (Boyden 1962) with chambers similar to those described by Keller et al (1975). Casein (5 mg ml^{-1}) was placed in the lower compartment as chemoattractant. Casein solution was prepared by dissolving it at 10 mg ml^{-1} in dil. NaOH (pH 11·5) and re-adjusting the pH to 7·2 with Na H_2PO_4, to this was added an equal volume of a solution of 9 ml distilled water, 1 ml Hanks solution and 0·1 ml $NaHCO_3$ (7·5% w/v). The filters (Millipore) of pore diameter 3·0 μm, were made of a mixture of cellulose esters. PMN were obtained from the pleural cavity of rats 4 h after the intrapleural injection of 1 ml isologous serum. The cells were washed three times with Hanks solution and their concentration was then adjusted to 5×10^6 cells ml^{-1} before incubation for 15 min at 37 °C in Hanks solution, cAMP, or cholera toxin. 100 μl of the cell suspension was then taken and added to the upper compartment of the chemotaxis chamber. The chambers were then incubated in air for 90 min at 37 °C. After incubation, ethanol was added to fix the cells onto the filter after which they were stained with haematoxylin. Cell migration in five high-power fields for triplicate filters was assessed under the light microscope using the leading front technique (Zigmond & Hirsch 1973).

Dibutyryl cAMP (db cAMP) at various doses was tested for an effect on cell migration by the method described above but was found to have no apparent

* Present address: Roche Products Limited, Welwyn Garden City, Hertfordshire, AL7 3AY.
† Correspondence.

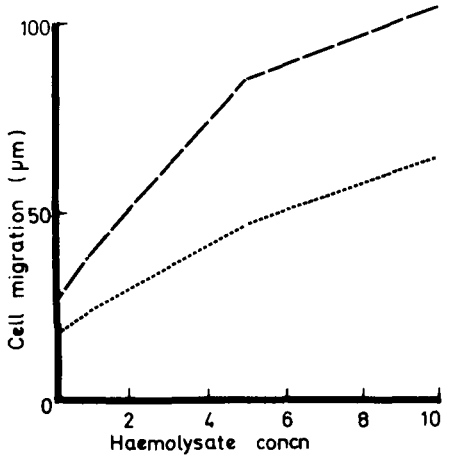

FIG. 1. Effect of cAMP on the haemolysate-stimulated migration of rat PMN (– –) Cell migration in the presence of haemolysate and dibutryl cAMP (10^{-3}M); (· · ·) cell migration in the presence of haemolysate only. Ordinate: cell migration (μm). Abscissa: haemolysate concentration (original = 1·0).

influence on the migration of rat PMN towards casein, there being no significant difference from the control value of 59·9 ± 1·7 μm mean ± s.e.m. from 5 fields for triplicate filters. To ensure that the lack of effect of db cAMP was not due to its immediate intracellular breakdown by phosphodiesterase, theophylline was added to inhibit this enzyme. The addition of theophylline (1 or 2 mg ml^{-1}) either alone or in conjunction with db cAMP caused a variable inhibition of cell migration, though the inhibition produced in the presence of exogenous cAMP was never greater than that produced by theophylline alone. This suggests that the inhibition produced was perhaps the results of some effect of theophylline other than phosphodiesterase inhibition.

Cholera toxin was tested at doses varying from 1 pg to 100 mg ml^{-1} and there appeared to be no significant effect of this substance on the cell migration (control value 63·3 ± 2·3 μm, conditions as before).

Since these results were in direct conflict with those obtained with the microscopic technique an explanation for the discrepancy was sought. One of the major differences between the two techniques is the use of a different chemoattractant, therefore in addition to casein we tested (i) the exudate from an acute pleurisy induced in rats by the intrapleural injection of 1 ml 1% calcium pyrophosphate, (ii) the exudate resulting from a similar injection of 0·1 ml 1% carrageenan, (iii) supernatants resulting from the lysis of erythrocytes with hypotonic saline (100 μl whole blood ml^{-1} 0·6% NaCl).

While db cAMP had no effect on the migration of rat PMN towards a pyrophosphate exudate, migration towards a carrageenan exudate was slightly enhanced and that towards an erythrocyte lysate was markedly

Table 1. Effect of cAMP on cell migration towards various chemoattractants.

Chemoattractant	Migration of cells into into filter (μm)* control	db cAMP (10^{-3} M)
Pyrophosphate exudate	121·7 ± 2·1	124·1 ± 2·4 N.S.
Carrageenan exudate	118·4 ± 5·3	133·4 ± 4·0 $P < 0.05$
Haemolysate	89·7 ± 2·0	128·0 ± 5·1 $P < 0.001$

* mean ± s.e.m. from 5 fields for 3 filters.

stimulated (Table 1). This stimulation was evident for various dilutions of haemolysate (Fig. 1) indicating that the lack of inhibitory effect of cAMP is not due to a supra-maximal stimulation of the cells by the chemoattractants.

It would appear therefore that the effect of cAMP on cell migration towards various substances varies according to the nature of these substances, suggesting that they may be promoting cell migration via different mechanisms. Preliminary experiments suggest that these different mechanisms may be represented by the processes of chemokinesis and chemotaxis respectively. That the effect of cAMP varies according to the stimulus could well account for the observed differences of the effect of this substance when observed with microscopic and filter techniques of migration assessment. The use of different chemoattractants by separate groups of workers may then account for the inconsistency of results between these groups with regard to the effect of cAMP on cell migration.

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