Br. J. Pharmac. (1979), **66**, 161–163

MECHANISM OF INHIBITION OF PROSTAGLANDIN BIOSYNTHESIS BY HYDROCORTISONE IN RAT LEUCOCYTES

M. DI ROSA & P. PERSICO

Institute of Experimental Pharmacology, Faculty of Pharmacy, University of Naples, via L. Rodinò 22, 80138 Naples, Italy

Hydrocortisone (10 μg/ml) greatly inhibits the prostaglandin release by rat peritoneal leucocytes phagocytosing killed bacteria. The inhibition, which occurs after an initial latency of 30 min, is completely reversed by either actinomycin D (0.5 μg/ml) or cycloheximide (1 μg/ml). Since these antibiotics are known inhibitors of DNA-dependent RNA synthesis and protein synthesis respectively, it appears that the mechanism of inhibition of prostaglandin biosynthesis by hydrocortisone in rat leucocytes involves stimulation of transcription and induction of protein synthesis.

Introduction The inhibition of prostaglandin biosynthesis or release by anti-inflammatory steroids has been demonstrated in a variety of tissues and cultured cells such as rabbit mesenteric artery and guinea-pig lungs (Gryglewski, Panczenko, Korbut, Grodzinska & Ocetkiewicz, 1975), rabbit adipose tissue (Lewis & Piper, 1975) human and rat inflamed synovia (Kantrowitz, Robinson, McGuire & Levine, 1975; Floman & Zor 1976), mouse fibrosarcoma cells (Tashjian, Voelkel, McDonough & Levine, 1975), guinea-pig and mouse macrophages (Bray & Gordon, 1976; Glatt, Kälin, Wagner & Brune, 1977), human fibroblasts (Yaron, Yaron, Gurari-Rotman, Revel, Lindner & Zor, 1977) and rat leucocytes (Parente, Ammendola, Persico & Di Rosa, 1978).

It has been suggested that anti-inflammatory steroids prevent the activation of phospholipase A_2, possibly by a direct interaction with biomembranes, thus limiting the availability of arachidonic acid, i.e. the substrate for prostaglandin biosynthesis (Gryglewski, 1976). However, it has been shown in recent years that steroid hormones act primarily by stimulating transcription, thus controlling the rate of synthesis of certain key proteins (Thompson & Lippman, 1974). Moreover, recent findings have shown that corticosteroids inhibit prostaglandin output from rat renal papillae by a mechanism involving RNA and protein synthesis (Danon & Assouline, 1978).

In the light of the above considerations we decided to explore further the mechanism of the inhibitory effect exhibited by hydrocortisone on prostaglandin biosynthesis by rat leucocytes. Such a mechanism appears relevant for a better understanding of the mode of action of anti-inflammatory steroids because of the central role played by leucocytes in inflammation.

Methods The methods were those previously described by Parente *et al.*, (1978). Male Wistar rats (weighing 150 to 200 g) were killed by exposure to ether and bled. The peritoneal cavity was washed with 20 ml of heparinized Krebs solution. The cell-rich fluid was removed and centrifuged (50 *g*). The cells from 8 to 10 animals were pooled and washed twice by resuspending in Krebs solution enriched with bovine serum albumin (100 μg/ml). The final suspension contained 3 to 4×10^6 cells per ml (80% mononuclears and 20% polymorphonuclears).

Samples (2 ml) of the cell suspension were incubated for various times (30, 60, 90 or 120 min) in a metabolic shaker at 37°C with killed bacteria (*Bordetella pertussis*) in a ratio of 1000 bacteria per cell (Higgs, McCall & Youlten 1975). Hydrocortisone sodium phosphate, indomethacin, actinomycin D, and cycloheximide were added, separately or in various combinations (see Results), to the medium when incubation was started. After the incubation the cells were removed by centrifugation. The supernatant was acidified to pH 3.0 with 0.1 N HCl, extracted twice with an equal volume of ethyl acetate and evaporated. The residue was dissolved in 1 ml of Krebs solution and biossayed on rat stomach strips suspended in Krebs solution containing a mixture of antagonists to prevent the action of other mediators (Gilmore, Vane & Willie, 1968). The recovery of this procedure, evaluated with synthetic prostaglandin E_2 (PGE_2, Upjohn) was about 80%.

