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Prostaglandin biosynthesis in the human fetal adrenal gland: Regulation by glucocorticosteroids

(prostanoid/corticotropin/cortisol/inhibition)

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ABSTRACT **Human fetal adrenal (HFA) tissue was maintained in organ culture to evaluate the biosynthesis of prostaglandins and hormonal regulation of prostaglandin formation by this tissue. The HFA tissue secreted substantial amounts of prostaglandin E_2, prostaglandin $F_{2\alpha}$, 13,14-dihydro-15-ketoprostaglandin $F_{2\alpha}$, 6-ketoprostaglandin $F_{1\alpha}$, and thromboxane B_2; secretion of prostaglandin D_2 could not be demonstrated. Prostaglandin biosynthesis in HFA tissue was inhibited in a time-dependent manner by corticotropin (ACTH; 0.4 μM); by the fourth day of culture, the extent of inhibition of biosynthesis of each prostaglandin was 60–90%. Progesterone (1 μM), cortisol (1 μM), and dexamethasone (1 μM) inhibited prostaglandin biosynthesis whereas estradiol (1 μM) did not. Of the compounds tested for inhibitory activity, dexamethasone was the most potent. An inhibitor of 11β-hydroxylase activity (metyrapone; 0.1 mM) effectively eliminated the inhibition of prostaglandin biosynthesis caused by corticotropin and progesterone. Metyrapone treatment alone caused a 3-fold increase in prostaglandin biosynthesis by fetal adrenal tissues. Similar stimulatory effects resulted from treatment with inhibitors of (*i*) 3β-hydroxysteroid dehydrogenase (cyanoketone; 15 μM), (*ii*) steroid 17α-hydroxylase (SU 10603; 19 μM), and (*iii*) cholesterol side-chain cleavage (aminoglutethimide; 1 mM). Inhibition of prostaglandin biosynthesis by dexamethasone in the presence or absence of metyrapone was concentration dependent and 50% inhibition could be demonstrated at 1 nM. A competitive inhibitor of the binding of glucocorticosteroids to cytoplasmic receptors (cortisol 21-mesylate; 1 μM) significantly reduced the inhibition of prostaglandin biosynthesis effected by dexamethasone (10 nM). These findings suggest that prostaglandin biosynthesis in the HFA gland is regulated by endogenously synthesized glucocorticosteroids, the actions of which are mediated by a glucocorticosteroid receptor. Such glucocorticosteroids induce the synthesis of a substance that inhibits prostaglandin biosynthesis.**

In most, if not all, mammalian species, the fetal adrenal glands serve an important role in the maturational processes that ensure the timely birth of a viable fetus (1). An understanding of the regulation of hormone secretion by the human fetal adrenal (HFA) gland is therefore of signal importance to understanding the development of the human fetus. The HFA gland at term can secrete up to 200 mg of steroids daily (2, 3); the principal product is dehydroisoandrosterone sulfate, although significant quantities of cortisol also are produced (4–6). In rat adrenocortical cells, corticotropin (ACTH) causes the hydrolysis of cholesterol esters, resulting in increased availability of precursors for prostaglandin (PG) biosynthesis (7). It has been shown that PGs act to modulate steroid secretion by adult human adrenal gland tissue (8) and in particular HFA tissue (9). There is appreciable evidence that corticotropin and PGs act to modify steroid secretion by the adrenal gland by way of a mechanism(s) that involves activation or inhibition of adenylate cyclase (10–12). Hence, it seemed reasonable that PGs synthesized *in situ* might act as mediators or modulators of corticotropin action on the HFA. PG synthesis by the HFA, however, has not been described. Yet several lines of evidence support a role for the HFA as a source of PGs. For example, PGs are present in fetal plasma and concentrations increase with gestational age (13). Moreover, under conditions in which the human fetal adrenal has atrophied, such as fetal anencephaly, there is circumstantial evidence that fetal production of PGs is greatly reduced, as shown by undetectable levels in amniotic fluid (14). In addition, the rapid involution of the fetal zone of the HFA suggests an acute change in vascular tone that results in reduced blood flow to the cells of the fetal zone, an event that may be due to an alteration in local PG synthesis. A similar explanation has been invoked to account for the closure of umbilical vessels at birth (15).

