Human synoviocytes: Activation and desensitization by prostaglandins and 1-epinephrine
(cyclic AMP/hormone refractoriness/cAMP phosphodiesterase)

DAVID S. NEWCOMBE, CARL P. CIOSEK, JR., YOSHINORI ISHIKAWA, AND JOHN V. FAHEY

University of Vermont, College of Medicine, Rheumatology Unit, Department of Medicine, Burlington, Vt. 05401

Communicated by James B. Wyngaarden, April 17, 1975

ABSTRACT The human synoviocyte increases its intracellular adenosine 3':5'-cyclic monophosphate (cAMP) concentration significantly after incubation with prostaglandin E₁, prostaglandin E₂, or 1-epinephrine. The cells can be desensitized to these same hormones. Hormone-induced desensitization is receptor site specific and associated with a significant increase in intracellular 3':5'-cyclic AMP phosphodiesterase (EC 3.1.4.17; 3':5'-cyclic AMP 5'-nucleotidohydrolase) activity, whereas cAMP-induced desensitization is not hormone specific.

Cyclic nucleotides play a key role in certain hormone-mediated, differentiated cell functions (1) and in cell growth (2, 3). Some cell lines, after an initial exposure to an adenylate cyclase [EC 4.6.1.1; ATP pyrophosphate-lyase (cyclizing)] activating hormone, have been shown to be refractory to subsequent hormone stimulation (4–10). The regulatory functions of cAMP catabolism under conditions of adenylate cyclase activation have received less attention than the activation process (11). Many workers would assume that the initial increment in cAMP concentration mediated via hormone activation of adenylate cyclase is the primary message for those differentiated cell functions controlled by hormones, and the refractory phase represents a homeostatic mechanism to return the intracellular milieu to its pre-hormone status.

In this paper, we demonstrate in a normal nonestablished human cell in culture (synoviocyte) that a specific and complete 1-epinephrine or prostaglandin E₁ (PGE₁) refractory state (desensitization) is induced by hormone preincubation (prostaglandins are considered to be local or tissue hormones). We also show that PGE₁-desensitized cells have a significant increase in their cAMP phosphodiesterase (EC 3.1.4.17; 3':5'-cyclic AMP 5'-nucleotidohydrolase) activity. The specificity of the desensitization and the long duration of the refractory period in synoviocytes lead us to postulate that cyclic nucleotide catabolism and its control may represent not only a homeostatic mechanism to return intracellular cyclic nucleotide concentrations to prestimulated levels, but also may have a definite role in certain delayed, hormone-induced functions such as cell growth and proliferation, or other functions which more directly correlate with low intracellular cAMP levels.

MATERIALS AND METHODS

Cell Culture. Human synovial membrane or tendon sheath was obtained and processed as described (12). Cells were grown in minimal essential medium with Earle’s salts (Gibco) containing L-glutamine and supplemented with 20% fetal calf serum (Reheis Chemical Co.), 100 units/ml of pen-
icillin, and 100 μg/ml of streptomycin. After 18 hr of incubation at 36.5° in 5% CO₂ in humidified room air, 2.5 ml of fresh growth medium per plate was added to the tissue fragments. Within 7–14 days sufficient outgrowth had occurred, and subcultures were prepared by washing with 1:5000 EDTA (Gibco) and dissociated using 0.1% trypsin. The medium was changed every 3–4 days, and after subcultures had reached confluence the cells were split routinely 1 to 2. Cells between passage 3 and 17 were used in these studies.

Treatment of Cells. Reagents used for various treatments were made as 10X preparations in growth medium, after being solubilized. Prostaglandins were solubilized in ethanol. The final concentration of ethanol was always less than 0.5%, which alone has no significant effect on intracellular cAMP concentrations (12). Catecholamines and dl-propranolol were solubilized in 0.01 M HCl. Theophylline was solubilized directly in growth medium. All incubations were carried out in 15 × 60 mm tissue culture dishes at 36.5° in humidified air supplemented with 5% CO₂. To terminate incubations, the cell monolayers were washed quickly with 3.0 ml of ice-cold growth medium without serum, and the cell protein was precipitated with 0.5 ml of 5% trichloroacetic acid. The cells were then scraped from the plates and processed for protein and intracellular cAMP determinations as described (4).

cAMP Assay. Intracellular cAMP concentrations were determined by the Gilman binding-protein assay without the protein kinase inhibitor (13). Binding protein was prepared from calf brain as described by Weinryb (14). Data are represented as means plus or minus the standard deviation. Treatment of several samples with purified beef heart phosphodiesterase results in 90–95% reduction in the kinase binding activity of these samples.

