Decreased hyaluronic acid synthesis, a sensitive indicator of cortisol action on fibroblast

HEIKKI SAARNI*, MARKKU TAMMI, NIALL S. DOHERTY**, *Department of Medical Chemistry, University of Turku, Kiinamyllynkatu 10, SF-20520 Turku 52, Finland*

Cortisol decreases the formation of connective tissue by inhibiting fibroblast proliferation (Pratt & Aronow, 1966; Berliner & Nabors, 1967), and synthesis of both collagen (Uitto & Mustakallio, 1971; Blumenkrantz & Asboe-Hansen, 1976) and glycosaminoglycans (GAGs) (Castor & Dorstewitz, 1966; Tessler & Salmon, 1975). We have now compared the sensitivities of these parameters to cortisol action. To avoid species differences (Claman, 1975) and differences between normal and 'transformed' cells (Thrash & Cunningham, 1973) in response to corticosteroids all experiments were carried out with normal human cells.

Early passage human foetal skin fibroblasts (5th–12th passage) were grown to confluency in Dulbecco's modification of Eagle's minimal essential medium, buffered with 20 mM HEPES (Gibco Biocult, Paisley, Scotland) and 23 mM sodium bicarbonate to pH 7·3. When the cells had formed a confluent monolayer, a fresh medium, containing both cortisol (hydrocortisone sodium succinate, Orion, Mankkaa, Finland) and a labelled compound (methyl[^{3}H]thymidine, spec. act. 5 Ci mmol^{-1}, or [^{3}H]glucosamine hydrochloride, spec. act. 3·2 Ci mmol^{-1}, or L-[G-^{3}H]proline, spec. act. 653 mCi mmol^{-1}, The Radiochemical Centre, Amersham, U.K.) was added. The incubations (10 h) were carried out with 10% foetal calf serum (Flow laboratories, Irwine, U.K.) except in experiments on collagen and mucopolysaccharide syntheses, where additional incubations in serum-free medium were also used. DNA-synthesis was estimated by measuring the incorporation of [^{3}H]thymidine (1 μCi ml^{-1}) into cell nuclei as described earlier (Rönnemaa & Doherty, 1977). The cells were cultured on tissue culture plates with 0·3 cm^2 growth area (Falcon Microtest II, B–D, U.K., Ltd., Wembley, U.K.) containing 200 μl of medium per well. The synthesis of GAGs was determined as the incorporation of [^{3}H]glucosamine (3 μCi ml^{-1}) into medium hyaluronic acid and sulphated GAGs by the method of Saarni & Tammi (1977). The cells were grown on cell culture plates similar to those in the DNA synthesis experiments, except that the effect of serum was studied in 5·5 cm^2 cell culture tubes (Nunc, Roskilde, Denmark) with 2 ml of medium. Collagen synthesis was measured by the incorporation of [^{3}H]-proline (2·5 μCi ml^{-1}) into the medium hydroxyproline in 5·5 cm^2 cell culture tubes as described earlier (Doherty & Saarni, 1976). The data were analysed by one way analysis of variance and Newman-Keuls test (Zivin & Bartko, 1976).

The synthesis of DNA decreased only at 1×10^{-4} M cortisol (Fig. 1). The synthesis of sulphated GAGs remained unchanged with all the cortisol concentrations used, consistent with the results of Ebert & Prockop (1967). Synthesis of collagen was not inhibited at cortisol concentrations up to 1×10^{-4} M, rather a stimulation at 1×10^{-5} M was observed (Fig. 1). Omission of serum from the medium reduced the synthesis of collagen by 40% and no stimulation at 1×10^{-5} M cortisol was then found (Table 1). It is not possible at present to explain the stimulation of collagen synthesis by cortisol observed in this and earlier studies (Harvey, Grahame & Panayi, 1974; Nakagawa, Ikeda & Tsurufuji, 1975; Doherty & Saarni, 1976). There are many factors present in the serum which stimulate cell metabolism (Hoffman, Ristow & others, 1973) and at least one of them synergizes with corticosteroids (Gospodarowicz & Moran, 1974). Interaction with one or more of these factors may be the explanation of the stimulation seen here.