To test the hypotheses that HFA tissue synthesizes PGs and that corticotropin may influence the rate of PG synthesis, we maintained HFA tissue in organ culture (6) and investigated PG biosynthesis by the tissue in the absence and presence of corticotropin. We found that corticotropin treatment of HFA tissue caused a time-dependent inhibition of PG biosynthesis. The findings of a detailed study of this phenomenon are presented.

MATERIALS AND METHODS

Source of Tissue. Human fetal adrenal glands were obtained from first and second trimester abortuses; abortions were accomplished by dilatation and extraction. Tissues were obtained in accordance with the Donors Anatomical Gift Act of the State of Texas after obtaining written consent from the women to be aborted by using a consent form and protocol approved by the Human Research Review Committee of the University of Texas Health Science Center at Dallas.

Preparation and Culture of Tissue. Within 1 hr of fetal death, the adrenal glands were removed and minced into pieces of approximately 1 mm^3. Four fragments of adrenal tissue (approximately 1 mg of tissue protein) were placed on a lens paper supported by a stainless steel grid in each organ culture dish (60 × 15 mm; Falcon Plastics, Cockeysville, MD). The medium used consisted of 50 vol of Waymouth's MB 752/1 medium, 40 vol of Gey's balanced salt solution (GIBCO), 1 vol of a 1% solution of antibiotics and antimycotics (GIBCO), and 10 vol of whole human serum (6). The medium (1 ml) was changed every 24 hr for 4 days and stored at −20°C. At the end of the culture period, the tissue fragments were rinsed thoroughly and homogenized in 0.5 ml of 0.15 M sodium chloride. Aliquots of the

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Abbreviations: HFA, human fetal adrenal; PG, prostaglandin; PGE_2, $PGF_{2\alpha}$, etc., PG E_2, PG $F_{2\alpha}$, etc.; TXB_2, thromboxane B_2.

homogenates were taken for measurement of protein by the method described by Lowry *et al.* (16).

Each experimental condition was replicated in quadruplicate or quintuplicate. Results are expressed as pg of PG secreted into the culture medium per mg of tissue protein per 24 hr (mean ± SEM).

Materials. Synthetic $ACTH_{1-24}$ (Cortrosyn) was obtained from Organon (W. Orange, NJ). Progesterone, 17β-estradiol, cortisol, and dexamethasone were obtained from Sigma. Metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone) was purchased from Aldrich. Aminoglutethimide and SU 10603 [7-chloro-3,4-dihydro-2-(3-pyridyl)-napthalen-1-one] were provided by Ciba-Geigy. Cyanoketone (2α-cyano-4,4,17α-trimethyl-17β-hydroxyandrost-5-en-3-one) was a gift of Sterling Winthrop (Rensselaer, NY), and cortisol 21-mesylate was a gift of S. Stoney Simons, Jr., National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases.

Measurements of PGs. PGs were measured by sensitive and specific radioimmunoassays that have been fully described and validated elsewhere (17–19). The PGs measured were PGE_2, $PGF_{2\alpha}$, 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM, a major metabolite of $PGF_{2\alpha}$), PGD_2, and 6-keto-$PGF_{1\alpha}$, the degradation product of prostacyclin. Thromboxane B_2 (TXB_2, the degradation product of thromboxane A_2) was also measured (20). The lower limits of sensitivity of the assays are in the range 1–5 pg per tube and intra-assay coefficients of variation are less than 10%. Crossreactivities of the antisera with the other PGs measured are less than 1%.

RESULTS

HFA tissue was maintained in organ culture for 4 days in the absence or presence of corticotropin (0.4 μM). In the absence of corticotropin, the tissue secreted a variety of PGs and TXB_2 throughout the 4-day period. Secretion of PGD_2 by HFA tissue could not be detected. Corticotropin inhibited the biosynthesis of all PGs and TXB_2 with maximal inhibition occurring on the later days of culture; the rates of synthesis on the final day of culture are shown in Fig. 1. Basal rates of PG and TXB_2 production were 1,000–2,000 pg/mg of protein per 24 hr. Treatment with corticotropin for 4 days reduced rates of PG and TXB_2 production by 60–90%. The finding that PGFM production was also decreased by corticotropin treatment indicates that the reduction in PG secretion observed was not the result of an enhanced rate of catabolism. Since the secretion rates of all PGs and TXB_2 were affected similarly by corticotropin treatment and the effects observed were greatest toward the end of the culture period, we have chosen to present the results obtained for the rate of production of one PG (PGE_2) on the final day of culture in subsequent experiments. It should be noted, however, that data on rates of production of at least one and usually two other PGs were obtained in these experiments and always paralleled data on PGE_2.

