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Induction of Peroxidase in Corpora Lutea of Rat Ovary by Lutropin

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The lutropin-induced depletion of ascorbate in corpora lutea of albino-rat ovary is shown to be associated with the induction of peroxidase in corpora lutea. An inverse relationship between ascorbate depletion and peroxidase activity was established in a time-course study with lutropin. Analyses made at different phases of the reproductive cycle are in accord with this relationship. It is suggested that ascorbate, which is a well-established donor in peroxidase reactions, undergoes rapid oxidation in the presence of this enzyme, producing an intermediate free radical which, if coupled with pregnenolone, might produce progesterone in the corpora lutea. The exact role of peroxidase in steroidogenesis, however, remains to be elucidated and established.

Lutropin (luteinizing hormone), is specifically known to cause depletion of ovarian ascorbate in the pseudopregnant rat (Parlow, 1958, 1961). This is reported to occur in corpora lutea within minutes of lutropin injection and exhibits a characteristic time sequence (Goldstein & Sturgis, 1961). The biochemical mechanism of this depletion and its physiological significance in luteal functions remain obscure. Rhythmic changes in ascorbate content during the oestrous cycle (Deane, 1952; Foreman, 1963) have also been reported, but the regulatory mechanism remains to be elucidated.

The present paper describes the lutropin-induced changes in the enzyme peroxidase, which is known to oxidize ascorbate, acting as direct donor (Yamazaki & Souzu, 1960), or in coupled oxidations with other donors (Laloraya *et al.*, 1972).

Materials and Methods

Colony-bred albino rats (Wistar strain) maintained under laboratory conditions were used in the present study. The reproductive stage was identified by examination of vaginal smears. The mature female rats used for the study showed a regular 4–5 days oestrous cycle.

For experiments with lutropin, immature female rats (50–60 days old), were made pseudopregnant by vaginal cervical stimulation, by mating them with mature proven males (De Feo, 1966). Pseudopregnancy, which lasted 10–12 days, was verified by studying vaginal smears. Confirmed pseudopregnant rats were injected with 25i.u. of human chorionadotropin (1i.u. is equal to 1.3μg of a standard preparation) and were used after 48h for studying the effect of lutropin.

Purified bovine lutropin (NIH-LH-B9; 20μg), dissolved in 1.0ml of 0.9% NaCl, was administered to the rats in a single dose by subcutaneous injection. Rats were killed at different periods by cervical dislocation, dissected and the ovaries free of adhering fat were used for analysis. They were weighed quickly and processed for the enzyme assay or the determination of ascorbate. Of the two ovaries in each animal, the left ovary was analysed for peroxidase and the right ovary for ascorbate.

Peroxidase activity was measured with guaiacol as donor by the method of Maehly & Chance (1954). The ovary was quickly frozen at solid-CO_2 temperature, homogenized with 5ml of 0.2M-sodium acetate/acetic acid buffer, pH5.0, in a ground-glass homogenizer at 0–4°C and suspended at the same temperature for 30min before use for enzyme assay. This allowed complete extraction of the enzyme. The extract was centrifuged at 3000*g* for 10min and the supernatant was used as enzyme.

The reaction mixture for determination of peroxidase activity consisted of 2ml of 0.2M-sodium acetate/acetic acid buffer, pH5.0, 1ml of aq. 0.22mM-guaiacol and 2ml of enzyme extract. The activity was measured by recording the time taken for a fixed absorption change (0.1) in the Klett Summerson colorimeter, by using a blue filter (400–465nm), after initiating the reaction by the addition of 0.02ml of 10-volume H_2O_2 to the reaction mixture. A blank was run by preparing the reaction mixture as above but without H_2O_2. During the time of measurement ranging between 10 and 30s this was negligible.

Relative changes in peroxidase activity are expressed as absorption changes/min per fresh wt. of the ovary.

Ascorbate was determined by the colorimetric

method of Mindlin & Butler (1938) by following the decolorization of 2,6-dichlorophenol-indophenol in metaphosphoric acid. The ovaries were crushed in a ground-glass homogenizer in 5ml of cold buffered metaphosphoric acid, pH3.6, at 0–4°C, centrifuged at 3000*g* for 10min and the supernatant used for the reduction of the dye, observed by using a green filter (500–570nm) in the Klett Summerson colorimeter. Values are expressed as mg of ascorbate per g fresh wt. of ovary.

