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HYDROCORTISONE-INDUCED INHIBITOR OF PROSTAGLANDIN BIOSYNTHESIS IN RAT LEUCOCYTES

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Rat peritoneal leucocytes incubated with hydrocortisone (10 μg/ml) release a factor which inhibits prostaglandin generation. The steroid-induced inhibitor, which mediates the anti-phospholipase effect of anti-inflammatory steroids, may be a protein or a polypeptide since its formation is blocked by cycloheximide, a known inhibitor of protein synthesis.

Introduction We have recently shown that the mechanism of inhibition of prostaglandin biosynthesis by hydrocortisone in rat leucocytes depends on transcription and protein synthesis (Di Rosa & Persico, 1979). Furthermore the existence of specific steroid binding sites as well as a close correlation between receptor affinity and ability to inhibit prostaglandin generation by various steroids have been demonstrated (Flower & Blackwell, 1979).

These findings support the hypothesis that the mechanism of inhibition of prostaglandin biosynthesis by anti-inflammatory steroids is in good agreement with the classical mode of action of steroid hormones which act primarily by stimulating transcription, thus controlling the rate of synthesis of certain key proteins (Thompson & Lippman, 1974).

A steroid-induced factor which mimics the anti-phospholipase effect of anti-inflammatory steroids has been discovered in the effluent of guinea-pig lungs perfused with dexamethasone (Flower & Blackwell, 1979).

In this paper we present evidence that hydrocortisone induces in leucocytes the release of a factor which inhibits prostaglandin generation.

Methods *Generator leucocytes.* Leucocytes were collected from the peritoneal cavity of male Wistar rats (weighing 150 to 200 g) as previously described (Di Rosa & Persico, 1979). Cells from 10 to 15 animals were pooled and suspended in Krebs solution enriched with bovine serum albumin (100 μg/ml). The final suspension (10 to 20 ml), containing 5 to 10×10^6 cells per ml, was divided into two samples. Hydrocortisone sodium phosphate (10 μg/ml) was added to one sample. No drug was present in the other sample. Incubation was carried out for 90 min at 37°C in a metabolic shaker.

After the incubation, cells were removed by centrifugation and the supernatants were separately dialyzed overnight at 4°C against a large volume of Krebs solution replaced three times (supernatant/Krebs final ratio 1:300).

Prostaglandins in the supernatants were occasionally determined before and after the dialysis. In some experiments a small amount of labelled hydrocortisone ([1,2,6,7 (n) ^{3}H]-hydrocortisone, 90 mCi/mmol, C.E.A., France) was added to supernatants and the radioactivity measured before and after the dialysis.

Test leucocytes. A second pool of freshly collected leucocytes was suspended in Krebs-albumin. A series of 3 ml samples was prepared by mixing 1 ml of cell suspension with 2 ml of dialyzed supernatant from control or steroid-treated leucocytes and incubated as above for 120 min with killed bacteria (*Bordetella pertussis*) in a ratio of 1000 bacteria per cell (Higgs, McCall & Youlten, 1975).

After the incubation, cells were removed by centrifugation and prostaglandins in the supernatant were extracted and bioassayed as previously described (Di Rosa & Persico, 1979).

In some experiments cycloheximide (1 μg/ml) or arachidonic acid (1 μg/ml) were also added to the incubation medium of either generator or test leucocytes (see Results).

Results Rat peritoneal leucocytes incubated in Krebs-albumin generated small amounts of prostaglandins (mean value 3.2 ± 0.12 ng prostaglandin E_2 (PGE_2) equivalents per 10^6 cells at 90 min). Hydrocortisone (10 μg/ml) reduced this synthesis by 50 to 60%.

After dialysis, supernatants of incubated cells did not contain detectable amounts of prostaglandins. Dialysis was also able to remove hydrocortisone from the supernatant for the initial radioactivity of labelled hydrocortisone was reduced by 99% after dialysis.

The effect of dialyzed supernatants from either control or steroid-treated cells (generator leucocytes) on prostaglandin production by leucocytes phagocytosing killed bacteria (test leucocytes) are summarized in Table 1.