The effects of progesterone and 17β-estradiol (two steroids present in high concentrations in fetal plasma and known to alter PG production *in vivo*) on PG biosynthesis in HFA tissue were investigated. The results are shown in Fig. 2. Progesterone alone inhibited PG biosynthesis by HFA tissue whereas 17β-estradiol alone was without effect. Neither steroid influenced corticotropin inhibition of PG production and the degree of inhibition when progesterone and corticotropin were combined did not differ from that with corticotropin alone.

We hypothesized that the inhibitory action of corticotropin was mediated by way of stimulation of the synthesis of a steroid by the HFA tissue and that progesterone acted to inhibit PG biosynthesis by providing a greater pool of substrate for the

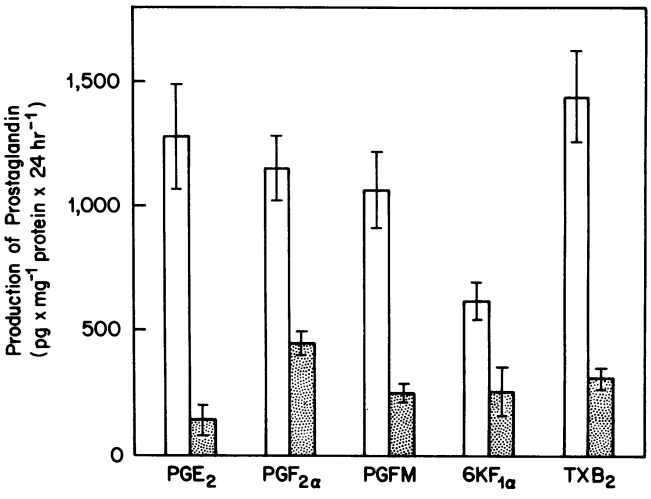

FIG. 1. Effect of $corticotropin_{1-24}$ on rates of production of prostanoids by HFA tissue. Fragments of HFA tissue maintained in organ culture were incubated in medium containing whole human serum alone (□) or in medium containing whole human serum and 4 μM $corticotropin_{1-24}$ (▨). The media were changed every 24 hr and prostanoids were assayed in the media collected on the fourth day of culture. Results represent mean ± SEM of values obtained from media of four replicate dishes. $6KF_{1\alpha}$, 6-keto-$PGF_{1\alpha}$.

HFA tissue to convert to such a steroid. Cortisol seemed a logical candidate for this steroid since cortisol is known to inhibit PG biosynthesis in certain other tissues (21, 22). Hence, we investigated the effects of cortisol and dexamethasone on PG production by HFA tissue and the effects of two inhibitors of steroid biosynthesis (23)—i.e., an inhibitor of steroid 17α-hydroxylase activity (SU 10603) and an inhibitor of 3β-hydroxysteroid dehydrogenase activity (cyanoketone). The results are shown in Fig. 3. Inhibition of steroid biosynthesis resulted in 3- to 4-fold stimulation of the rate of PG biosynthesis, sug-

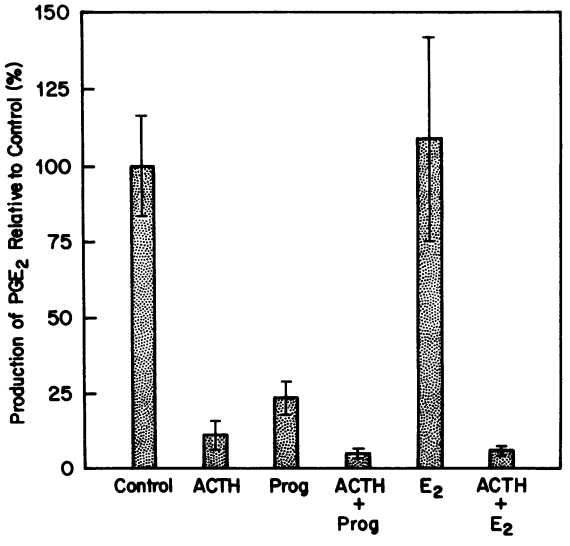

FIG. 2. Effects of various combinations of hormones on the rate of production of PGE_2 by HFA tissue. Fragments of HFA tissue maintained in organ culture were incubated in medium containing whole human serum alone (control) or in medium containing whole human serum together with 0.4 μM $corticotropin_{1-24}$ (ACTH), 1 μM progesterone (Prog), 1 μM 17β-estradiol (E_2), or combinations thereof. The media were changed every 24 hr and PGE_2 was assayed in the media collected on the fourth day of culture. Results represent mean ± SEM of values obtained from media of four replicate dishes and are normalized to control = 100%.