Results

Fig. 1 shows changes in ascorbate and peroxidase activity with time after lutropin treatment. As the depletion of ascorbate ensues within 15min after lutropin injection, the peroxidase activity tends to increase. A peak in peroxidase activity is observed between 2 and 3h after lutropin injection, when the ascorbate content is the lowest. Peroxidase activity falls rapidly at 4h when the ascorbate content begins to recover. At 24h, when ascorbate content has returned close to the initial value, the peroxidase exhibits low activity. An inverse relation between peroxidase activity and ascorbate content is clearly evident.

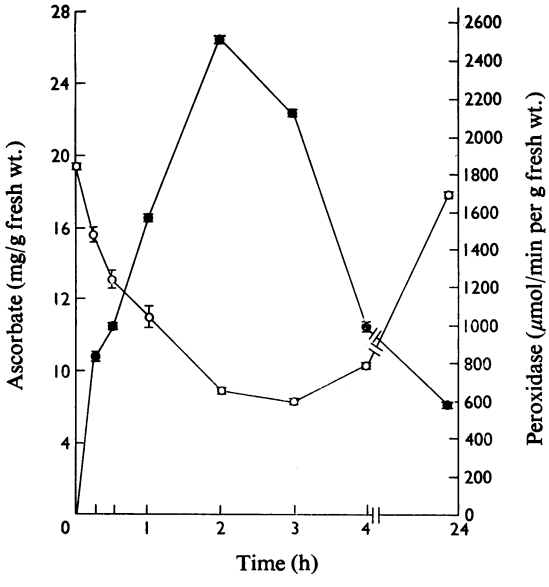

Fig. 1. *Lutropin-induced changes in ascorbate and peroxidase in immature pseudopregnant rats*
The Figure demonstrates changes in rat ovarian ascorbate concentration and peroxidase activity that take place within 24h of subcutaneous injection of lutropin. Rats were killed at 15 and 30min and 1, 2, 3, 4 and 24h after lutropin injection; control rats received only NaCl and were killed at the same time intervals. Experimental details are given in the Materials and Methods section. Values are the means±S.D. for five animals. Peroxidase activity was calculated by the method of Maehly & Chance (1954). ●, Peroxidase; ○, ascorbate.

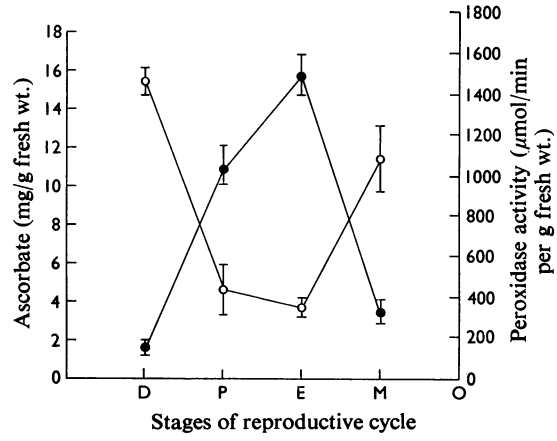

Fig. 2. *Changes in ascorbate and peroxidase activity during different stages of reproductive cycle of albino rats*
Mature female rats showing a regular 4- or 5-day oestrus cycle were used for the study and the stages of the oestrus cycle were determined by vaginal smear. Experimental details are given in the Materials and Methods section. Values are the means±S.D. for five animals. ●, Peroxidase; ○, ascorbate; D, dioestrus; P, proestrus; E, oestrus; M, metoestrus.

Fig. 2 shows changes in peroxidase activity and ascorbate content at different stages of the reproductive cycle. A similar correlation is seen. At oestrus the peroxidase activity is highest and the ascorbate content is very much depleted. At dioestrus ascorbate content is high and peroxidase activity is very low.