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Table 1 Effect of dialyzed supernatant from rat leucocytes incubated with hydrocortisone (generator leucocytes) on the prostaglandin release by rat leucocytes phagocytosing killed bacteria (test leucocytes)

Generator leucocytes[a]	*Test leucocytes*[b]	*Prostaglandin release* (ng)[c]
Control	—	11.4 ± 0.41 (12)
Hydrocortisone (10 μg/ml)	—	5.1 ± 0.32 (12)
Cycloheximide (1 μg/ml)	—	10.8 ± 1.09 (4)
Hydrocortisone (10 μg/ml) + cycloheximide (1 μg/ml)	—	12.7 ± 0.71 (4)
Hydrocortisone (10 μg/ml)	Cycloheximide (1 μg/ml)	4.9 ± 0.17 (3)
Hydrocortisone (10 μg/ml)	Arachidonic acid (1 μg/ml)	10.7 ± 0.51 (3)

[a] Cells (5–10 × 10^6 per ml) were incubated for 90 min at 37°C. At the end of the incubation, cells were removed by centrifugation and supernatants dialyzed overnight at 4°C against Krebs solution.
[b] One ml of cell suspension (5 to 10 × 10^6 cells) and 2 ml of dialyzed supernatant from corresponding generator leucocytes were incubated for 120 min at 37°C with killed bacteria.
[c] Results are expressed in terms of PGE_2 equivalents per 1 × 10^6 cells (mean ± s.e. of (*n*) values). Test leucocytes without addition of any supernatant released 11.2 ± 0.60 ng (10).

Test leucocytes released substantial amounts of prostaglandins (mean value 11.2 ± 0.60 ng PGE_2 equivalents for 10^6 cells at 120 min). Such a release was not affected by the dialyzed supernatant from generator leucocytes incubated in Krebs-albumin (mean value 11.4 ± 0.42 ng) while it was greatly reduced (5.1 ± 0.32 ng) when the dialyzed supernatant from generator leucocytes incubated with 10 μg/ml hydrocortisone was present in the medium.

When generator leucocytes were incubated with hydrocortisone in the presence of cycloheximide (1 μg/ml) the supernatant did not inhibit the prostaglandin formation by test leucocytes.

In contrast cycloheximide, added to the medium of test leucocytes, failed to modify the inhibition of prostaglandin formation exhibited by the supernatant from generator leucocytes incubated with hydrocortisone. On the other hand the inhibition exhibited by this supernatant did not occur when arachidonic acid (1 μg/ml) was present into the medium of test leucocytes.

Discussion Anti-inflammatory steroids inhibit prostaglandin biosynthesis by preventing the release and/or the activation of phospholipase A_2, the enzyme which releases fatty acids from phospholipids and therefore supplies the substrates for cyclo-oxygenase (Flower & Blackwell, 1976).

Recent findings have shown that the inhibition of phospholipase A_2 by anti-inflammatory steroids works through the same mechanism exhibited by steroid hormones on their target cells, i.e. stimulation of transcription and consequent induction of specific proteins (Danon & Assouline, 1978; Flower & Blackwell, 1979; Di Rosa & Persico, 1979).

Our results demonstrate that rat peritoneal leucocytes incubated with hydrocortisone release a non-dialyzable inhibitor which mediates the anti-phospholipase effect of anti-inflammatory steroids. Arachidonic acid is in fact able to reverse the inhibition of prostaglandin biosynthesis exhibited by the hydrocortisone-induced inhibitor in rat leucocytes. The generation by leucocytes of the steroid-induced inhibitor is blocked by cycloheximide which is unable to modify the effect exhibited by the pre-formed inhibitor.

These data strongly suggest that the steroid-induced inhibitor may be a protein or a polypeptide since its formation does not occur when protein synthesis is inhibited.

In conclusion, in rat leucocytes hydrocortisone induces the synthesis of a factor, presumably a protein or a polypeptide, which inhibits phospholipase A_2.

The discovery of a steroid-induced inhibitor of prostaglandin biosynthesis in leucocytes, because of the key role played in inflammation by both prostaglandins and leucocytes, appears of great interest in the light of a possible explanation of the mode of action of anti-inflammatory steroids.

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