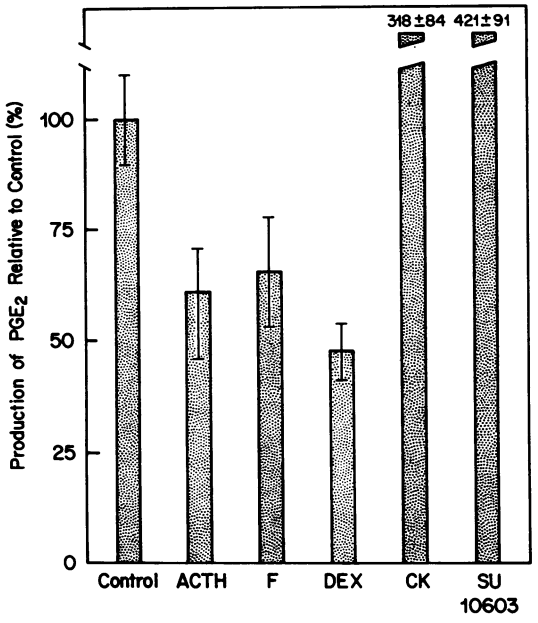

FIG. 3. Effect of hormones and drugs on the rate of production of PGE_2 by HFA tissue. Fragments of HFA tissue maintained in organ culture were incubated in medium containing whole human serum alone (control) or in medium containing whole human serum together with 0.4 μM corticotropin$_{1-24}$ (ACTH), 1 μM cortisol (F), 1 μM dexamethasone (DEX), 15 μM cyanoketone (CK), or 19 μM SU 10603. The media were changed every 24 hr and PGE_2 was assayed in the media collected on the fourth day of culture. Results represent mean ± SEM of values obtained from media of five replicate dishes and are normalized to control = 100%.

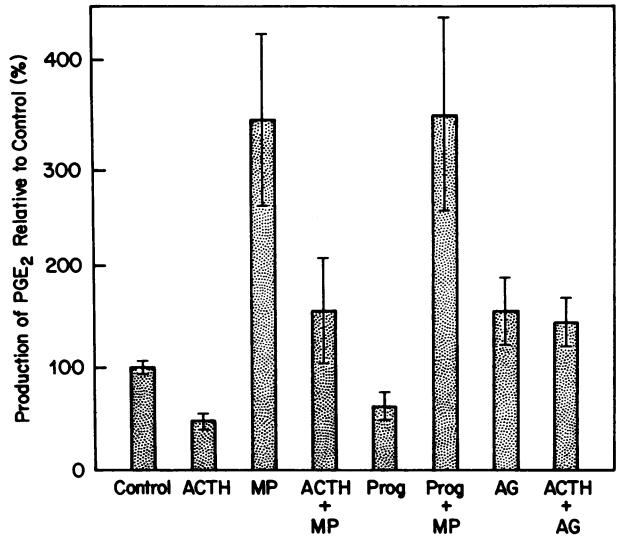

FIG. 4. Effects of hormones and drugs on the rate of production of PGE_2 by HFA tissue. Fragments of HFA tissue maintained in organ culture were incubated in medium containing whole human serum alone (control) or in medium containing whole human serum together with 0.4 μM corticotropin$_{1-24}$ (ACTH), 0.1 mM metyrapone (MP), 1 μM progesterone (Prog), 1 mM aminoglutethimide (AG), or combinations thereof. The media were changed every 24 hr and PGE_2 was assayed in the media collected on the third day of culture. Results represent mean ± SEM of values obtained from media of five replicate dishes and are normalized to control = 100%.

gesting that basal PG production in HFA tissue is inhibited in a tonic manner by an endogenously produced steroid. Furthermore, since cortisol and dexamethasone inhibited PG biosynthesis at least as effectively as did corticotropin, it seemed likely that the inhibitory steroid produced in response to corticotropin was cortisol.