Phosphodiesterase. For assay of phosphodiesterase activity, the cells from 1 or 2 plates were washed three times with ice-cold growth medium without serum, and once with 0.05 M Tris-HCl buffer (pH 8.0), containing 3.75 mM mercaptoethanol and 0.25 M sucrose. The cells were then scraped from the dishes and homogenized at 4° in 0.3–0.5 ml of the same buffer using a ground glass homogenizer. Phosphodiesterase activity was determined by a modification of the Thompson and Applemann method (15). One unit of phosphodiesterase activity is defined as the amount of enzyme required to hydrolyze 1 pmol of cAMP per min at 30°. Specific activity is expressed as enzyme units per mg of protein. Protein was measured in the cell homogenate after precipitation with 10 volumes of 5% trichloroacetic acid and resublimation in 1 M NaOH. Protein concentration was determined by the Lowry procedure (16).

Chemicals. The prostaglandins were supplied by Dr. John E. Pike, Upjohn Co. l-Epinephrine, 3-isobutyl-1-methylxan-
RESULTS

Epinephrine and Prostaglandin Responsiveness of Human Synoviocytes. Human synoviocytes respond to l-epinephrine, PGE₁, and PGE₂ with an increment in intracellular cAMP concentrations (Figs. 1 and 2). The magnitude of the cAMP response to PGE₁ is variable but ranges between 20 and 100 times the basal intracellular cAMP concentration. In all experiments, including some not shown, the order of potency of these compounds is PGE₁ > PGE₂ > l-epinephrine. The time-response curves (Figs. 1 and 2) demonstrate the transient nature of human synoviocyte response both to prostaglandins and l-epinephrine. With both compounds, the downslope of the response curve is biphasic, with a rapid phase returning to nearly normal intracellular cAMP concentrations in 1–2 hr followed by a much slower phase until basal or subbasal intracellular cAMP concentrations are attained within 4–6 hr. After synoviocytes are exposed to hormone, the time required for reestablishment of basal intracellular cAMP concentrations is dependent upon the magnitude of the maximal intracellular cAMP response. For example, l-epinephrine (10⁻⁸ M) results in a maximal intracellular cAMP concentration that is one-third to one-fourth the maximal intracellular cAMP concentration produced by PGE₁ (3 µg/ml), and the epinephrine-stimulated cell attains basal intracellular cAMP concentrations more rapidly than the PGE₁-stimulated cell (Figs. 1A and 2).

The presence of a β-catecholamine receptor in human synoviocytes is supported by the fact that these cells respond with an increment in intracellular cAMP to L-isoproterenol treatment as well as l-epinephrine (data not shown). The L-isoproterenol activation is unaffected by phentolamine and completely blocked by dl-propranolol.

Epinephrine- and Prostaglandin-Desensitized Human Synoviocytes. The data in Figs. 1 and 2 show the time course of desensitization to PGE₁, PGE₂, and l-epinephrine. Experiments were conducted with matched controls so that intracellular cAMP concentrations of control and hormone-rechallenged cells could be compared. With both PGE₁ and l-epinephrine, cells begin to demonstrate unresponsiveness (desensitization) within 1 hr after initial hormone exposure by demonstrating an attenuated response to rechallenge with the hormone. Complete hormonal unresponsiveness was usually demonstrable between 1 and 3 hr later (Figs. 1A and 2). A tenfold increment in PGE₁ dose at 2 hr failed to elicit a normal response in PGE₁-desensitized cells (Fig. 1A). The time course for the development of desensitization to PGE₂ is qualitatively similar to that shown for PGE₁ (Fig. 1B); however, the magnitude of the initial increase in intracellular cAMP concentration is considerably lower.

