IN VITRO RESPONSES OF FOCAL HYPERPLASTIC TISSUE OF THE HUMAN ADRENAL ZONA FASCICULATA TO ACTH

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ABSTRACT

The temporal cAMP, cortisol and aldosterone responses to ACTH of focal hyperplasia of the zona fasciculata and of normal human adrenocortical tissue were investigated. ACTH significantly increased cAMP levels (1 min) and cortisol output (2 min) in normal adrenal tissue but not in hyperplastic tissue. However, following ACTH treatment cortisol and aldosterone production were depressed in the abnormal adrenal tissue below the untreated or the ACTH stimulated normal adrenal tissue. In addition, basal cortisol and aldosterone production of the hyperplastic adrenal tissue was elevated above that of the normal adrenal tissue. These findings suggest that the cAMP second messenger concept may be only one of several mechanisms in the modulation of human adrenocortical function.

ACTH may have little or no stimulatory effect on steroidogenesis in many adrenocortical carcinomas (Lipsett et al. 1963) and adenomas (Saez et al. 1975), however, the mechanism of this tropic failure is unknown. The biochemical abnormalities in rat adrenocortical tumours include failure to increase cAMP levels, lowered ATP levels (Ney et al. 1969), and an inhibitory effect of ACTH on the formation of deoxycorticosterone from pregnenolone (Sharma 1973). The latter ACTH effect does not appear to be mediated via cAMP. Interest-

This work was supported in part by Clinical Cancer Research, Grant CA 07177 and Grant TA 12 CA 08096 from the National Cancer Institute, U.S. Public Health Service.

363
ingly, exogenous cAMP produced only slight inhibition of the pregnenolone to deoxycorticosterone conversion (Sharma 1973).

Recently, high levels of cytochromes P_{150} and a_{3} were observed in human adrenal focal hyperplasia of the zona fasciculata (deAlvare et al. 1977) which suggested elevated metabolic activity and steroid biosynthesis. As human adrenal nodular hyperplasia is reported to possess some degree of autonomy (Neville 1969), the in vitro effects of ACTH on cAMP, cortisol and aldosterone levels in normal and abnormal human adrenocortical tissue were evaluated.

**MATERIALS AND METHODS**

Four human adrenal glands were obtained at surgery from two women aged 64 years (60.9, 70.5 kg) with breast carcinoma who received no previous antimitotic or X-ray therapy. The adrenals were immediately placed in cold (0–4°C) Krebs' Ringer bicarbonate buffer, KRBGA (pH 7.4, 200 mg glucose/100 ml, 0.5% serum albumin fraction V). The left adrenal of one patient was found to contain a well circumscribed coarsely nodulated mass approximately 1 x 1.5 cm (180 mg). The mass was identified as an area of focal hyperplasia of the zona fasciculata. This area was dissected free of adherent normal adrenal tissue, diced, pre-incubated and exposed to ACTH as described below. The normal contralateral (right) adrenal was treated similarly. Glands were diced (2 x 3 mm) and pre-incubated (37°C) in KRBGA for 45 min. These dice then were incubated (1 ml KRBGA; 37°C; 95% O_{2} + 5% CO_{2}) in a Dubnoff metabolic shaker for 1–32 min. The dice were exposed to porcine ACTH (100 mIU/ml; chromatographically pure; 150 IU/mg) or KRBGA alone. Adrenal incubates were quenched in liquid nitrogen and analyzed for cAMP by RIA (Honn & Chavin 1975a). Cortisol and aldosterone secretion into the incubation medium was quantitated by RIA (Foster & Dunn 1974; McKenzie & Clements 1974). Proteins were determined (Honn & Chavin 1975b) and the data expressed as pmole cAMP or ng steroid per mg protein, x ± sem and % of control. A minimum of four tissue replicates were used per datum point. Data were analyzed by analysis of variance and Student's t-test. Differences were accepted as significant when P < 0.05.

**RESULTS**

As the cAMP, cortisol and aldosterone responses of the normal adrenal tissue of both patients were not significantly different, the results were combined and compared to the responses of the hyperplastic tissue. ACTH (100 mIU/ml) significantly elevated normal adrenal cAMP levels (1 min) above the control levels with a peak response at 8 min (Fig. 1). These levels remained significantly elevated for 32 min. In contrast, the hyperplastic tissue did not respond to ACTH. cAMP levels were not significantly different between normal adrenal controls, hyperplastic controls and the ACTH treated hyperplastic groups (Fig. 1).
Basal and post-ACTH (100 mIU/ml) cAMP levels in normal and hyperplastic human adrenocortical tissue. Closed circle, normal adrenal control; Square, hyperplastic adrenal control; Open circle, normal adrenal + ACTH; Triangle, hyperplastic adrenal + ACTH. Standard error indicated as vertical bar except when smaller than symbol.