To test the hypothesis that PG biosynthesis by HFA tissue is inhibited by endogenously synthesized cortisol and that the inhibitory actions of corticotropin and progesterone are mediated by way of cortisol production, we investigated the effects of an inhibitor of steroid 11β-hydroxylase activity [metyrapone (23)] on HFA tissue production of PG in the absence and presence of corticotropin or progesterone. Also, to evaluate further whether the effects of cyanoketone and SU 10603 were due to inhibition of cortisol biosynthesis or to concomitant accumulation of other steroidal intermediates, we tested the effect of an inhibitor of cholesterol side-chain cleavage activity [aminoglutethimide (23)]. The data are shown in Fig. 4. Both aminoglutethimide and metyrapone alone enhanced PG production by HFA tissue, the former by 50% and the latter by 300%. Progesterone did not inhibit PG biosynthesis in HFA tissue in the presence of metyrapone, although corticotropin somewhat inhibited the effect of metyrapone.

To further assess the nature of glucocorticosteroid inhibition of PG biosynthesis by HFA tissue, the effects of various concentrations of dexamethasone were investigated. PG biosynthesis was inhibited by 30% in the presence of 1 nM dexamethasone and 90% by 10 nM dexamethasone (data not shown). A similar experiment was conducted in which the dexamethasone was added in the presence of metyrapone to inhibit endogenous cortisol secretion and the results are shown in Fig. 5. Inhibition of PG biosynthesis in HFA tissue by dexamethasone was concentration dependent with 50% inhibition attained at 1 nM dexamethasone. Inhibition of PG biosynthesis was also time dependent, maximal inhibition usually being attained by the third day of culture (data not shown).

To evaluate the possibility that the glucocorticosteroid receptor may be essential in mediating the response to dexamethasone, the effect of cortisol 21-mesylate on the dexamethasone-induced inhibition of PG biosynthesis was examined.

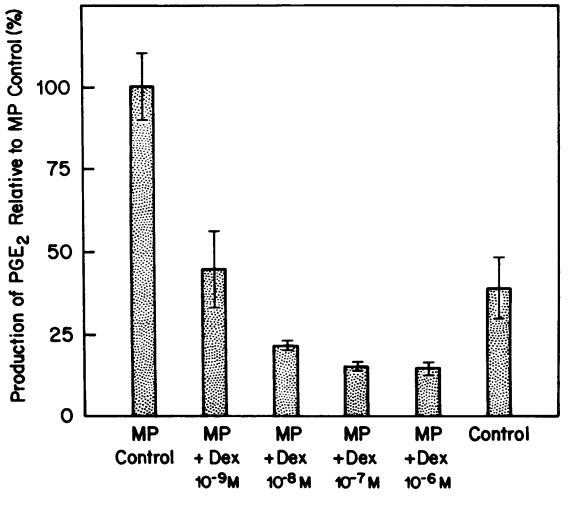

FIG. 5. Effects of various concentrations of metyrapone and dexamethasone on the rate of production of PGE_2 by HFA tissue. Fragments of HFA tissue maintained in organ culture were incubated in medium containing whole human serum alone (control), in medium containing whole human serum/0.1 mM metyrapone (MP control), or in medium containing whole human serum, 0.1 μM metyrapone, and various concentrations of dexamethasone (Dex). The media were changed every 24 hr and PGE_2 was assayed in the media collected on the fourth day of culture. Results represent mean ± SEM of values obtained from media of four replicate dishes and are normalized to MP control = 100%.

