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PARADOXICAL STIMULATION OF BOTH LIPOCORTIN AND PROSTAGLANDIN PRODUCTION IN HUMAN AMNION CELLS BY DEXAMETHASONE

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Summary. Glucocorticoids inhibit prostaglandin biosynthesis by inducing the formation of lipocortins. In human amnion cells dexamethasone elicited a concentration-dependent increase in prostaglandin production and raised intracellular lipocortin 1 concentrations. Dexamethasone could also potentiate the epidermal growth factor (EGF)-induced stimulation of prostaglandin production. EGF alone or in combination with dexamethasone increased lipocortin 1 formation in amnion cells. Human amnion cells may provide a unique insight into interactions between glucocorticoids, lipocortin and eicosanoid biosynthesis.

Introduction. An increase in the rate of prostaglandin E_2 (PGE_2) biosynthesis by amnion is considered to be an important part of the mechanism of human parturition (1). Glucocorticosteroid production is also increased during labor (2). This had seemed paradoxical since glucocorticosteroids inhibit prostaglandin biosynthesis by inducing the biosynthesis of lipocortins (3,4,5,6) which have antiphospholipase A2 activities although this has been disputed (7). Recent studies have demonstrated that PGE_2 production by human amnion cells is not inhibited by dexamethasone treatment (8). The present study was undertaken to evaluate this phenomenon in more detail. We have evaluated the effect of glucocorticosteroids on epidermal growth factor (EGF) action. It is known that EGF stimulates PGE_2 production (9,10) an action that has been

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reported to be abrogated by dexamethasone treatment (11). Moreover EGF can induce phosphorylation of lipocortin (12), an action that may attenuate the antiphospholipase activity of lipocortin (13).

Methods. Human amnion cells were grown in primary monolayer culture to confluence as described previously (14). Under these conditions the cells secrete principally PGE_2 and do not metabolize prostaglandins (14). Experiments were conducted with cells derived from amnions of at least 4 individual pregnancies. The cells were incubated with dexamethasone (10^{-10}M to 10^{-6}M) in the presence and absence of EGF (0.1 ng/ml) for 16h. All experiments were conducted with replicates of 4-6 wells of amnion cells; results are presented as mean ± SE. Protein determinations were performed by the method of Lowry et al (15). PGE_2 was measured in the media by a specific radioimmunoassay (14) and lipocortin I was measured in cell lysates using an immunoblotting technique (6). Cell extracts of equivalent amounts of protein were added to 5x SDS sample buffer containing SDS 0.4 M tris-HCl pH 6.8 and 2-mercaptoethanol and boiled for 2 min. The samples were subjected to 10% SDS polyacrylamide gel electrophoresis with 0.4% methyl bisacrylamide. For Western blot analyses immunoreactive proteins were detected with a rabbit polyclonal antibody raised against human cloned lipocortin I protein expressed in E. coli. Iodinated protein A was hybridized with the nitrocellulose for 2 hr at room temperature, the blots were washed with TBS containing 0.5% Tween 20, and dried. Autoradiograms of the blots were obtained after 24 to 36 hr of exposure in the presence of enhancing screen at -70C. All samples were processed in duplicate or triplicate.

Results. Dexamethasone alone caused a variable but concentration-dependent increase in PGE_2 production; up to 4.7-fold stimulation being observed at 10^{-6}M (Figure 1). Dexamethasone

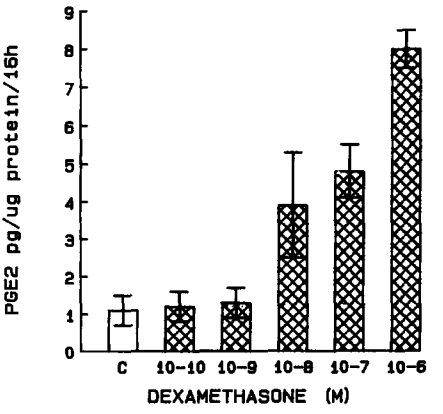

Figure 1. The effects of dexamethasone at various concentrations on prostaglandin E_2 production (mean ± S.E., n=4) by confluent human amnion cells.

