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THE EFFECT OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS ON ADENOSINE TRIPHOSPHATE CONTENT AND HISTAMINE RELEASE FROM RAT PERITONEAL CELL SUSPENSIONS RICH IN MAST CELLS

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1 Non-steroidal anti-inflammatory drugs (NSAID) suppressed compound 48/80-induced histamine release from rat peritoneal cells *in vitro* in a dose-dependent manner.

2 NSAID suppressed the adenosine triphosphate (ATP) content of rat peritoneal cells *in vitro* and this correlated strongly with the suppression of compound 48/80-induced histamine release.

3 The correlation demonstrated suggests that the mechanism of action of NSAID in the rat peritoneal cells is via depletion of cellular ATP.

Introduction

Mast cells, which are rich in histamine and other vasoactive factors, are involved in the immediate hypersensitivity reaction (Mongar & Schild, 1962; Austen, 1974) and probably in the early stages of acute inflammation (Keller, 1968). The release of these factors can be initiated by antigen directed against specific cell bound antibodies, some products of activated complement (Dias da Silva & Lepow, 1968; Cochrane & Muller-Eberhard, 1968) ATP (Diamant & Kruger, 1967) compound 48/80, various basic drugs (Goth, 1967) and dextrans (Goth, 1966; Baxter, 1972). These agents can release without concomitant cell disruption or loss of intracellular constituents, such as potassium ions or lactic dehydrogenase (Johnson & Moran, 1969). The binding of endogenous or exogenous releasing agents to specific surface receptors on the mast cell membrane (Bach & Brashler, 1973; Read, Ortner, Hino & Lenney, 1974) initiates a sequence of ill-defined intracellular events culminating in granule exocytosis. This exocytosis requires energy (Diamant & Uvnäs, 1961; Uvnäs, 1963) and generally calcium (Foreman & Mongar, 1972); it may be modulated by intracellular levels of cyclic 3',5'-AMP (Sullivan, Parker, Eisen & Parker, 1975). In a second phase histamine and other factors are displaced from the released granules by cations from the extracellular fluid (Thon & Uvnäs, 1967).

Non-steroidal anti-inflammatory drugs (NSAID) such as indomethacin, aspirin and phenylbutazone, suppress the release of histamine from rat mast cells by compound 48/80 and antigen (Yamasaki & Saeki, 1967; Norn, 1971). This paper describes the relationship between histamine release and ATP content in rat peritoneal cells incubated with several NSAID.

Methods

Male Fullensdorf Albino rats weighing 200–300 g were used in all experiments. Mixed peritoneal cells were collected by the method of Johnson & Moran (1966). Mast cells comprised $7.2 \pm 0.8\%$ (mean $\pm$ s.e. mean) of the mixed peritoneal cell population. They were not separated from other cells as the histamine from rat peritoneal cells is contained in the mast cells (Norn, 1968; Herzig & Kusner, 1975). Pooled cells from six rats were resuspended in the buffered salt solution to give 100,000–200,000 mast cells/ml. All experiments were performed both with and without dextrose (5.6 mM) in the media. Aliquots of the cell suspension (0.5 ml) were pre-incubated at 37°C with the drug to be tested which was added in 0.25 ml of the buffered salt solution at pH 7.0. Aliquots (50 µl) of the incubation suspension were added to 1.95 ml of TRIS (hydroxymethyl) -methyl-amine buffer (0.02 M, pH 7.75) at 100°C for 15 s, then plunged into an ice bath and later stored at −20°C until assayed for ATP. Various concentrations of compound 48/80 (Burroughs Wellcome & Co.) dissolved in 0.25 ml of the buffered salt solution were incubated with the cell suspension for 5 minutes. The suspension was then centrifuged at 300 *g* for 5 min at 4°C. Histamine in the supernatant was assayed by the method of Shore,

Burkhalter & Cohn (1959) as modified by Kremzner & Wilson (1961) omitting the butanol extraction procedure for histamine which is unnecessary in this system (Loeffler, Lovenberg & Sjoerdsma, 1971). The total histamine content was measured by disrupting the cells (0.5 ml cell suspension diluted to 0.95 ml) by boiling for 10 min and then assaying the histamine in the supernatant. Histamine release was expressed as a percentage of the histamine released on cell disruption. Oxyphenbutazone was the only drug examined that affected the histamine assay and histamine standards were assayed in the presence of appropriate concentrations of oxyphenbutazone.