Cortisol secretion by the normal adrenal in response to ACTH was significantly elevated at 2 min and continued to increase throughout the study period (Fig. 2). Basal cortisol secretion by the hyperplastic tissue was elevated above the normal control level at 2 and 4 min (41% and 24%, respectively) although such was not statistically significant. However, at 32 min a significant elevation (31%) in cortisol production was observed (Fig. 2). ACTH significantly depressed cortisol secretion by the hyperplastic tissue during the initial 16 min period. However, at 32 min the cortisol output of the abnormal tissue was not
Basal and post-ACTH (100 mIU/ml) aldosterone production by normal and hyperplastic human adrenocortical tissue. Closed circle, normal adrenal control; Square, hyperplastic adrenal control; Open circle, normal adrenal + ACTH; Triangle, hyperplastic adrenal + ACTH. Standard error indicated as vertical bar except when smaller than symbol.

Different from the basal secretory level of normal tissue. In addition, the cortisol output of the abnormal tissue with ACTH was significantly depressed below the basal secretory level of the hyperplastic tissue (Fig. 2).

ACTH significantly elevated normal adrenal aldosterone output above the control level from 2–32 min. However, ACTH depressed aldosterone output by the hyperplastic tissue below normal (8–32 min) and hyperplastic (1–32 min) control levels (Fig. 3). Basal aldosterone secretion in the hyperplastic control group was significantly elevated above the normal control group (16–32 min) and not significantly different from ACTH treated normal adrenal from 2–32 min (Fig. 3).

Finally, it should be noted that the patient with adrenal focal hyperplasia did not present clinical signs of excessive glucocorticoid or mineralocorticoid production.

**DISCUSSION**

ACTH (100 mIU) rapidly and significantly elevates normal human adrenal cAMP levels preceding or coexistent with increased cortisol secretion. This agrees with the basic findings in the lower forms studied (Garren et al. 1971) and represents the first report of the simultaneous temporal responses to ACTH of both parameters by the normal human adrenal. In addition, ACTH stimulates *in vivo* aldosterone secretion in normal human subjects (Tucci et al. 1967). In the present report this response is confirmed *in vitro*.

Basal cAMP levels are similar in normal and hyperplastic human adreno-
cortical tissue. These findings agree with similar reports for rat adrenocortical tumours (Ney et al. 1969). Basal cortisol and aldosterone secretion however, are significantly elevated in the hyperplastic tissue. As the final hydroxylation reactions for both cortisol and aldosterone occur intramitochondrially, this finding is predictable from the elevated mitochondrial cytochrome P450 levels observed by deAlvare et al. (1977) in similar hyperplastic tissue. Surprisingly, ACTH depresses in vitro cortisol and aldosterone production by the hyperplastic tissue of the zona fasciculata although the cortisol depression appears to be incomplete or transient in nature. This unusual response to ACTH may aid in the explanation of the failure of the dexamethasone suppression and ACTH-stimulation test to influence cortisol production by nodular hyperplastic tissue of the human adrenal (Neville 1969).

ACTH fails to stimulate cAMP production in the hyperplastic adrenocortical (zona fasciculata) tissue. A number of explanations is possible, including increased phosphodiesterase activity, abnormal adenylate cyclase and/or decreased substrate (ATP) availability, modification of the ACTH receptor, etc. Saez et al. (1975) examined several human adrenocortical carcinomas and adenomas and reported heterogenous responses to ACTH including complete failure to stimulate adenylate cyclase. The results of receptor binding studies prompted these investigators to postulate the existence of two distinct binding sites for the ACTH molecule in the normal human adrenal. One binding site related to the ACTH1-10 sequence and another related to the ACTH11-24 sequence. The existence of dual ACTH receptors also has been suggested previously (Moyle et al. 1973). As ACTH1-10 stimulates cAMP production and steroidogenesis, the failure of ACTH to stimulate cAMP production by hyperplastic tissue and in the other human adrenal tumours studied (Saez et al. 1975) may be interpreted as an alteration in or loss of the 1-10 binding site. Nevertheless, in the present report ACTH specifically interacted with the abnormal adrenocortical cells, as cortisol and aldosterone production are depressed. As this depression is not the result of cAMP alterations, the existence of another effector, possibly antagonistic to or modulating cAMP, is suggested.

The existence of a ‘unitary’ second messenger in the mechanism of ACTH action in the adrenocortical cell has been questioned (Moyle et al. 1973; Sharma 1974; Honn & Chavin 1975a). In addition, ACTH has been demonstrated to alter both adrenocortical cAMP and cGMP levels in opposite directions (Honn & Chavin 1975a; Whitely et al. 1975). Therefore, the possible role of cGMP in the modulation of cortisol and aldosterone production by human adrenocortical tissue requires investigation. Further, a number of prostaglandins alter human adrenal cyclic nucleotide levels and steroid output directly or indirectly by modulating ACTH action (Honn & Chavin 1976a,b). Therefore, several factors in addition to cAMP emerge to form a potential complex in the control of cortisol and aldosterone production by the human adrenal gland.
REFERENCES


Received on December 20th, 1976.