Effects of prostaglandins E₁, E₂, and F₂α, on N-acetyl-β-glucosaminidase activities of human synovial cells in culture

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SUMMARY The acid hydrolase N-acetyl-β-glucosaminidase (NAG) was used to examine the effects of prostaglandins E₁ (PGE₁), E₂ (PGE₂), and F₂α (PGF₂α) on the lysosomal system of human synovial cells in vitro. A spontaneous release of the enzyme occurred from control cultures, which was accelerated by each of the prostaglandins in a concentration-dependent manner, within the range of 10⁻⁸–10⁻⁶ moles per litre (M). No clear order of potency could be established. The effects of the prostaglandins on release of NAG were less consistent and of smaller magnitude when human serum was replaced by bovine serum albumin in the medium. In the presence of serum small increases also occurred in intracellular NAG activity, but only the effect of PGE₂ was statistically significant. The prostaglandins did not appreciably affect the previously established pattern of increased intracellular activity of NAG and reduced release produced by sucrose.

The synovial intima includes at least 3 cell types in a ground matrix of reticulin fibres, collagen, and hyaluronic acid. Type A synoviocytes have ultrastructural and functional characteristics of macrophages. Type B synoviocytes more closely resemble connective tissue fibroblasts and are probably the major source of the hyaluronic acid in the matrix and the synovial fluid. A third (type C) form has mixed properties of the A and B cells. During rheumatoid arthritis (RA) the synovial membrane hypertrophies and appears to play a direct part in the erosion of cartilage which occurs in advanced disease. However, there is still uncertainty about the relative roles of the component cell types of the synovial tissue. Certain prostaglandins accumulate in the synovial fluid during rheumatoid inflammation, but the contribution of these and related bioactive metabolites of arachidonic acid to RA is also still obscure. Primary cultures of synovial cells dispersed by trypsin from rheumatoid-affected synovial tissue secrete large quantities of PGE₂. Since prostaglandin production is a common property of macrophages, the type A synovial cells are likely to be a principal source of this PGE₂. In RA, PGE₂, or other prostanooids might exert their effects at distant sites, as in the resorption of bone, but might also act within the synovium on intimal cells. To examine this possibility, we studied the effects of PGE₁, PGE₂ and PGF₂α on the lysosomal enzyme activity of established lines of synovial cells from nonarthritic donors, using the enzyme N-acetyl-β-glucosaminidase as the marker.

Materials and methods

Synovial cell culture. Isolations were made from intact, nonarthritic joints of cadavers as described previously. Methods of cultivation and establishing fibroblast-like cell lines from primary cultures were also as in previous communications. Human serum (HS) was obtained by venesection of fasting, nonarthritic donors and heat-inactivated at 56°C for 30 min before use. Fetal bovine serum (FS) was used for the initial attachment and spreading of cells in culture vessels but not for experiments, because it contributes a high background in the assay of N-acetyl-β-glucosaminidase. HS was replaced by lipid-free bovine serum albumin (BSA) from the Sigma Chemical Company, USA, when serum-free conditions were required. Media were prepared with Eagle's basal medium (EBM) from Commonwealth Serum Laboratories, Parkville, Victoria, Australia.

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Prostaglandins. These were obtained from Upjohn Diagnostics, Michigan, USA. PGE₁ and PGE₂ were the free acids and PGF₂α the tromethamine salt. Stock solutions (10 μg per ml) of PGEs were prepared in 95% ethanol and stored at −70°C. PGF₂α was dissolved in EBM and stored as for PGE₁ and PGE₂.

Experiments. Replicate cultures were prepared as monolayers in 50 ml polystyrene flasks (approximately 3 × 10⁶ cells in 4 ml medium). After 24 h to allow complete attachment and spreading, the cultures were randomised and the prostaglandins added in fresh medium, with an intervening rinse to remove FS. Experimental media consisted of EBM + 10% HS (EBM-S) or EBM + 4 mg/ml BSA (EBM-B). Initial and final cell numbers were determined with a Coulter counter (Model B) after dispersal of the cells with 0.5% trypsin in Ca++/Mg++-free Dulbecco phosphate buffered saline.

Assays. The activity of N-acetyl-β-glucosaminidase was determined in 0.5 ml aliquots of supernatant media and in 0.5 ml of cellular extracts as described previously.9 The extracts were prepared by freezing and thawing the pellets obtained by centrifugation after the suspensions, derived from trypsinised cultures, had been counted.

Lactic acid dehydrogenase (LDH) was assayed by the Department of Biochemistry in the Royal Melbourne Hospital.

Results

intracellular NAG
Table 1 summarises the effects of PGE₁, PGE₂, and PGF₂α on the intracellular activity of NAG in synovial cultures. In EBM-S PGE₁ produced an increase in activity in 6 separate experiments, and though the mean percentage increase was only 17.8% the effect was statistically significant. PGE₂ and PGF₂α also produced slight increases in NAG activity in the majority of experiments, but these responses were not significant. None of the prostaglandins produced any appreciable effect in serum-free medium.