ATP levels were measured by a modification of the method of Cheer, Gentile & Hegre (1974). Buffered firefly lantern extract (Sigma Chemical Company) reconstituted with double distilled water was placed in an ice bath 30–60 min before use. The samples for ATP assay were thawed and also kept at 0°C. Using uniformly chilled glass scintillation vials containing a glass reaction cuvette, 200 µl of the unknown samples were quickly added to 150 µl of firefly tail extract suspension which had been standing in the cuvette for 60 s exactly. After 30 s, the light emitted was measured for 60 s in a 'tray loaded' Phillip's Liquid Scintillation Analyser. (The accurate timing described was necessary to standardize the temperature of the luciferin-luciferinase reaction and because of the mechanics of the counter.) ATP dissolved in the TRIS buffer and boiled for 15 s was used for standards. To correct for drug effects on this assay, ATP standards were measured in the presence of the drugs at each concentration studied. Log-log plots gave linear standard curves.

The percentage reduction of ATP content (A. Sup.) of the mixed peritoneal cells induced by the various concentrations of drugs and the percentage suppression of histamine release (H. Sup.) were calculated from the equations:

$$\text{H. Sup.} = \frac{A_H - B_H}{A_H} \times 100$$

and,

$$\text{A. Sup.} = \frac{A_{ATP} - B_{ATP}}{A_{ATP}} \times 100$$

where: A_H = maximum percentage release of histamine by compound 48/80, B_H = maximum percentage release of histamine by the same concentration of compound 48/80 from cells preincubated with a NSAID, A_{ATP} = concentration of ATP in ng per ml of the peritoneal cell suspension incubated at 37°C and B_{ATP} = concentration of ATP in ng per ml of the peritoneal cell suspension preincubated with a NSAID at 37°C.

The NSAID studied were all obtained as pure substances. Indomethacin was donated by Merck, Sharpe & Dohme (Australia), Ro 20–5720 by Roche Products (Australia), ibuprofen and fluorbiprofen by the Boots Company (Australia), and naproxen by Syntex Corporation.