To determine whether an inhibitor, present in the medium of these desensitized cells, is responsible for their refractoriness to hormone rechallenge, the medium containing the hormone (PGE₁ or l-epinephrine) was removed from desensitized cells and incubated with untreated synoviocytes. The medium from PGE₁-desensitized cells elicits a maximal cAMP response in all instances, and in some cases a stimulation greater in magnitude than had occurred initially is observed (Fig. 1A). However, when the medium from l-epinephrine-desensitized cells was used, a less than maximal response is observed (Fig. 2). Since relatively high doses of l-epinephrine (10⁻⁵ M) were used, it seems unlikely that this attenuated response results from destruction of the hormone. A possible explanation for this attenuated response might be the presence of an antagonist present in the medium of l-epinephrine-activated cells, as proposed by Ho and Sutherland for a similar phenomenon occurring in rat adipocytes (10).

To evaluate the duration of the PGE₁-desensitized state, human synoviocytes were desensitized and then assayed for intracellular cAMP concentrations at various time intervals after the initial hormone treatment. In these experiments, the medium containing the initial hormone stimulant was not changed. These synoviocytes do not respond with an increment in intracellular cAMP when rechallenged with hor-
mone for periods up to 72 hr. The data suggest that the presence of PGE₁ in the medium after the initial stimulus maintains the refractory state. In other experiments, desensitized human synoviocytes were washed three times with culture medium free from the desensitizing hormone and then reasayed for their response to this hormone at various time intervals. These experiments define both the capacity of the cell to regenerate its responsiveness to the desensitizing hormone and the time for complete regeneration to occur. As shown in Fig. 3, the human synoviocyte can regenerate its responsiveness to PGE₁. The duration of the complete refractory period of human synoviocytes, after hormone removal, is approximately 8 hr, and the recovery phase begins between 8 and 12 hr after the hormone is removed. In this experiment, full hormone responsiveness comparable to the initial hormone activation was reached approximately 36 hr after hormone removal.

In an experiment to determine the minimal concentration of PGE₁ capable of inducing desensitization, cells were treated with PGE₁ for 6 hr at concentrations of 0.1, 0.01, and 0.001 μg/ml and were then rechallenged with PGE₁ (0.3 μg/ml) for 15 min. In this experiment cells desensitized with the highest concentration of PGE₁ were totally unresponsive to rechallenge (desensitized); cells treated with the intermediate concentration responded half-maximally (partially desensitized); and cells treated with the lowest concentrations were completely responsive.

cAMP-Induced Desensitization. The increase in intracellular cAMP concentration observed with the treatment of cells for 1 hr with either cAMP alone, with 3-isobutyl-1-methylxanthine, or with both results in the desensitization of human synoviocytes to both l-epinephrine and PGE₁. Peak intracellular cAMP concentrations at 1 hr for the l-epinephrine desensitization experiments were 396.9 ± 14.9, 70.5 ± 11.9, and 264.5 ± 128.9 pmol/mg of cell protein for cAMP (10⁻³ M), isobutyl methylxanthine (10⁻³ M), and both combined, respectively. The cells were washed four times to remove cAMP or isobutyl methylxanthine at 1 hr, and 5 hr later the intracellular cAMP concentrations had returned to nearly basal levels (40-55 pmol/mg of cell protein). Rechallenge with l-epinephrine at 6 hr showed no increment in intracellular cAMP concentrations as compared to a control that responded to l-epinephrine with a 300% increase in cAMP. Comparable data were observed when PGE₁ was used to rechallenge cells treated with cAMP, isobutyl methylxanthine, or both.

Mechanism of PGE₁ Desensitization: cAMP Phosphodiesterase or Adenylate Cyclease. Two characteristics of the synoviocyte cAMP response were examined with respect to the mechanism of desensitization: hormone receptor specificity and phosphodiesterase activity. The data in Table 1 demonstrate that the desensitization process has hormone specificity. The PGE₁-desensitized cell is not responsive to PGE₂, a hormone of the same class as PGE₁, but is activated by l-epinephrine, a hormone of a different class. Similar results were obtained when an l-epinephrine-desensitized cell was challenged with PGE₁. Since the normal synoviocyte is responsive both to PGE₁ and PGE₂, our results suggest that (i) PGE₁ and PGE₂ act at the same receptor site and (ii) the hormone-induced desensitization process is either hormone class or receptor site specific. Since desensitized synoviocytes are able to accumulate cAMP in response to heterologous hormone, desensitization does not simply result from the inability of the cells to retain elevated intracellular levels of cAMP unless hormone-specific compartmentalization exists.

