Glucocorticoids: Effects on Prostaglandin Release, Cyclic AMP Levels and Glycosaminoglycan Synthesis in Fibroblast Tissue Cultures

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Summary. Glucocorticoids (GCs) reduced cyclic AMP levels and inhibited glycosaminoglycan (GAG) synthesis in secondary embryonic mouse fibroblast cultures, when cells were incubated for short periods (30 min). The order of potency was dexamethasone > prednisolone > hydrocortisone. The effect was more marked, when cyclic AMP levels and GAG synthesis were increased by addition of PGE₁.

Glucocorticoids exerted no longer an inhibitory effect on cyclic AMP and GAG synthesis in cultures pretreated for 48 h with the steroids. Addition of PGE₁ caused a stronger rise in cyclic AMP and GAG synthesis than in controls without GC-preincubation. This enhancement was even more pronounced, when PGE₁ was added together with the GCs.

The reversal of the inhibitory effect of the GCs into a potentiating effect following preincubation correlated to a reduction of endogenous PGE formation in the cultures. Short-term treatment with GCs did not reduce endogenous PGE levels, but prolonged incubation markedly decreased PGE levels. PGE formation recovered following addition of fresh medium after the 48 h incubation with the steroids, but the amount of PGE formed remained significantly lower than in untreated cultures. Non-glucocorticoid steroid hormones did not decrease PGE levels.

The results indicate that the apparent loss of inhibitory activity of GCs on cyclic AMP and GAG synthesis observed after prolonged incubation may result from a reduction of endogenous PGE formation which renders the cells more sensitive to the stimulatory effect of exogenous PGE₁.

Key words: Glucocorticoids – PGE release – Cyclic AMP levels – Glycosaminoglycan synthesis – Fibroblast cultures.

INTRODUCTION

The anti-inflammatory properties of glucocorticoids (GCs) are clinically well established. They inhibit the inflammatory responses induced by dissimilar causes such as trauma, chemical agents, infectious or immunological processes (Cope, 1972). Suppression of the inflammatory response which occurs as a consequence of immunological hypersensitivity reactions has been implicated with the therapeutic efficacy of GCs in cell-mediated immune reactions, since GCs do not interfere with the processes governing the development of cell-mediated immunity (Cohen, 1971); Balow and Rosenthal, 1973). The mechanism or mechanisms by which GCs suppress the inflammatory responses are not yet fully understood. Schayer (1967) proposed in an “unified” hypothesis that the inhibitory effects of GCs in inflammatory reactions may be linked to a vasoconstrictor effect. This effect was found after a lag period of 1 h and more following application of the hormones both in normal and traumatized capillaries and decreased the extravasation of fluids and cells, thus antagonizing the vasodilator effects of mediators of inflammation (for review see Thompson and Lippman, 1974).

Prostaglandins (PGs) were implicated with the vasoconstrictor effect of GCs by the observation that the functional vasodilatation following stimulation of lipolysis in subcutaneous fat tissue was effected by the release of PGs, most likely PGE₂, and that GCs inhibited this functional vasodilatation by interfering with the release of PGs (Lewis and Piper, 1975). Under these conditions inhibition of the effects of PGs was not due to an inhibition of PG-synthetase, a blockade of PG action at the vascular cells or a reduction of ACTH-induced lipolysis as the functional trigger for PG release. Further evidence for the involvement of PG release in the action of GCs was reported by Gryglewski and coworkers (1975). They
found that the rise in perfusion pressure in isolated mesenteric vascular preparations induced by nor-epinephrine was associated with the release of PG-like substances and that GCs inhibited the norepinephrine-induced release of PGs. Contrary to the in-hibition of PG synthesis effected by indomethacin, infusion of arachidonic acid was able to overcome the reduction of PG release effected by the GCs. Therefore, the authors concluded that GCs may reduce PG release by impairing the availability of sub-strate for the PG synthetase.