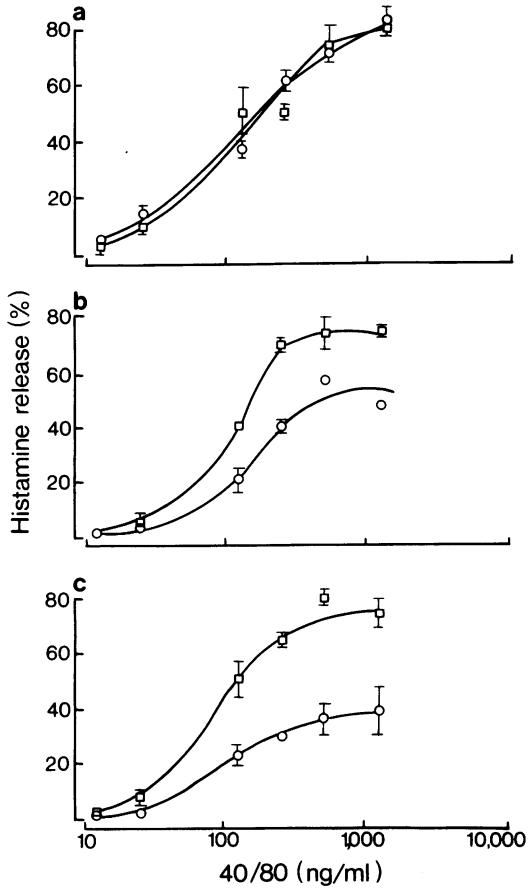

Figure 1 The effect of increasing concentrations of indomethacin on dose-response curves of histamine release induced by compound 48/80. In this representative experiment, dextrose was absent from the media. The cells were incubated with (a) indomethacin (○) 0.85 mM (b) 1.0 mM and (c) 1.4 mM or without indomethacin (□). Compound 48/80 was added after 30 min incubation. Each point is the mean of three histamine estimations. Vertical lines show s.e. means. There were significant differences ($P < 0.05$, unpaired Student's t test) between the test cells in experiments (b) and (c) and their respective control cells for all concentrations of 48/80 greater than 100 ng/ml.

Results

Histamine release and ATP content of rat peritoneal cells incubated with NSAID

NSAID suppressed the 48/80-induced release of histamine from rat peritoneal cells in a dose-dependent

fashion (Figure 1). The percentage suppression of histamine release and the corresponding percentage reduction in ATP content at each concentration of NSAID (Table 1) showed a high degree of correlation ($r=0.82$, $P<0.001$) when all experiments were considered together. This correlation remained highly significant when incubations with or without dextrose were examined separately (dextrose 5.6 mM, $r=0.74$, $P<0.001$: no dextrose, $r=0.85$, $P<0.001$).

The absence of dextrose from the media generally

Table 1 The mean percentage suppression of histamine release (±s.e. mean) induced by compound 48/80 (300 ng/ml) and the mean percentage reduction of ATP content (±s.e. mean) of rat peritoneal cells preincubated with various non-steroidal anti-inflammatory drugs for 30 minutes

	Dextrose (5.6 mM)		*No dextrose*	
Drug (mM)	*% Histamine suppression*	*% ATP reduction*	*% Histamine suppression*	*% ATP reduction*
Flufenamic acid				
0.05			52.0±3.7	72.1±2.1
0.07	32.0±0.1	41±11.8		
0.09			100±0.03	82±6.2
0.10	100±0.05	84±1.0		
Ibuprofen				
1.0			24±2.3	6±9.0
1.5			96±2.3	74.7±2.1
2.2	35±4.3	29.6±23.8	100±1.3	100±0.2
2.4	89±3.1	51±3.0		
Indomethacin				
0.7	18±4.2	33.1±5.6	−7.0±11.4	20.1±5.3
1.0	32±0.8	61.5±9.7		
1.4			32±4.9	38.4±8.5
RO 20–5720				
0.09			3.0±8.5	14.8±8
0.18	35.1±0.1	32±5.8	69.6±2.0	62±1.1
0.36	72.6±0.1	48.5±11.3	75.8±1.0	76±7.0
Oxyphenbutazone				
0.15	6.7±0.1	−12±12.3	1.1±0.1	−18±1.1
0.30	51.3±5.0	48.1±4.5	46.2±0.1	12±5.2
Phenylbutazone				
0.4			−0.1±0.3	53.8±3.9
0.7			98.0±1.2	74±1.9
1.3	6.8±0.05	45±4.4		
1.6	58.0±0.4	49±9.9		
Fluorbiprofen				
0.6			8.8±5.9	20.4±6.7
1.0	46.7±2.3	56.8±10.6	100±0.2	83.7±7.9
Acetylsalicylic acid				
2.8			−0.7±4.6	−18±9.2
3.3	1.9±0.5	2.4±6.5	61.3±1.0	49±2.5
5.0	19.4±0.1	8.8±2.7		
5.7	23.1±0.7	−1.0±8.2		
Salicylic acid				
7.3			36.2±0.7	40±4.7
10.8	11.7±0.5	23±5.8		
Naproxen				
2.2	2.5±0.8	0.05±0.06	20.2±0.5	12.6±0.04
3.3	19.8±3.6	17.5±6.0	41.7±0.5	23.6±4.7
4.4			25.8±0.04	24.0±4.0
5.4	50.6±3.8	18.0±4.4		

made the mast cells more sensitive to the inhibitory effects of NSAID, particularly, of phenylbutazone, flufenamic acid, ibuprofen, acetylsalicylic acid and salicylic acids (Table 1).