The use of inhibitors also confirms the specificity of the hormone-induced desensitization response. dl-Propranolol, a β-receptor antagonist, has no effect on the intracellular cAMP concentration, yet it completely blocks the responsiveness of the PGE₁-desensitized cell to l-epinephrine (Table 1). The converse experiment, using a l-epinephrine-desensitized cell, demonstrates the lack of effect of dl-propranolol on the response to PGE₁ in these cells. Thus, experiments with the β-receptor antagonist, dl-propranolol, confirm the specificity of the hormone-induced desensitization process, since this antagonist, known to bind specifically to the β-adrenergic receptor in other systems, blocks only the

![Graph](attachment:image.png)

**Fig. 3.** Recovery of PGE₁ responsiveness. All cells were treated with PGE₁ (3.0 μg/ml) and assayed for cAMP at indicated times (Q). Several plates were rechallenged with PGE₁ (0 μg/ml), and cAMP was assayed (△). At 4 hr, the PGE₁-containing media were removed from a set of plates, the cells were washed, and medium minus PGE₁ was added. These plates were again incubated and rechallenged with PGE₁, and cAMP was assayed (Δ).

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Unchallenged</th>
<th>PGE₁</th>
<th>10⁻⁴ M EPI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE₁, 3 μg/ml</td>
<td>732 ± 144</td>
<td>126 ± 5</td>
<td>—</td>
</tr>
<tr>
<td>PGE₁, 3 μg/ml</td>
<td>286 ± 67</td>
<td>94 ± 15</td>
<td>—</td>
</tr>
<tr>
<td>PGE₁, 3 μg/ml</td>
<td>32 ± 3</td>
<td>27 ± 7</td>
<td>72 ± 27</td>
</tr>
<tr>
<td>PGE₁, 3 μg/ml</td>
<td>519 ± 54</td>
<td>34 ± 3</td>
<td>463 ± 144</td>
</tr>
<tr>
<td>EPI 10⁻⁴ M</td>
<td>226 ± 30</td>
<td>148 ± 13</td>
<td>66 ± 9</td>
</tr>
<tr>
<td>EPI 10⁻⁴ M</td>
<td>30 ± 6</td>
<td>31 ± 7</td>
<td>—</td>
</tr>
<tr>
<td>PGE₁, 3 μg/ml</td>
<td>68 ± 15</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>EPI 10⁻⁴ M</td>
<td>100 ± 26</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PRO 5 μg/ml</td>
<td>28 ± 6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PRO + EPI†</td>
<td>37 ± 5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PGE₁, 3 μg/ml</td>
<td>1148 ± 147</td>
<td>772 ± 198</td>
<td>—</td>
</tr>
<tr>
<td>PRO 5 μg/ml</td>
<td>—</td>
<td>40 ± 4</td>
<td>—</td>
</tr>
<tr>
<td>PRO + PGE₁†</td>
<td>—</td>
<td>770 ± 95</td>
<td>—</td>
</tr>
</tbody>
</table>

* Desensitized cells (EPI-D and PGE₁-D) are assayed 6 and 2 hr after initial hormone and epinephrine (EPI) treatment, respectively. Subsequent hormone treatment was 15 min for PGE₁, PGE₂, dl-propranolol (PRO), and PRO + PGE₁ 1 min for EPI, PRO, and PRO + EPI.
† The concentrations of mixed agents are identical to those where they are used alone.

---

**Table 1.** Specificity of PGE₁- and epinephrine-desensitized synoviocytes (pmol/mg of protein)

---

3126 Cell Biology: Newcombe et al.

**Table 2. Effect of theophylline on the PGE₁ response of PGE₁-desensitized synoviocytes**

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Time of exposure (min)</th>
<th>cAMP (pmol/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>PGE₁-D* (3 µg/ml)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>—</td>
<td>15</td>
<td>50 ± 15</td>
</tr>
<tr>
<td>PGE₁ 3 µg/ml</td>
<td>15</td>
<td>1859 ± 221</td>
</tr>
<tr>
<td>Theophylline 1 mM</td>
<td>20</td>
<td>49 ± 0.5</td>
</tr>
<tr>
<td>Theophylline 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGE₁†</td>
<td>15</td>
<td>2300 ± 139</td>
</tr>
</tbody>
</table>

*PGE₁-D refers to PGE₁-desensitized cells.
†The concentrations of theophylline and PGE₁ are identical to those where they are used alone. Theophylline is incubated with the cells for 5 min before PGE₁ is added.