Results The results are summarized in Figure 1. Rat peritoneal leucocytes phagocytosing killed bacteria generated substantial amounts of prostaglandins (ranging from 3.8 ± 0.3 ng PGE_2 equivalents per 10^6 cells at 30 min to 9.9 ± 0.6 ng at 120 min).

Hydrocortisone (10 μg/ml) did not affect the prostaglandin production occurring at 30 min, while it completely abolished the prostaglandin generation at subsequent times. Indomethacin (1 μg/ml) greatly suppressed prostaglandin formation throughout the time course considered. Actinomycin D (0.5 μg/ml) did not change prostaglandin production while it was able to reverse the inhibition produced by hydrocortisone.

0007-1188/79/060161-03 $01.00

© Macmillan Journals Ltd 1979

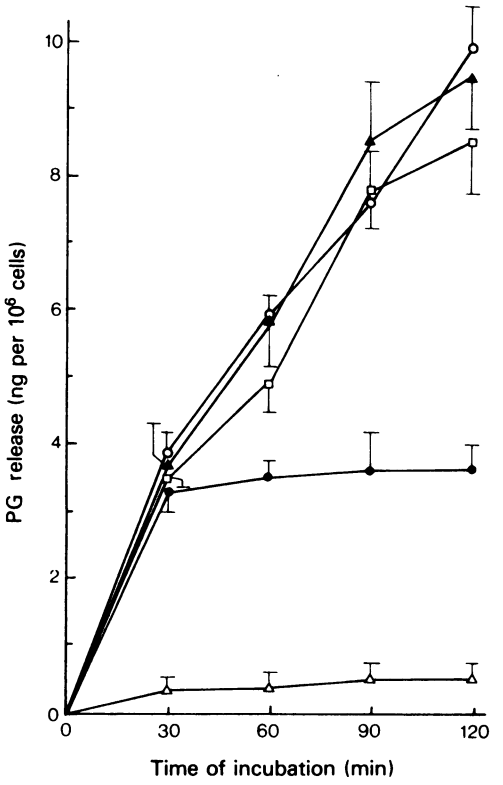

Figure 1 Reversal by actinomycin D of the inhibitory effect of hydrocortisone on prostaglandin (PG) release by rat peritoneal leucocytes phagocytosing killed bacteria. Indomethacin has been used as a reference inhibitor. Drugs were added to the cell medium (Krebs solution enriched with 100 μg/ml bovine serum albumin) when incubation was started. Results are expressed in terms of PGE_2 equivalents. Each point represents the mean value ± s.e. (vertical bars) of 4 to 8 experiments. In general, differences of at least 25% attained a level of $P < 0.05$ (Student's t test); (○) control incubation; (●), hydrocortisone 10 μg/ml; (□), actinomycin D 0.5 μg/ml; (▲) hydrocortisone 10 μg/ml plus actinomycin D 0.5 μg; (△) indomethacin 1 μg/ml.

The same effect was exhibited by 1 μg/ml cycloheximide (data not shown).

Discussion Corticosteroids are known to stabilize biomembranes and this property has been proposed as the basis of their inhibition of prostaglandin biosynthesis (Gryglewski, 1976). According to this hypothesis, anti-inflammatory steroids may induce a shortage of prostaglandin precursors by preventing the release and/or the activation of phospholipase A_2, the enzyme which releases arachidonic acid from membrane phospholipids and therefore supplies the substrate for cyclo-oxygenase (Flower & Blackwell, 1976).

Our results demonstrate that prostaglandin generation by rat peritoneal leucocytes phagocytosing killed bacteria is greatly reduced by hydrocortisone and confirm previously reported findings (Parente, *et al.*, 1978). The time course of hydrocortisone inhibition shows that the inhibition occurs after an initial latency of 30 min. A similar lag period was also shown to occur in the corticosterone-induced inhibition of prostaglandin formation by rat inflamed synovia (Floman & Zor 1976). The latency period, which is not exhibited by indomethacin, could depend on the availability of preformed stores of arachidonic acid which could initially sustain prostaglandin biosynthesis despite the presence of the steroid.

However, the latency period may well be due to the time required for protein synthesis. This view is supported by experiments showing that hydrocortisone inhibition is completely reversed by either actinomycin D or cycloheximide, known inhibitors of DNA-dependent RNA synthesis and protein synthesis respectively.

The hydrocortisone inhibition of prostaglandin output from rat renal papillae appears to involve a similar mechanism as it requires RNA and protein synthesis (Danon & Assouline, 1978). However, in this system the effect of hydrocortisone could only be demonstrated when incubation was carried out in tissue culture medium (Delbecco's modified Eagle's medium, DMEN), not in Krebs buffer, while in our experiments hydrocortisone inhibition occurred in Krebs solution enriched with albumin.