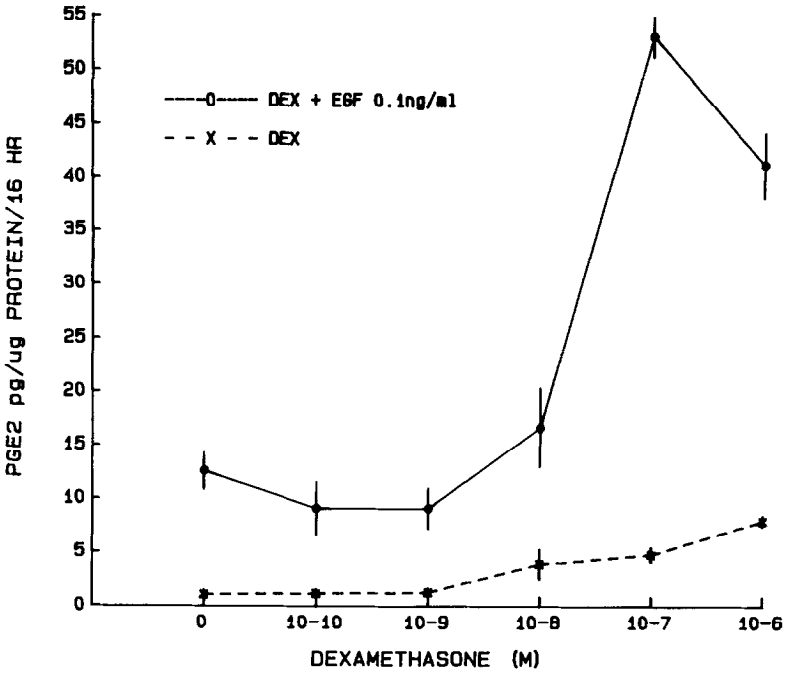

Figure 2. The effects of dexamethasone at various concentrations on prostaglandin E_2 production (mean $\pm$ S.E., n=4) by confluent human amnion cells in the absence (--x--) and presence (--o--) of epidermal growth factor (0.1 ng/ml).

could potentiate the stimulatory action of epidermal growth factor (0.1 ng/ml) on amnion PGE_2 biosynthesis (Figure 2); although only an additive effect was obtained on several occasions. Lipocortin I was found to be formed constitutively by human amnion cells and increased amounts (113 to 220% of control) were observed upon treatment with dexamethasone (10^{-7}M) in 3 of 4 experiments (Figure 3). EGF (5ng/ml) in the presence or absence of dexamethasone also increased the level of lipocortin I protein (maximally 217%) in all 4 experiments.

Discussion. The stimulatory action of dexamethasone on amnion cell prostaglandin biosynthesis is remarkable and rare. It does, however, explain how glucocorticosteroid and prostaglandin secretion can both increase during labor; amnion being a major site of prostaglandin biosynthesis. The lack of an inhibitory action of dexamethasone on amnion cell prostaglandin

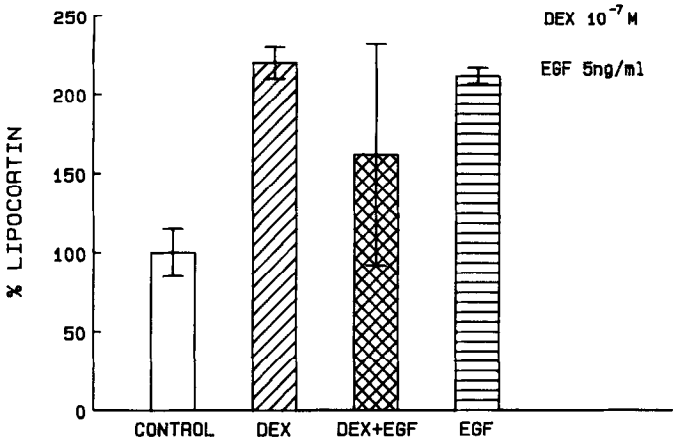

Figure 3. The effects of dexamethasone (DEX, 10^{-7}M) and epidermal growth factor (EGF, 5 ng/ml) alone or in combination on lipocortin I formation (mean ± S.E., n=3) in confluent human amnion cells.

biosynthesis is not due to a defect in lipocortin formation. It is possible, however, that the lipocortin formed is somehow inactive. Alternatively, the arachidonic acid to be used for prostaglandin biosynthesis may be liberated from glycerophospholipid stores by the actions of phospholipase C and diacylglycerol and monoacylglycerol lipases (lipocortin is considered to be specific for phospholipase A_2) or be provided by another source. Amnion contains a very active phospholipase C (16). An interesting observation is that lipocortin 1 (approximately 1uM) can stimulate phospholipase C activity (Bleasdale, J.E., personal communication). Hence it is possible that dexamethasone increases lipocortin 1 formation which causes enhanced phospholipase C activity leading to greater release of arachidonic acid that serves as substrate for increased prostaglandin biosynthesis. It should be noted that EGF action has been linked to calcium mobilization and phosphatidylinositol metabolism (17). The mechanisms both of stimulatory action of dexamethasone on amnion cell prostaglandin biosynthesis and its potentiation of the EGF stimulatory effect are unknown.

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