Discussion

This study and those of Norn (1971) and Yamasaki & Saeki (1967) indicate that NSAID suppress 48/80 and antigen-induced histamine release from rat mast cells. The rank order of potency of NSAID in this system is similar to that found using various *in vitro* and *in vivo* tests for anti-inflammatory activity (Wax, Winder, Tessman & Stephens, 1974). One discrepancy was the finding that oxyphenbutazone was more potent than phenylbutazone in this cell system, although these drugs have similar anti-inflammatory potencies *in vivo*. This phenomenon has been noted in other test systems (Takagi & Fukao, 1971) but is unusual (Ignarro, 1971; Clark, 1972).

The concentrations of some of the NSAID required to inhibit histamine release from the peritoneal cells are higher than the presumed effective concentrations *in vivo*. However, these drugs accumulate at sites of inflammation (Graf, Glatt & Brune, 1975). Also the sensitivity of tissue mast cells to NSAID may be greater *in vivo* than *in vitro*. It appears that the rat peritoneal cell system can give useful information on new anti-inflammatory compounds and on the mode of action of NSAID.

The observed correlation between the suppression of histamine release and the reduction in peritoneal cell ATP suggests that NSAID may act in this system by lowering cellular ATP levels. The liberation of histamine by antigen or compound 48/80 from rat mast cells and other tissues such as guinea-pig lung is an energy-dependent process which can be blocked by anoxia and metabolic inhibitors such as 2,4-dinitrophenol, oligomycin and cyanide (Parrat, 1942; Mongar & Shild, 1957). This blocking effect is antagonized by glucose. Using metabolic inhibitors to manipulate cellular ATP levels, Johansen & Chakravarty (1972; 1975) have shown that the amount of histamine released from rat mast cells by compound 48/80 or by the anaphylactic reactions, bears a linear relationship to the ATP content of the cells prior to challenge with either of these histamine releasers. Peterson & Diamant (1973) demonstrated that exposure of rat mast cells to compound 48/80 consumed 25–30% of their ATP content. There is no conclusive evidence yet that ATP is utilized in the histamine release from sensitized mast cells initiated by antigen (Diamant, 1975).

The NSAID, although chemically heterogeneous, have the common biochemical action of uncoupling oxidative phosphorylation (Whitehouse, 1968). This effect is possibly a consequence of drug binding to essential amino groups in the mitochondrial energy chain (Whitehouse, 1964; 1968) and its potency *in vitro* parallels the anti-inflammatory potency of NSAID *in vivo*. Additionally, the inhibitory actions of NSAID in the rat peritoneal cell system were enhanced when dextrose, a major source of cellular energy, was absent. Therefore, it seems likely that NSAID reduced the ATP content of the cells by uncoupling oxidative phosphorylation. The ATP content probably determined the amount of histamine released by compound 48/80. ATP levels may similarly influence the release by antigen.

It should be noted that the changes in ATP levels measured in this study relate to the total peritoneal cell population and possibly may not reflect the ATP levels of the mast cell subpopulation. However, it seems unlikely that the NSAID would differentiate between the mast cells and the other peritoneal cells since the NSAID uncouple oxidative phosphorylation in many *in vitro* systems and are well known to interact with a wide variety of cell and organelle membranes (Famey & Whitehouse, 1975).

The role of the uncoupling of oxidative phosphorylation (Kalben & Domenjoz, 1968) or inhibition of histamine release in the actions of NSAID *in vivo* is not yet clear. However, since all NSAID tested produced these effects the rat peritoneal cell model may be a useful method for the study of compounds with anti-inflammatory activity.

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