**l-epinephrine response in the PGE₁-desensitized cells and not the PGE₁ response in the l-epinephrine-desensitized cell.** A comparison of the l-epinephrine-induced increase in cAMP in control and PGE₁-desensitized cells shows that in the latter there is a diminished response. For example, in Table 1 the control cell has an intracellular cAMP concentration of 226.1 ± 29.5 pmol/mg of protein after treatment with 10⁻¹⁴ M l-epinephrine, whereas the PGE₁-desensitized cells under the same conditions have an intracellular cAMP concentration of 148.3 ± 13.3 pmol/mg of protein. Similar data are seen for the l-epinephrine-desensitized cell and control cell response to PGE₁. Thus, desensitization to one hormone has modified the capacity of a second unrelated hormone to raise the intracellular level of cAMP.

Since phospholipids have a critical role in the hormone responsiveness of broken cell preparations (17, 18), we have examined the effect of phospholipid mixtures on the intracellular cAMP concentration and the duration of the unresponsiveness in the l-epinephrine-desensitized cell. A phospholipid mixture containing 100 µg/ml of phosphatidyl serine, phosphatidyl inositol, and phosphatidyl ethanolamine had no effect on these processes.

Since the physiological characteristics of desensitized synoviocytes suggest that increased phosphodiesterase activity is an important factor in the desensitization process, theophylline (1 mM) has been used to determine if an effective desensitization is possible in cells in which the phosphodiesterase activity is significantly inhibited. An increase in PGE₁ responsiveness is observed in the PGE₁-desensitized cells in the presence of theophylline (Table 2). However, these cells still demonstrate a reduced response to hormone compared to control cells; this, combined with other experiments already described using isobutyl methylxanthine (a more potent inhibitor of phosphodiesterase), which can induce hormone refractoriness, suggest that the increment in phosphodiesterase activity observed at 4–8 hr in PGE₁-desensitized cells (Fig. 4A) is not the complete mechanism for the desensitization. The change in PGE₁ responsiveness attributable to the presence of theophylline is approximately 10% greater than the PGE₁-desensitized cell incubated without theophylline. The slight cAMP increment to rechallenge with PGE₁ in the presence of theophylline is comparable to the magnitude of the depression of the cAMP response shown for l-epinephrine on the PGE₁-desensitized cells (Tables 1 and 2); this small decrease in responsiveness may be due to increased phosphodiesterase activity.

**FIG. 4. Phosphodiesterase activity in prostaglandin-treated synoviocytes: time course.** Confluent cells were treated with PGE₁ (3.0 µg/ml) and the intracellular cAMP concentrations were determined (O). Plates were rechallenged with PGE₁ (3.0 µg/ml), and cAMP was assayed (●). Duplicate sets of plates were also assayed for phosphodiesterase specific activity ( ○). In experiment (A), PGE₁ was present for the duration of the experiment. In experiment (B), the PGE₁ was removed after 3.75 hr, the cells were washed three times, and medium without hormone was added.

In order to evaluate the role of phosphodiesterase in the desensitization process we have examined the time course of desensitization relative to increased phosphodiesterase activity. Enzyme activity begins to rise 4–6 hr after treatment with PGE₁ (3.0 µg/ml), reaches a maximum level (100% above base) at 8–12 hr, and then begins a very slow decline (Fig. 4A). At 4 hr the cells are resistant to rechallenge with additional PGE₁ (3.0 µg/ml), but the phosphodiesterase activity is not significantly elevated. Therefore, desensitization has developed before a significant increase in phosphodiesterase activity becomes evident. A second experiment in which the hormone is removed 3.75 hr after its addition shows that the phosphodiesterase activity remains high even during redevelopment of PGE₁ responsiveness (Fig. 4B).

**DISCUSSION**

In this paper we have shown that human synoviocytes respond to PGE₁ with an increase in intracellular cAMP concentration. These cells also respond to PGE₂ and l-epinephrine, but the resultant increments in intracellular cAMP are less dramatic. The cAMP responses generated by these hormones are transient, returning to nearly basal levels in 2–4 hr, and rechallenges with the same hormone within 1 hr after the initial exposure demonstrate a significant decrease in the cell's ability to again raise its intracellular level of cAMP (desensitized cell).