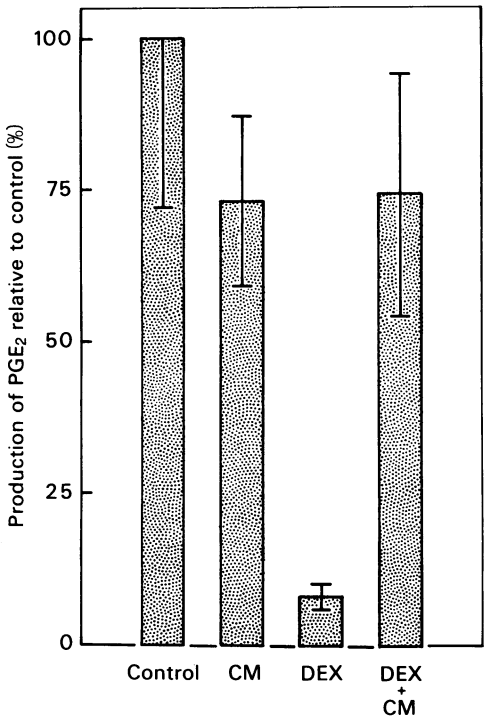

FIG. 6. Effects of hormones and drugs on the rate of production of PGE_2 by HFA tissue. Fragments of HFA tissue maintained in organ culture were incubated in medium containing whole human serum alone (control) or in medium containing whole human serum together with 10 nM dexamethasone (DEX), 1 μM cortisol mesylate (CM), or a combination of the two. The media were changed every 24 hr and PGE_2 was assayed in the media collected on the fourth day of culture. Results represent mean ± SEM of values obtained from media of four replicate dishes and are normalized to control = 100%.

Cortisol 21-methylate is believed to be an antagonist of the binding of glucocorticosteroids to cytosolic receptors (24). The results are shown in Fig. 6. The dexamethasone-induced inhibition of PG biosynthesis by HFA was attenuated greatly by cortisol 21-mesylate. In the absence of dexamethasone, cortisol 21-mesylate did not affect PG biosynthesis by HFA.

DISCUSSION

We have shown that the HFA gland synthesizes and secretes a variety of PGs and TXB_2. Moreover, the production of PGFM by this tissue suggests the presence of 15-hydroxy-PG dehydrogenase activity. The finding that corticotropin treatment reduces the rate of biosynthesis of PGs while stimulating steroidogenesis is not consistent with the hypothesis that PGs are mediators of the effects of corticotropin on steroidogenesis. Moreover, our findings do not support the view that corticotropin may induce the hydrolysis of cholesterol esters and liberate arachidonic acid to serve as a substrate for PG biosynthesis in HFA tissue, as occurs in rat adrenocortical cells (7).

Because the degree of inhibition of biosynthesis of prostanoids by HFA tissue in response to corticotropin (or glucocorticosteroids) was similar for all prostanoids investigated, the inhibitory effect likely occurs at the level of the fatty acid cyclooxygenase or earlier in the biosynthetic pathways for prostanoid formation. The mechanism(s) whereby glucocorticosteroids inhibit PG biosynthesis is believed to include induction of the synthesis of a protein(s) that inhibits the action of phospholipase A_2 (25–27) or other phospholipases (28). A prerequisite for this action is the binding of glucocorticosteroids to cytoplasmic receptors (25, 27). It is possible that, in HFA tissue, glucocorticosteroids act to induce the synthesis of an inhibitor of phospholipase A_2 action akin to macrocortin (25, 26) or lipomodulin (27, 28). This proposition is based primarily on the fact that interference with the action of phospholipase A_2 would result in reduced availability of arachidonic acid (the rate-limiting step in PG biosynthesis) and hence lower the rates of production of all prostanoids. This was the finding of the present study. However, at this time, we cannot exclude the possibility that glucocorticosteroids act to inhibit PG biosynthesis by causing a decrease in cyclo-oxygenase activity or by stimulation of arachidonate lipoxygenase activities. Our findings that the inhibitory effect of glucocorticosteroids is retarded by an antagonist of receptor binding (cortisol 21-mesylate) and that 50% inhibition is observed at 1 nM dexamethasome strongly suggest that the actions of glucocorticosteroids to inhibit PG secretion are mediated by binding to a glucocorticosteroid receptor. Whether the cells that synthesize prostanoids in the HFA gland are the same as those that synthesize steroids remains to be determined.

A wide variety of properties are attributable to prostanoids but these compounds are particularly potent in action on vascular smooth muscle (29). Hence, depending on the predominant prostanoid formed, a vascular bed may be constricted or dilated. In HFA tissue, the inner (fetal) zone involutes rapidly after birth at a time when concentrations of adrenal steroids in the circulation are reduced markedly in comparison with plasma concentrations during fetal life. Thus, it is possible that, in HFA tissue at birth, a significant increase in the local formation of prostanoids may commence and, if vasoconstrictor prostanoids predominate, the fetal zone of the adrenal may atrophy as a result of the reduced blood supply.

In summary, we have demonstrated that HFA tissue synthesizes a variety of prostanoids and that the synthesis of the prostanoids appears to be inhibited by glucocorticosteroids that are produced in response to corticotropin.

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