Extracellular NAG
To examine the effects of PGE₁, PGE₂, and PGF₂α on the amount of NAG liberated into the medium, synovial cultures were treated for 48 h with increasing concentrations of the prostaglandins in EBM-S, and NAG activity was measured in the supernatant media. The dose responses shown in Fig. 1 were from experiments in which the 3 prostaglandins were used simultaneously. Each of the prostaglandins produced a concentration-dependent release of NAG. In general little or no response could be detected at prostaglandin concentrations below 10⁻⁸ M, though there was considerable variation in the lower threshold between individual experiments. The slight inhibitory effect at the lower concentrations was not a consistent feature of the responses. No definite order of potency was apparent for the 3 prostaglandins.

The absolute values of enzyme activity and both the magnitude and consistency of the effects were greater in EBM-S than in EBM-B (Table 2). In the presence of HS, PGE₁ produced increases in NAG ranging from 10.1 to 92.1% (mean = 44.1%), and the corresponding ranges for PGE₂ and PGF₂α were 17.5 to 62.5% (mean = 29.3%) and 8.0 to 53.6% (mean = 29.5%) respectively. In EBM-B the effects due to PGE₁ varied from 11.7 to 50.7% (mean = 24.9%), compared with 1.1 to 74.5% (mean = 29.7%) for PGE₂ and 7.4 to 28.1% (mean = 18.0%) for PGF₂α.

The cumulative effect on release of NAG with time

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>10% HS</th>
<th>BSA (4 mg per ml)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment* No. of experiments</td>
<td>No with increased NAG</td>
<td>Mean NAG activity (±SD)†</td>
<td>p‡</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>6</td>
<td>103.4 ± 35.1</td>
</tr>
<tr>
<td>PGE₁</td>
<td>112.1 ± 35.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>5</td>
<td>70.8 ± 32.1</td>
</tr>
<tr>
<td>PGE₂</td>
<td>73.0 ± 30.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>5</td>
<td>72.9 ± 14.9</td>
</tr>
<tr>
<td>PGF₂α</td>
<td>75.6 ± 15.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In each experiment cellular extracts were prepared from 3-5 cultures per treatment.

*Prostaglandins were each 10⁻⁸ M.
†Enzyme activity is expressed as picogram of paranitrophenol released from 3·6 mM paranitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside per cell per hour of incubation.
‡Student’s paired t test. NS = not significant (p > 0.05).
SI conversion: mg/ml = g/l.
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Fig. 1 Effect of increasing concentrations of PGE$_1$ (○), PGE$_2$ (■), and PGF$_{2\alpha}$ (▼) on release of NAG from synovial cells. (a) and (b) are separate experiments with different cell lines. The medium was EBM with 10% HS (EBM-S) and the duration of experiments was 48 h. NAG activity was measured as paranitrophenol released per cell per h from the substrate (see footnote (†) Table 1). Points in (a) are means from 4 cultures (shown with SE of mean), and in (b) are means from duplicate cultures.

is shown by the example in Fig. 2, in which the synovial cultures were treated continuously with $10^{-6}$ M PGE$_2$. There was a spontaneous release of NAG from control cultures, which was accelerated by the prostaglandin. Increase in extracellular NAG activity could often be detected within 4 h, and a significant response was sustained throughout the 48 h of treatment (Table 3). Levels of LDH did not increase in supernatant media from either control or prostaglandin-treated cultures.

In previous work sucrose was found to be a potent activator of intracellular lysosomal activity in synovial cells, but had either no effect on release of enzyme or was inhibitory. A further experiment was carried out to determine whether the prostaglandins could cause liberation of NAG from the enlarged lysosomal reservoir induced by sucrose. Synovial cells were treated with 0.08 M sucrose alone and together with $10^{-6}$ M PGE$_1$ for 48 h (Fig. 3). The PGE$_1$ produced no change in intracellular NAG activity compared with that due to sucrose alone. The amount of NAG released by sucrose-treated cells was

**Table 2** Effect of PGE$_1$, PGE$_2$, and PGF$_{2\alpha}$ on release of N-acetyl-$\beta$-glucosaminidase from human synovial cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>10% HS</th>
<th>BSA (4 mg per ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>PGE$_1$</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>PGE$_2$</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>PGF$_{2\alpha}$</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

In each experiment supernatants from 3–5 replicate cultures were assayed. Other footnotes as for Table 1.
Table 3  Rate of release of NAG from prostaglandin-treated synovial cells

<table>
<thead>
<tr>
<th>Interval (h)</th>
<th>Rate of release of NAG (units per h in culture*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PGE1</td>
</tr>
<tr>
<td>0—</td>
<td>3.1</td>
</tr>
<tr>
<td>4—</td>
<td>2.2</td>
</tr>
<tr>
<td>24—28</td>
<td>2.1</td>
</tr>
</tbody>
</table>

*1 unit = 1 pg paranitrophenol released per cell per h. The prostaglandins were studied separately, with different cell-lines, so that rates for each prostaglandin are not directly comparable.