The hormone-desensitized cells demonstrate receptor site specificity based on the following data. Cells desensitized to PGE₁ do not respond to PGE₂, but do demonstrate responsiveness to l-epinephrine. Similar experiments show that cells desensitized to l-epinephrine also respond to PGE₁. However, in specificity experiments, the response to hetero-
ous hormone is submaximal, suggesting some overlap in receptor site topography. Several explanations can be postulated for the modulating effects observed with heterologous hormones: (i) altered receptor-adenylate cyclase complexes, (ii) increased cAMP catabolism, (iii) a change in cAMP secretion, or (iv) combinations of the above. It seems possible to have some overlap between the prostaglandin and epinephrine receptor site, and therefore, a cell desensitized to PGE₁ might have an altered epinephrine response dependent upon the degree of shared receptor site. Assuming the β-antagonist, dl-propranolol, has absolute identity with the epinephrine receptor and has the same binding characteristics in PGE₁- and l-epinephrine-desensitized cells, the experiments showing no effect of dl-propranolol on the PGE₁ response in the l-epinephrine-desensitized cells support the absence of receptor site overlap. The reverse experiments, demonstrating a complete block of the effect of l-epinephrine in PGE₁-desensitized cells by dl-propranolol, suggest that receptor overlap, if it exists, does not involve a critical dl-propranolol binding site. However, definitive answers to these alterations in responsiveness in the hormone-desensitized cells must await kinetic analyses of the adenylate cyclase and receptor binding sites in desensitized cells.

The changes in phosphodiesterase specific activity observed in association with the desensitization process suggest that hormone regulation of cellular events may be biphasic. The desensitized synoviocyte is PGE₁ unresponsive prior to the increment in phosphodiesterase specific activity, and the enzyme specific activity is still increased at a time when the PGE₁-desensitized cell has partially recovered its responsiveness to PGE₁. This evidence, combined with the data showing both the hormone-induced desensitization process to be receptor site specific and the desensitized cell to remain relatively unresponsive in the presence of phosphodiesterase inhibition, implies that the intracellular changes in phosphodiesterase associated with desensitization play a minor role in the mechanism of desensitization in our system. Furthermore, the apparent control of this increased phosphodiesterase activity by a number of different hormones (4, 8, 19–23) suggests that phosphodiesterase has an important regulatory role in hormone action. Experiments using phosphylline suggest that the submaximal cAMP response to rechallenge with heterologous hormone may be due to increased phosphodiesterase activity. These observations provide a possible mechanistic hypothesis for the modulating effects of prostaglandins on other hormones involving increased phosphodiesterase activity as the factor responsible for altered heterologous hormone response.

Some groups have undertaken, with some difficulties, the measurement of prostaglandin concentrations in synovial fluid (24, 25). In the laboratories that have measured PGE₁ and PGE₂ concentrations in the synovial fluid from patients with untreated arthritis, the mean concentration given as PGE₂ equivalents is 18.8 ± 5.3 ng/ml (24). This concentration, as shown in this paper, is sufficient to result in at least the partial desensitization of human synoviocytes in vitro. Thus, we propose that prostaglandin desensitization may occur in vivo in states of severe inflammation and altered cell proliferation.

The hormone-induced desensitization process in human synoviocytes has the following characteristics: (i) desensitization is present for as long as the hormone is present; (ii) regeneration of hormone responsiveness begins approximately 8 hr after hormone removal and is not complete until approximately 36 hr after hormone removal; (iii) PGE₁ desensitization is dose-dependent, with complete desensitization occurring at 2.8 × 10⁻⁷ M, partial desensitization occurring at 2.8 × 10⁻⁸ M, and no desensitization at 2.8 × 10⁻⁹ M PGE₁; (iv) desensitization cannot be overcome by increasing the concentration of hormone 30-fold over the desensitizing dose; (v) associated with the PGE₁ desensitization process, there is an increment in phosphodiesterase activity; and (vi) the desensitization process is hormone receptor site specific. In contrast to these features of hormone-induced desensitization, cAMP-induced desensitization does not demonstrate hormone specificity with l-epinephrine and PGE₁.

Drs. Philip Davis and Robert Johnson provided the surgical specimens for the synovial cell cultures. The work was supported by grants from the National Institutes of Health (AM 16151) and The Arthritis Foundation.