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Acth Induced Changes In Ascorbate And Activity In The Adrenalof Hypophysectomized Rat

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Abstract: ***The possibility of ACTH being involved in the peroxidase-ascorbate system for the synthesis of progesterone. Rapid depletion of AA under the action of ACTH is known to be a donor in peroxidase reaction.***

Keywords: Adrenal, ACTH, Hypophysectomy, Peroxidase-Ascorbate System, Progesterone

I. INTRODUCTION

ACTH has a major role in the synthesis of progesterone which is known to be a precursor of several steroid hormones including androgens, estrogens and corticoids (Gorbman & Bern, 1974). ACTH is also known to cause depletion of adrenal ascorbate and cholesterol in the hypophysectomized rat (Tyslowitz, 1943; Sayers et al., 1946) which is shown to occur within minutes of ACTH injection and to exhibit a characteristic time sequence. Administration of ACTH also stimulates adrenal secretion of progesterone as well as corticosterone (Resko, 1969; Feder et al., 1969; Feder et al., 1971; Piva et al., 1973). Thus, the study emphasized on the possibility of ACTH being involved in the peroxidase-ascorbate system for the synthesis of progesterone.

II. MATERIAL & METHODS

Colony-bred albino rats (Wistar Strain) of our departmental colony maintained on a regimen of 12hrs.light/12 hrs. dark in a temperature controlled room ($25^0 - +1^0$ C) were used in this study. They received food & water ad libitum.

The method followed for the priming of rats was that of Fisher et al. (1962). Hypophysectomized immature female rats, 80-100 gm weight were used in this study. 20µ of purified ACTH (Porcine-ACTH, Sigma Chemical Co., USA) dissolved in 0.5ml of physiological saline was administered to rats in a single dose by sub-cutaneous injection. The control group received physiological saline (0.9%) only which was administered to rats in a single dose by sub-cutaneous injection. Rats were sacrificed at different periods by cervical dislocation, dissected and the adrenals free of adhering fat were used for analysis. The tissues were stored at -20^0C after weighing whenever necessary. Out of the two adrenals in each animal, the left adrenal was analysed for peroxidase and the right for AA. Until & otherwise mentioned, five replicates at a time were used for each analysis.

TOTAL PROTEINS

Total proteins was estimated by the method of Lowry et al. (1951) after proceeding for calibration of caesin).

ASCORBIC ACID

Ascorbate was determined by the colorimetric method of Mindlin and Butler (1938) by following the decolorization of 2,6 dichlorophenolindophenol in metaphosphoric acid after proceeding for calibration of Ascorbic Acid.

PEROXIDASE ACTIVITY

Total peroxidase activity was measured using guaiacol as donor by the method of Maehly and Chance (1954).

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RESULTS

The effect of ACTH on hypophysectomized rats injected with 20µ of ACTH is shown in fig. 1. It is seen that as the depletion of AA ensures within 10 minutes after ACTH injection the peroxidase activity tends to show an increase. A marked increase in peroxidase activity is observed between 30-60 minute sof ACTH injection, the optimum being observed between 60-90 minutes when the ascorbate content is the lowest. Peroxidase activity falls rapidly at 2 hrs. when ascorbate level begins to recover. An inverse relationship between ascorbate content and peroxidase activity in the adrenal is clearly evident

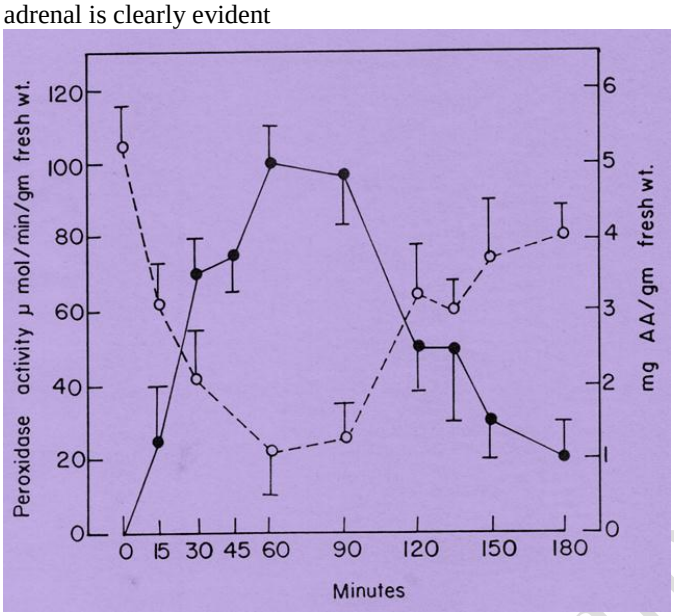

Figure 1: ACTH-induced changes in ascorbate and peroxidase activity in the Adrenal of hypophysectomized rats

III. DISCUSSION

Although the depletion of adrenal ascorbate by ACTH has been utilized as a bioassay of ACTH (Tyslowitz, 1943; Sayers et al., 1946; Long, 1947) and an indicator of increased secretion of hormones, no evidence for the participation of ascorbate in the actual formation or release of steroid hormones freom the adrenal gland has been shown or suggested.

ACTH induces the formation of peroxidase in the adrenal of hypophysectomized rats, which is associated with the depletion of asorbate. An inverse relationship is observed. Sayers et al., (1948) showed that depletion of ascorbate with ACTH in hypophysectomized rats exhibits a time pattern relationship; the depletion sets in within 5 minutes of ACTH injection,reaches a point of maximum depletion at about 1-2 hrs., whereafter the ascorbic acid concentration starts building up again, showing a rebound at about 20-24 hrs. High level of AA in adrenal has been suggested to act as a restraint factor on steroidogenesis particularly in the early reactions of the sequence involving cholesterol conversion to progesterone (Hayano,et al.,1956). It has been proposed that inhibition of steroidogenesis by AA is chiefly affected through hydroxylase system, which is relieved when stimulated by ACTH (Kitabachi, 1967).

ACTH depletes only the excess of ascorbate to retain a catalytic concentration that favours steroidogenesis, since it was foundthat ascorbate promotes the activity of cholesterol side-chain cleaving enzyme complex at lower concentration while inhibiting the same at high concentration (Sulimovici and Byod, 1969; Shimizu, 1970). At high concentration of AA, the free radical intermediate of pregnenolone is rapidly reduced by ascorbate, the latter getting oxidized; the onward oxidation of pregnenolone proceeds when AA content is depleted to the level that would fail to reduce the free radical form of pregnenolone and pregnenolone then is rapidly oxidized to progesterone. At low concentration ascorbate stimulates this conversion (Agrawal and Laloraya, 1977) Fig.2

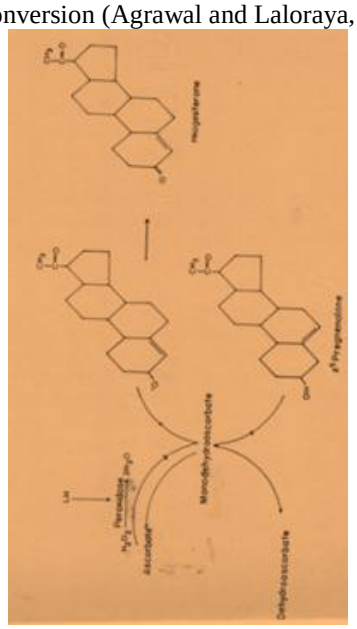

Figure 2: Postulated mechanism of peroxidase in luteal Steroidogenesis

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