ADENOSINE TRIPHOSPHATE-INDUCED CONTRACTILE PROCESS IN RAT LYMPHOCYTES AND ITS INHIBITION BY ANTI-INFLAMMATORY AGENTS

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Addition of adenosine triphosphate to glycerol-treated rat lymphocytes produced characteristic cytoplasmic movements. These were inhibited or prevented by low concentrations of acetylsalicylic acid, phenylbutazone and indomethacin.

Monocytes and lymphocytes play a fundamental role in the intermediate and chronic phases of inflammatory reactions and in the development of immune responses. Locomotion, adherence and diapedesis, phagocytosis and cell-division are all thought to be based on mechanisms of cytoplasmic contraction and relaxation.

Glycerol-extraction, a procedure originally used by Szent-Györgyi (1949) for the study of muscle fibre contraction has been adapted for the preparation of various contractile 'cell models'. The glycerol treatment of the cell removes or destroys soluble contents including enzymes, adenosine triphosphate (ATP), and membrane systems, but many microfilaments and microtubules are well preserved in their original condition. In this cell preparation, external ATP is able to penetrate into the exposed actomyosin system and induce contraction. Using glycerol-treated lymphocytes and monocytes from human blood, Norberg (1971) showed that in basically motionless mononuclear cells, directional movements could be induced by ATP in the presence of Mg^{++} and Ca^{++}. This finding suggested that the motions of lymphocytes are contractile processes.

We have shown earlier that some non-steroidal anti-inflammatory agents inhibit the contraction of natural actomyosin in striated muscle and vascular smooth muscle (Görög & Kovács, 1970, 1972). In the present study, the effect of these agents on ATP-induced cytoplasmic movements of glycerol-treated mononuclear cells was investigated.

Methods Sprague-Dawley rats were anaesthetized with pentobarbitone. Blood was collected from the abdominal aorta into a tube containing heparin (20 u/ml final concentration). Five ml of heparinized blood was mixed with 1.5 ml 3% gelatin, and the red cells allowed to sediment. The supernatant leucocyte and platelet-rich plasma was used.

The experimental conditions were the same as those used by Norberg (1970). Leucocyte-rich plasma was coagulated in the perfusion chamber. Perfusion solutions were introduced at one of the unsealed sides of the coverslip and absorbed by a piece of filter paper at the opposite side.

Cells were treated at 4°C with increasing concentrations of glycerol. After extraction, cells were rinsed in the extraction solution without glycerol (20 min) followed by perfusion with a solution containing (mM): KCl 140, $CaCl_2$ 2, $MgCl_2$ 1, disodium-edetate 1; tris-buffer pH 7.4. Cells were observed through a Zeiss phase-contrast microscope for 15 min to ensure that perfusion *per se* did not alter the shape of the selected cell chosen for further investigation. Perfusion with the solution containing the anti-inflammatory compound was then started, and followed after 10 min by the same solution containing in addition 2.5 mM ATP. Cytoplasmic contractions of the selected cell were scanned for 30 minutes. The scanning period included the perfusion with ATP. In some experiments microphotographs were taken.

Drugs were dissolved by addition of NaOH and applied in concentrations which did not alter the pH (7.4) of the perfusion solution.

Results No spontaneous cytoplasmic movements were seen before addition of ATP. Without ATP, the investigated compounds did not cause significant alterations in the cell-shape. Sometimes the cell became more relaxed and expansion of the area of cytoplasm was observed. On the basis of numerous control experiments, four characteristic cytoplasmic movements were selected for studying the effects of anti-inflammatory drugs: (1) hernia or 'hand mirror' formation; (2) amoeboid movements; (3) general cytoplasmic contraction; (4) formation of free cytoplasmic fragments. Drug concentrations were chosen on the basis of preliminary experiments. Table 1 shows that all four

types of movement induced by ATP were reduced by anti-inflammatory drugs.

Discussion Anti-inflammatory steroids, chloroquine and phenylbutazone potently inhibited the chemotactic response of neutrophils in the Boyden chamber, while acetylsalicylic acid had no such effect (Ward, 1971). Leucocyte migration was inhibited by indomethacin (Phelps & McCarthy, 1967) and phenylbutazone (Di Rosa, Papadimitriou & Willoughby, 1971). However, the hypothesis that the main mode of action of the non-steroidal anti-inflammatory drugs is in the inhibition of the migration of mono-nuclear cells into the inflamed site, is a new concept (Di Rosa *et al.*, 1971) and is supported by our present findings.

The counting of the four cytoplasmic movements established an order of effectiveness of the investigated compounds. The effective concentrations of all three drugs were less than the peak concentrations found in plasma after therapeutic dosage, even taking account of binding to plasma protein (Flower, Gryglewski, Herbaczynska-Cedro & Vane, 1972). It may be assumed that the effective concentrations were even lower, as near maximal inhibition was obtained and the plasma clot in the chamber may have bound some of the drug.

Evidence is accumulating that the actomyosin-like contractile microfilaments in the peripheral cytoplasm of various cells play an important role in inflammatory reactions. In certain cells, biochemical characterization of microfilaments has been achieved. Definite contractile proteins have been isolated from blood platelets (Lüscher, Probst & Bettex-Galland, 1972), from equine neutrophil leucocytes (Shibata, Tatsumi, Tanaka, Okamura & Senda, 1971) and from fibroblasts of granulation tissues (Gabbiani, Hirschel, Ryan, Statkov & Majno, 1972). Indirect evidence supports the view that contractile microfilaments play a role in all forms of endocytosis (Wessels, Spooner, Ash, Bradley, Luduenda, Taylor, Wrenn & Yamada, 1971), in the release of histamine from mast cells (Orr, Hall & Allison, 1972), in the increase of capillary permeability by the contraction of endothelial cells (Majno & Palade, 1961) and in the function of contractile ring filaments during cell division (Schroeder, 1973).

Inhibition of actomyosin-ATP interactions seems to be a common property by which the diverse effects of non-steroidal anti-inflammatory drugs can be explained.

Table 1 The effect of non-steroidal anti-inflammatory agents on ATP-induced cytoplasmic activity of glycerol-treated lymphocytes.

				Cytoplasmic movements		
Compounds	*Conc. (μM)*	*No. of expts.*	*Hernia formation*	*General contraction*	*Amoeboid movement*	*Free cytoplasmic fragment*
Control	–	10	10	7	8	2
Acetylsalicylic acid	50	10	0	0	0	0
Phenylbutazone	10	10	2	0	1	0
Indomethacin	1	10	1	2	1	0

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