Fig. 3  Effect of PGE1 (10^{-4} M) and sucrose (0.08 M) on (a) intracellular and (b) extracellular activity of NAG (measured as for Fig. 1). The medium was EBM-S and the duration of the experiment was 48 h. Bars represent means from determinations on 2 or 3 replicate cultures (shown with SE of mean).

Discussion

The principal finding in the present study was that release of N-acetyl-\( \beta \)-glucosaminidase from human synovial fibroblasts was increased by treatment with PGE1, PGE2, or PGF3a. The prostaglandins produced little net effect on intracellular activities of NAG, which contrasted with the effect of adenosine, the only other agent found so far to cause liberation of NAG without gross cytotoxicity.\(^6\) The response of the synovial cells to PGF3a was of particular interest, since in several other respects, notably its effects on morphology,\(^7\) glycolysis, hyaluronic acid synthesis, and intracellular levels of cAMP,\(^8\) and adenylate cyclase activity (B. J. Clarris, E. Baxter, V. P. Michelangeli, unpublished observations) of synovial fibroblasts, PGF3a was considerably less potent than the E-type prostaglandins.

Release of NAG from synovial cells might be a selective effect on living cells or a cytotoxic response in a susceptible subpopulation. In serum-supplemented medium we have found that synovial cells treated with the same prostaglandins used in the present study multiply at rates only slightly lower than those of untreated control cultures (unpublished data). Moreover we could find no morphological evidence of cell damage or death in synovial cells treated with prostaglandins in the presence of HS. The failure of any of the prostaglandins in these circumstances to cause the release of LDH from synovial cells was further argument against a cytotoxic mechanism. On the other hand replacement of serum with BSA usually results in partial contraction of some synovial cells, occasionally into rather bizarre shapes, and, depending on the concentration of BSA, the cells become quiescent or numbers decline. The greater variation in results obtained from cultures treated with the prostaglandins in serum-free medium might therefore largely reflect the overriding effects of inadequate cell nutrition.

PGE1, PGE2, and PGF3a produced an increase in the total activity of NAG without appreciable increase in the numbers of lysosomes. This suggested de-novo synthesis of the enzyme within existing lysosomes. Spontaneous release of NAG in controls could occur by passive exocytosis due to fusion of peripheral lysosomes with invaginations of the plasma membrane during pinocytosis. The amount liberated would be increased if individual lysosomes contained increased stores of NAG. The prostaglandins might also influence plasma membrane activity and the rate of pinocytosis, as seen in macrophages.\(^9\) None of the prostaglandins caused a further stimulation of NAG activity in sucrose-treated synovial cells, suggesting perhaps a direct inhibition of prostaglandin activity by sucrose, or an inability of activated lysosomes to respond to an additional stimulus, possibly due to exhaustion of substrate or alteration of membrane receptors. The low rates of release of NAG from sucrose-treated synovial cells on the addition of prostaglandin might be evidence of stabilisation of cytoplasmic membranes, as suggested previously.\(^6\)

An involvement of prostaglandins in rheumatoid inflammation has largely been inferred from
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increased levels in synovial exudates. On this basis PGE$_2$ is likely to be a principal agent, though recent studies suggest that prostacyclin and thromboxane might be of equal or even greater significance. A comparatively low level of PGF$_{2\alpha}$ in rheumatoid synovial fluids could indicate a lesser importance in the disease processes but might also reflect higher rates of turnover within the synovium. Little is known of prostaglandin concentrations or rates of metabolism at local tissue sites, and levels in synovial fluid might not be a reliable index of the activities of these substances in inflamed tissues. Thus, though in the present experiments it was found necessary to use prostaglandin concentrations at least an order of magnitude greater than those found in synovial fluids, in order to establish the effects clearly, we propose that our observations are valid evidence of the responses of cells to prostaglandins in the parent synovial tissue. Studies on phagocytes have shown that PGE$_1$ and PGE$_2$ inhibit the release of lysosomes, suggesting that these agents are anti-inflammatory. In contrast our findings indicate that PGE$_1$, PGE$_2$, and PGF$_{2\alpha}$ might have a proinflammatory role in the synovium. In rheumatoid disease excess prostaglandins secreted by inflammatory cells, or perhaps by a subpopulation of the synovial intimal cells, might act back within the synovial tissue to stimulate release of potentially destructive enzymes. Though NAG is unlikely to have any pathological significance, it is likely to be paralleled by liberation of other lysosomal enzymes, among which at least one example is capable of degrading collagen and activating neutral proteolytic enzyme.

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