Biochemical Changes In Peroxidase And Ascorbate In The Adrenal Of Rabbit During Pregnancy

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Abstract: 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 the level that would fail to reduce the free radical form of pregnenolone and and pregnenolone then is rapidly oxidized to progesterone.

Keywords: Adrenal, Peroxidase-Ascorbate System, Progesterone & Pregnancy

I. INTRODUCTION

ACTH has as 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). 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). ACTH is also the known to cause depletion of adrenal ascorbate and cholesterol in the in 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. Since ascorbate is known to be a donor in peroxidase reaction, the possibility of peroxidase system being involved in the rapid depletion of AA during pregnancy has been studied. A preovulatory surge of progesterone in the systemic plasma of adrenal origin preceeding the display of behavioural estrous in rodents has been reported (Feder et al., 1968). Prolactin and LH are the chief components of the pituitary luteotrophic hormone which maintains pregnancy up to day 11, and thereafter the function is taken over by the placenta maintaining high levels of progesterone and estrogen, thus replacing for the LH requirement which drop after this period. Towards the end of pregnancy, withdrawal of placental luteotrophic hormone or the increased release of pituitary LH, leads to a fall in ovarian progesterone secretion and to a rise in secretion of 20α-dihydropregesterone just before the onset of parturition and initiates lactation. Progesterone is both an obligatory intra-adrenal substrate for corticosterone production and a steroid essential for maintenance of pregnancy. Thus, the regulation of adrenal steroidogenesis during pregnancy has two potentially important aspects:
✓ Maintenance of optimal blood levels of corticosterone
✓ Contributing significant amounts of progesterone to the total maternal pool. Since the extended luteotrophic function of ovary in rat and mice during pregnancy is related to the peroxidase-ascorbate system (Agrawal & Ladoraya, 1979), it appears likely that synthesis of progesterone under the action of ACTH during pregnancy may be controlled by a similar mechanism as reported for LH in the ovary, thus causing increased synthesis and secretion of the progesterone and corticosteroids from the gland. The changes in the endogenous levels of peroxidase in the adrenal gland during fertilization, implantation and gestation period alongwith the ascorbate content in rat have been studied.

II. MATERIAL & METHODS

Colony bred mature female white rabbits of our departmental Colony were caged individually in a controlled environment with a light-dark cycle of 14:10 hours. Water and food were supplied ad libitum. They were mated twice with different bucks of proven fertility followed by i.v. injection of 100 i.u. ofhuman chorionic gonadotropin (CG-5, Sigma Chemicals Co., USA) to induce ovulation and were designated...
as pregnant rabbits. The pregnant females were anesthetized by i.v. injection of Sodium pentobarbitone at various stages of pregnancy. The dissected tissues i.e., adrenals were stored at -20°C and then subjected to biochemical studies.

Circadian rhythm in the secretions of the hypothalamo-hypophyseal-adrenal axis has been reported by a large number of workers (Ganong, 1963; Critchlow, 1963; Critchlow et al., 1963). Therefore, it follows that comparable results can be obtained by examination of animals killed at the same time of the day. Hence, keeping this in view, all experimental animals were sacrificed at one fixed time.

III. BIOCHEMICAL ANALYSIS

TOTAL PROTEINS: was estimated by the method of Lowry et al. (1951) after proceeding for calibration of casein.

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).

IV. RESULTS

Peroxidase activity and ascorbate content in the adrenal of rabbit during different days of pregnancy are shown in Fig. 1. Peroxidase activity is first discernible on day 1 of pregnancy, and after a peak is reached on day 6, activity falls sharply on day 9. An increase in the activity of peroxidase is again seen on days 10 and 11, which thereafter falls sharply on day 12, being barely detectable only up to the day 17. The ascorbate content which is high on the day of estrous, progressively declined on day 1 & 2. A sharp decline is seen on day 4 & 5 and thereafter the levels of AA remain almost unchanged till day 11. The ascorbate content begins to recover on day 12 and recovered close to the initial value by day 19. Thus it appears that with the establishment of the maternal pregnancy, the peroxidase activity is very high in the adrenals and this is accompanied by a depletion in ascorbate content.

V. DISCUSSION

The presence of high peroxidase activity, and the following depletion of ascorbate, during days 5-11 of pregnancy in the adrenal correlates well with stimulated steroid biogenesis during this period. 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 found that ascorbate promotes the activity of of cholesterol side-chain cleaving enzyme complex at lower concentration while inhibiting the same at high concentration (Sulimovici and Boyd, 1969). At low concentration the peroxidase mediated conversion of pregnenolone to progesterone stimulated in the presence of ascorbate in the rat and rabbit ovarian tissue has been demonstrated (Agrawal and Laloraya, 1977; Agrawal and Harper, 1982). A similar mechanism appears to operate in the adrenal gland. Since the 14C labeled ascorbate is not known to be incorporated into steroid hormones, though it does seem to regulate steroidogenesis (Datta and Sanyal, 1975), the induction of peroxidase by ACTH in the cortex of adrenal strongly suggests that the oxidation to the dehydroform, monodehydro-ascorbate MDHA), may indeed be involved as the first step in this process. That ascorbate begins to accumulate in the adrenal tissue as peroxidase activity starts to decline around 2 hrs. after ACTH injection, indicates that oxidation of ascorbic acid and its biosynthesis may be going on simultaneously but because of high rate of oxidation of ascorbate in presence of peroxidase, a rapid depletion is obtained. There are several other enzymes that can cause oxidation of ascorbate, namely ascorbate oxidase.
lactase and polyphenol oxidase. However, these enzymes have been found to be lacking in the adrenal of rat.

**Figure 1: Postulated mechanism of peroxidase in luteal Steroidogenesis**

**REFERENCES**


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