In contrast to the lack of inhibition in the other synthetic functions with low concentrations, cortisol was a potent inhibitor of hyaluronic acid synthesis (Fig. 1, Table 1). The degree of inhibition as compared to control, was similar with and without foetal calf serum, although the basal synthetic rate of hyaluronate

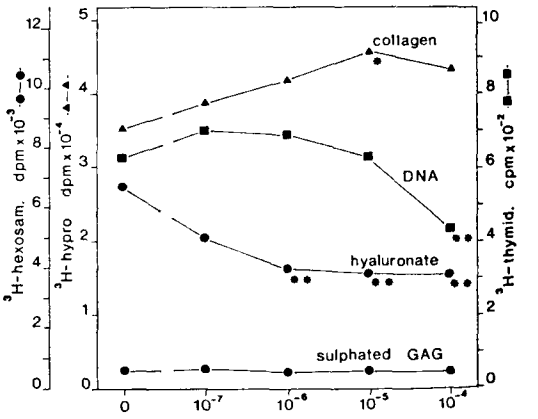

FIG. 1. Concentration dependence of cortisol action on DNA, collagen and glycosaminoglycan synthesis by cultured human skin fibroblasts in 10 h incubation with foetal calf serum. The radioactivities are expressed as d min^{-1}/culture, n = 5–6, *P < 0·05, **P < 0·01.

* Correspondence.

** Royal Society European Research Fellow. Present address: Roche Products Ltd., P.O. Box 8, Welwyn Garden City, Herts AL7 3AY, U.K.

Table 1. *Effect of foetal calf serum and cortisol on the synthesis of collagen and hyaluronic acid in human skin fibroblasts.*

Serum (%)	Collagen (d min^{-1} × 10^{-5})* Cortisol (M) 0	Collagen Cortisol (M) 10^{-5}	Hyaluronic acid (d min^{-1} × 10^{-5}) Cortisol (M) 0	Hyaluronic acid Cortisol (M) 10^{-5}
0	79·0 (74·2–82·1)**	80·3 (68·1–108·2)	12·3 (15·2–10·3)	4·8 (4·7–5·2)
10	132·3 (127·4–142·8)	148·5 (139·2–157·4)	124·4 (113·2–132·0)	57·7 (51·2–63·4)

* Measured as [^{3}H]hydroxyproline.
** Mean and range; n = 5–7 in collagen, n = 3 in hyaluronic acid.

was about ten times higher in the presence of 10% foetal calf serum.

Effects of cortisol on the cell layer hyaluronic acid, sulphated GAGs and collagen were not studied, because earlier experiments (Doherty & Saarni, 1976; Saarni & Hopsu-Havu, 1977) had shown that they are parallel to the effects of cortisol on the synthesis of these compounds in the medium. It thus appears that in short term incubations 1×10^{-6} M cortisol significantly inhibits the synthesis of hyaluronate, but does not change the synthesis of DNA, sulphated GAGs or collagen. This suggests that reduced hyaluronate synthesis is a sensitive indicator of glucocorticoid action on normal human fibroblasts.

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A potential interaction between gentamicin and cephalexin

JOAN E. WHITELEY, J. R. BROWN*, D. N. ELLIOTT, *Department of Pharmacy, University of Manchester, Manchester M13 9PL, U.K.*

Gentamicin is often administered with a cephalosporin, or a penicillin, as these combinations are considered to be synergistic (Watanakunakorn & Glotzebecker, 1974). But there is a possibility of an interaction, for example, visible precipitation in intravenous mixtures of gentamicin with cephalothin (Noone & Pattison, 1971) or with cloxacillin (Noone & Pattison, 1971) or with cephapirin (Prasad, Granatek & Mihotic, 1974) have occurred. Also, the antibacterial activity of gentamicin can be reduced by the presence of carbenicillin (Noone & Pattison, 1971; Riff & Jackson, 1972) and ticarcillin (Davies, Morgan & Anand, 1975; Ervin, Bullock & Nuttall, 1976) at high ratios (20:1 to 30:1) of β-lactam antibiotic to gentamicin. It is therefore necessary to ascertain if such an interaction is likely *in vivo*. The potential interaction between cephalexin and gentamicin has therefore been examined.

*Correspondence

Vapour pressure osmometry. Since the vapour pressure of a solution is dependent on the total number of molecules and ions of solute present, the technique provides a useful tool for the determination of the molecular weight of a solute in terms of all the species present (this is the number average molecular weight, $\overline{Mn}$). Thus the molecular weight of an ionized solute will be lower when determined by this technique than when