Fibroblasts play an essential role in the reparative phase of inflammatory processes by enhanced pro- liferation and synthesis of connective tissue. The increased synthetic activity of fibroblasts is reflected in a rise of glycosaminoglycan (GAG) formation and secretion and appears to be regulated—possibly in a bidirectional way—by cyclic nucleotides (Goggins et al., 1972; Peters et al., 1974; Peters and Schönöhfer, 1975). Putative mediators of inflammation such as PGE₁ and bradykinin increased cyclic adenosine 3’,5’-monophosphate (cyclic AMP) concentrations and GAG synthesis in fibroblasts (Schönöhfer et al., 1974). Non-steroidal anti-inflammatory drugs such as acetylsalicylic acid, indomethacin or phenylbutazone decreased cyclic AMP concentrations and GAG synthesis in fibroblast cultures aside from their well known inhibitory action on PG synthesis in in cultured cells (Levine et al., 1972; Burstein et al., 1976), since the inhibitory action on cyclic AMP levels and GAG synthesis was also found in the presence of high concentrations of PGE₁ added to the cultures (Peters et al., 1975). In a previous study it was shown that 30 min exposure of fibroblast cultures to prednisolone also caused a decrease in cyclic AMP concentrations and GAG synthesis (Schönöhfer et al., 1974). Therefore, the present experiments were designed to gain more detailed in-formations on the effects of GCs on cyclic AMP concen-trations and synthetic activity of the fibroblasts. In addition, the action of GCs was studied on the PG release from the fibroblast cultures in view of a possible explanation for the observed changes in cyclic AMP concentrations and GAG synthesis, since cultured cells were shown to produce and secrete measurable amounts of PGs into the medium (Hamprecht et al., 1973; Burstein et al., 1976) and since GCs were de-scribed to inhibit PG formation and secretion in fibrosarcoma cells (Tashjian et al., 1975) and in synovial tissue explants (Kantrowitz et al., 1975).

MATERIALS AND METHODS

Materials were obtained from following sources: Dulbecco’s mod-i-fication of Eagle’s basal medium (BME) (Flow Laboratories, Bonn); calf serum (Labsor service, München); hydrocortisone, pred-nisolone and testosterone (E. Merck, Darmstadt); dexamethasone (Synochem-Pr/iparate, Berlin); estradiol-17β (Serva, Heidelberg). PGE₁ was kindly supplied by Dr. John Pike (Upjohn, Kalamazoo, U.S.A.). All other chemicals used were obtained from the same sources as described in an earlier paper (Peters et al., 1975).

Tissue Culture. Secondary cultures of embryonic mouse fibroblasts were prepared by essentially the same procedure as described earlier (Peters et al., 1975) except for following modifications: Primary cultures were seeded at 2 - 3 x 10⁶ cells per flask and grown in plastic culture flasks (Falcon, 3024; growth area 75 cm²). Secondary cultures were seeded at 3 - 4 x 10⁵ cells per flask (Falcon, 3012; growth area 25 cm²). The cultures received a serum-restricted medium (1 % calf serum) 24 h before the experiments to achieve growth arrest in the G₁ phase of the cell cycle.

Glucocorticoids were dissolved in ethanol and added to the medium to give a final concentration of 0.01 % ethanol. Experiments were performed in cultures containing 10⁶ cells in 5 or 10 ml BME with only 2 % calf serum in order to reduce interferences from steroid hormones present in varying amounts in commercially available sera (Miło et al., 1976). Addition of 1 % serum was used in incubations for determinations of PG synthesis. Triplicate incubations were used for each experimental condition.

Determination of GAG-Synthesis. GAG synthesis was measured by determining the incorporation of ³⁵SO₄ into the acid-soluble GAG according to the methods of Fraiantoni et al. (1968). Cells were incubated in Dulbecco’s modification of BME in which MgCl₂ was substituted for MgSO₄. Carrier-free ³⁵SO₄ (1 - 5 μCi/ml) was added together with or after short-term drug treatment at the start of the experiments. GAG synthesis was determined by measuring the amount of GAG secreted into the medium. The medium was collected after removal of the cells and dialysed at 4°C for 24 h against 0.1 M ammonium sulfate (20 l, changed twice), followed by running tap water for another 48 h at room-temperature to remove labeled inorganic material. Aliquots (1.0 ml) of the dialysed material were dissolved in 1.0 ml hyamine and 5 ml scintillation fluid for counting of the radioactivity. Values are expressed as pg ³⁵SO₄ incorporated per mg protein.

Determination of Prostaglandins. Incubations were terminated by heating for 2 min at 95°C. Concentrations of PGE₂, PGF₂α, PGE₁ and the metabolites 13,14-dihydro-15-keto-PGE₂ and 13,14-dihydro-15-keto-PGF₂α were directly determined in the culture medium by radioimmunoassays, the sensitivity and specificity of which have been described previously (Jobke et al., 1973; Liebig et al., 1974; Peskar and Peskar, 1976). PGs were extracted from the cells and separated into the subgroups PGE, PGF and PGA using silicic acid chromatography exactly as described by Jaffe et al. (1973). The PGE found was not further identified as either PGE₁ or PGE₂. All the results are calculated as PGE₂.

Determination of Cyclic AMP was performed by the method of Gilman (1970) as modified by Peters et al. (1975).

RESULTS

Hydrocortisone, prednisolone and dexamethasone de-creased cyclic AMP concentrations in untreated as well as in PGE₁-treated fibroblast cultures, when cells were exposed to the GCs for 30 min (Table 1). In the absence of PGE₁ the effect was most marked with dexamethasone, while not significant with hydrocortisone. In the presence of PGE₁ all GCs signifi-