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PHAGOCYTOSIS STIMULATES THE RELEASE OF A SLOW REACTING SUBSTANCE IN CULTURED MACROPHAGES

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1 A slow-reacting substance (SRS) was released from non-elicited mouse peritoneal macrophages during phagocytosis of zymosan particles, whereas no detectable SRS was produced by resting cells.

2 The macrophage SRS induced a delayed and slow contraction of the guinea-pig ileum but not of the chick rectum.

3 The myotonic activity was antagonized by low concentrations of FPL 55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2 carboxylate) but was not affected by mepyramine or hyoscine, and was not associated with tachyphylaxis.

4 SRS release was increased by indomethacin and was abolished by the lipoxygenase and cyclo-oxygenase inhibitor, BW755C (3-amino-1-[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline).

5 Addition of exogenous arachidonic acid or cysteine enhanced SRS production.

Introduction

The generation of slow-reacting substance (SRS) has long been considered as characteristic for anaphylactic reactions. However, in recent years several authors have shown that SRS is also released from isolated cells by non-immunological stimuli (Conroy, Orange & Lichtenstein, 1976; Jakschik, Kulczycki, MacDonald & Parker, 1977; Bach & Brashler, 1978; Yecies, Wedner, Johnson, Jakschick & Parker, 1979).

In this paper, we show that SRS is released by mouse peritoneal macrophages as a consequence of phagocytosis. Since phagocytosis initiates macrophage activation (Schnyder & Baggiolini, 1978a, b) and plays an important role in inflammation, SRS deserves consideration as an inflammatory mediator.

Methods

Macrophage cultures and zymosan phagocytosis were performed essentially as described by Schnyder & Baggiolini (1978a, b). SRS in the cell-free culture media was assayed by the method of Engineer, Niederhauser, Piper & Sirois (1978). A preparation of SRS obtained from rat basophilic leukaemia (RBL-1) cells according to Jakschik *et al.* (1977) served as standard. Arbitrary units (u) of SRS are given, corresponding to 10 to 20 u as defined by Stechschulte, Austen & Bloch (1967). SRS stability was tested under the following conditions: 45 u of SRS/ml were incubated for 1 h at 37°C in the presence of either 0.1 M HCl, 0.1 M NaOH, 1 mg/ml chymotrypsin (from bovine pancreas, Type II, Sigma) in 0.1 M ammonium bicarbonate buffer, pH 8.3, or 10 mg/ml arylsulphatase (from limpets, Type V, Sigma) in 0.05 M sodium acetate buffer, pH 6.

Results

When non-elicited mouse peritoneal macrophages were allowed to phagocytose zymosan, a substance appeared in the culture medium which induced a delayed and slow contraction of the smooth muscle of guinea-pig ileum. In contrast, the medium of resting macrophages was always free of such activity.

The characteristics of this myotonic activity in the bioassay suggested that it could be ascribed to a slow-reacting substance (SRS): (1) It was insensitive to mepyramine and hyoscine. (2) It was completely blocked by the SRS-antagonist, FPL 55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate) (Augstein, Farmer, Lee, Sheard, & Tattersall, 1973) at 2 μM, a concentration which did not affect the contraction induced by prostaglandin E_2 (PGE_2) or $PGF_{2\alpha}$. (3) It did not contract the chick rectum which responded to prostaglandins but not to standard SRS. In parallel experiments, the threshold doses of prostaglandins for the bioassay tissues were found to be 2 to 5 ng for PGE_2 and 5 to 10 ng for $PGF_{2\alpha}$. The amounts of prostaglandins released by the activated macrophages were therefore below these levels.

As shown in Figure 1, agents which affect the ara-

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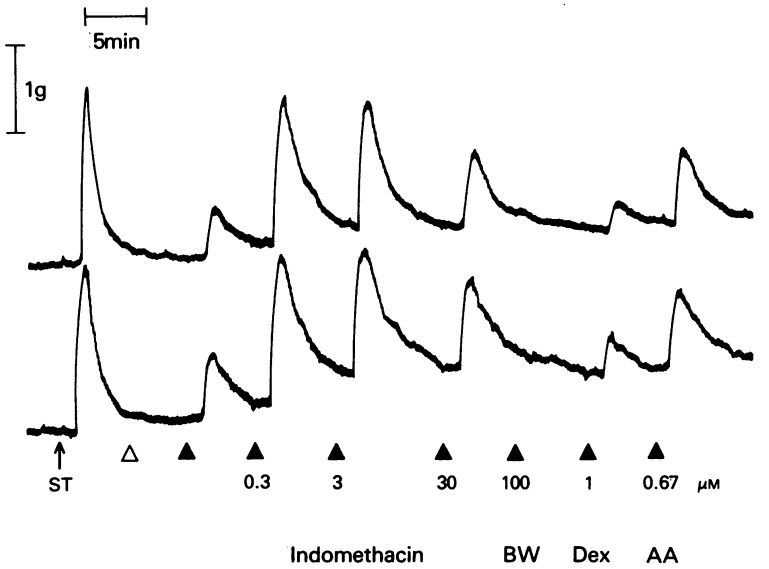