The difference may be explained by considering that both supplemented albumin and digestion products of phagocytosed bacteria may represent available substrates supporting protein synthesis by rat peritoneal leucocytes. Furthermore these cells are characterized by a large Golgi apparatus and abundant endoplasmic reticulum, both indicating an efficient synthetic capability (Spector, 1969).

In conclusion, hydrocortisone-induced inhibition of prostaglandin biosynthesis may require RNA synthesis and protein synthesis. Such a mechanism, which has been shown to occur in leucocytes for the first time, appears to be the same as the one exhibited by steroid hormones on their target cells, i.e. stimulation of transcription and consequent induction of specific proteins (Thompson & Lippman 1974). Experiments are in progress to clarify further the mechanism by which anti-inflammatory steroids inhibit prostaglandin synthesis in leucocytes, i.e. cells directly involved in inflammation.

References

BRAY M.A. & GORDON D. (1976). Effects of anti-inflammatory drugs on macrophage prostaglandin biosynthesis. *Br. J. Pharmac.*, **57**, 466–467P.

DANON A. & ASSOULINE G. (1978). Inhibition of prostaglandin biosynthesis by corticosteroids requires RNA and protein synthesis. *Nature*, **273**, 552–554.

FLOMAN Y. & ZOR U. (1976). Mechanism of steroid action in inflammation: inhibition of prostaglandin synthesis and release. *Prostaglandins*, **12**, 403–413.

FLOWER R.J. & BLACKWELL G.J. (1976). The importance of phospholipase A_2 in prostaglandin biosynthesis. *Biochem. Pharmac.*, **25**, 285–291.

GILMORE N., VANE J.R. & WYLLIE J.H. (1968). Prostaglandin release by the spleen. *Nature*, **218**, 1135–1140.

GLATT M., KÄLIN H., WAGNER K. & BRUNE K. (1977). Prostaglandin release from macrophages: an assay system for anti-inflammatory drugs in vitro. *Agents & Actions*, **7**, 321–326.

GRYGLEWSKI R.J. (1976). Steroid hormones, anti-inflammatory steroids and prostaglandins. *Pharmac. Res. Commun.*, **8**, 337–348.

GRYGLEWSKI R.J., PANCZENKO B., KORBUT R., GRODZINSKA L. & OCETKIEWICZ A. (1975). Corticosteroids inhibit prostaglandin release from mesenteric blood vessels of rabbit and from perfused lungs of sensitized guinea pig. *Prostaglandins*, **10**, 343–355.

HIGGS G. S., MCCALL E. & YOULTEN L.J.F. (1975). A chemotactic role for prostaglandins released from polymorphonuclear leucocytes during phagocytosis. *Br. J. Pharmac.*, **53**, 539–546.

KANTROWITZ F., ROBINSON D.R., MCGUIRE M.B. & LEVINE L. (1975). Corticosteroids inhibit prostaglandin production by rheumatoid synovia. *Nature*, **258**, 737–739.

LEWIS G.P. & PIPER P.J. (1975). Inhibition of release of prostaglandins as an explanation of some of the action of anti-inflammatory corticosteroids. *Nature*, **254**, 308–311.

PARENTE L., AMMENDOLA G., PERSICO P. & DI ROSA M. (1978). Glucocorticosteroids, prostaglandins and the inflammatory process. *Pol. J. Pharmac. Pharm.*, **30**, 141–155.

SPECTOR W.G. (1969). The granulomatous inflammatory exudate. *Int. Rev. exp. Path.*, **8**, 1–55.

TASHJIAN A.H., VOELKEL E.F., MCDONOUGH J. & LEVINE L. (1975). Hydrocortisone inhibits prostaglandin production by mouse fibrosarcoma cells. *Nature*, **258**, 739–741.

THOMPSON B.E. & LIPPMAN M.E. (1974). Mechanism of action of glucocorticoids. *Metabolism*, **23**, 159–202.

YARON M., YARON I., GURARI-ROTMAN D., REVEL M., LINDNER H.R. & ZOR U. (1977). Stimulation of prostaglandin E production in cultured human fibroblasts by poly(I).poly(C) and human interferon. *Nature*, **267**, 457–459.

(*Received January 5, 1979.*)