Figure 1 Bioassay of macrophage slow reacting substance (SRS) on the superfused smooth muscle strip of guinea-pig ileum. Non-elicited mouse peritoneal macrophages were allowed to adhere to plastic petri dishes for 3 h and were subsequently incubated for an additional hour in the absence (△) or presence (▲) of zymosan (8 particles per cell) in the serum-free medium described by Jakschik *et al.* (1977b). Indomethacin and BW755C (BW) were added at the indicated concentrations 15 min and arachidonic acid (AA) 2.5 min before zymosan, while dexamethasone (Dex) was present during the adherence and phagocytosis period. For bioassay, 100 μl of the cell-free medium (corresponding to 2×10^5 cells) was injected into the superfusing Tyrode solution containing mepyramine (1 μM) and hyoscine (1 μM). ST: SRS standard (0.6 u) prepared from RBL-1 cells.

chidonic acid metabolism had a pronounced effect on SRS release. Indomethacin (0.3 to 30 μM) markedly increased SRS production. At 0.3 μM this increase was 212 ± 16% of controls (mean ± s.e. mean, $n = 5$). BW755C (3-amino-1-[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline), an inhibitor of both lipoxygenase and cyclo-oxygenase (Higgs, Flower & Vane, 1979), at 100 μM completely inhibited SRS production ($n = 5$). This inhibition was reversible. In separate experiments with macrophages which had incorporated [1-^{14}C]-arachidonic acid into their phospholipids, it was shown that both indomethacin and BW755C at the concentrations used above, fully blocked PGE_2 synthesis induced by the phagocytic stimulus. Dexamethasone at 1 μM decreased SRS release to 71 ± 7% ($n = 3$) of controls, while supplementation of the culture medium with arachidonic acid (0.67 μM) or cysteine (100 μM) resulted in an increase to 130 ± 6% ($n = 4$) and 148% ($n = 2$), respectively. None of these compounds influenced phagocytosis or induced SRS release in macrophages which were not subjected to phagocytosis. Furthermore, none of them induced by itself a contraction of the assay tissues or influenced the response to standard SRS.

When SRS released from phagocytosing macrophages was compared with standard SRS from RBL-1 cells, some differences were observed. As shown in Figure 1 the macrophage product induced a slower and more protracted contraction than standard SRS. Furthermore, while standard SRS was labile at acid and stable at alkaline pH and was inactivated by treatment with arylsulphatase, the macrophage product was labile at acid and alkaline pH and resisted arylsulphatase treatment under the conditions described in the methods section. Digestion with chymotrypsin, on the other hand, neither affected the myotonic activity from macrophages nor standard SRS.

In quantitative terms, the amount of SRS released by phagocytosing macrophages was considerable. As estimated on the basis of peak height in the guinea-pig ileum bioassay, macrophages produced 0.9 ± 0.1 unit/10^6 cells ($n = 7$) during the 60 min phagocytosis period or about 1/3 of the amount released by RBL-1 cells stimulated with the ionophore, A23187.

Discussion

That phagocytes release SRS upon stimulation with the ionophore, A23187, has been shown previously in

human neutrophils (Conroy *et al.*, 1976) and rat peritoneal mononuclear cells (Bach & Brashler, 1978). In addition, Borgeat & Samuelsson (1979) demonstrated that arachidonic acid is converted to leukotrienes by neutrophils.

Working with well-defined populations of non-activated macrophages (Schnyder & Baggiolini, 1978a, b) we have now found that phagocytosis induces the release of significant amounts of an agent which was identified as SRS on the basis of the following evidence: (1) the contractile activity on the guinea-pig ileum which is antagonized by FPL 55712; (2) the enhancing effect of indomethacin and inhibiting effect of BW755C on its production and release indicating that it is a metabolite of arachidonic acid generated by the lipoxygenase pathway. The difference observed between the macrophage product and standard SRS is not incompatible with the SRS nature of the product. In fact, Bach & Brashler (1979) have reported that rat peritoneal cells produce substances with SRS character which differ in their chemical stability. Different leukotrienes have been shown to induce contractions with different time courses (Örning, Hammarström & Samuelsson, 1980). At this stage, we cannot exclude the possibility that the macrophage media contain other lipoxygenase products.

Since it has been shown that activated macrophages convert C_3 into C_{3a} (Fergula, Schorlemmer, Baptista & Allison, 1978), the possibility had to be considered that anaphylatoxins could be responsible for the myotonic activity. However, a role of these agents could be ruled out because the macrophage product did not produce tachyphylaxis, and was insensitive to chymotrypsin.

Phagocytosis is the main function of macrophages. It is also a powerful stimulus of macrophage activation (Schnyder & Baggiolini, 1978b) as shown by the greatly increased oxygen metabolism and hexose monophosphate shunt activity (Karnovsky, Lazdins & Simmons, 1975), release of products of arachidonic acid metabolism (Humes, Bonney, Dahlgren, Sadowski, Kuehl & Davis, 1977; Brune, Glatt, Kälin & Peskar, 1978), and induction of secretion of lysosomal hydrolases and neutral proteinases (Schnyder & Baggiolini, 1978b). Our data indicate that, in macrophages activated by phagocytosis, arachidonic acid is metabolised to a significant extent through the lipoxygenase pathway. Since macrophages are prominent in chronic inflammation, SRS and possibly other arachidonic acid lipoxygenase products deserve consideration as inflammatory mediators.

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