Discussion

The depletion of ovarian ascorbate by lutropin has been utilized as a sensitive bioassay of lutropin (Parlow, 1958, 1961) and ascorbate changes in the rat ovary during the oestrous cycle have been investigated by many (Deane, 1952; Foreman, 1963). There is general agreement that ascorbic acid is at its lowest concentration just before oestrus and reaches its peak at dioestrus. Our work on cyclic changes in ascorbate during oestrus confirms these earlier reports. Goldstein & Sturgis (1961) showed that depletion of ascorbate with lutropin in pseudopregnant immature female rats exhibits a characteristic time relationship; the depletion sets in within 15min of lutropin injection, reaches a point of maxi-

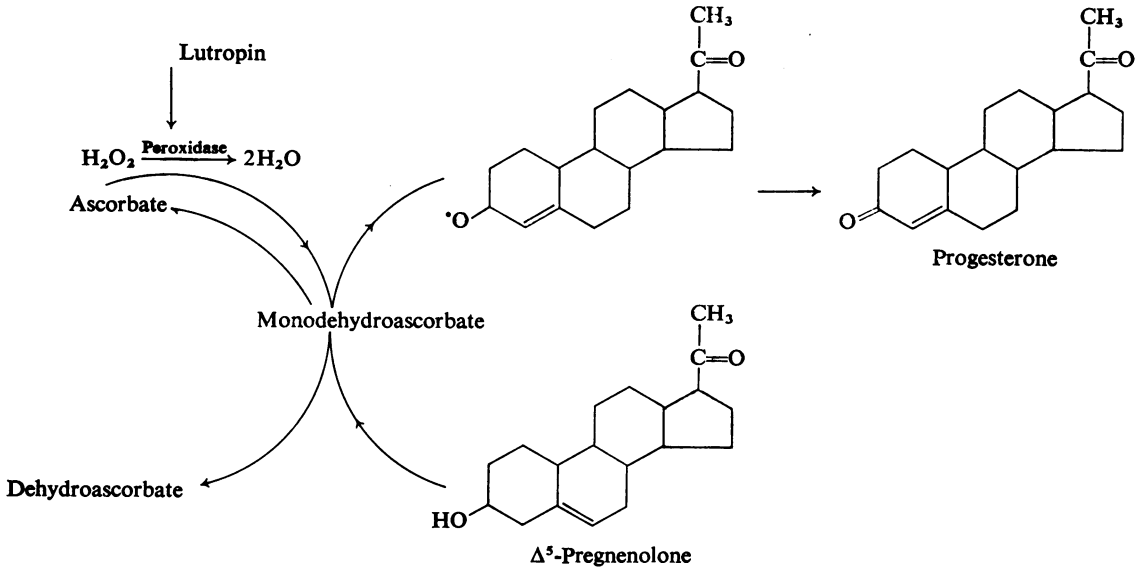

Fig. 3. *Postulated mechanism of peroxidase in luteal steroidogenesis*
Lutropin-mediated depletion of ascorbate is brought about by the induction of peroxidase enzyme, ascorbate acting as a direct donor. The free radical of ascorbate may trigger oxidation of pregnenolone through a free-radical mechanism, thus bringing about rapid formation of progesterone accompanying luteal steroidogenesis.

mum depletion at about 3 h, after which the ascorbate concentration starts to increase again, showing a rebound at about 20 h and attaining normal values at 24 h. Goldstein & Sturgis (1961) had suggested that the depletion of ascorbate by lutropin could be brought about by its release from the ovary via venous blood, by actual utilization in the process of steroidogenesis or by oxidation to the dehydro form. Although there is no evidence yet for the first two possibilities, and ^{14}C-labelled ascorbate is not known to be incorporated into steroid hormones, though it does seem to regulate steroidogenesis (Datta & Sanyal, 1975), the induction of peroxidase by lutropin in the corpora lutea of the ovary strongly suggests that the oxidation to the dehydro form may indeed be involved as the first step in this process. The inverse correlation obtained with ascorbate and peroxidase activity (highest peroxidase activity at 3 h after lutropin injection, when ascorbate is lowest) lends strong support to this view. That ascorbate begins to accumulate in the ovarian tissue as peroxidase activity starts to decline indicates that oxidation of ascorbate and its biosynthesis may be going on simultaneously, but because of the high rate of oxidation of ascorbate in the 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, no study has yet been made on the changes in activities of these enzymes during lutropin-induced depletion of ascorbate, and more work is needed along these lines to throw light on the exact nature of biochemical events associated with the depletion of ascorbate.

Another important biochemical event that takes place under the action of lutropin is marked synthesis of progesterone in the corpora lutea. The biochemical mechanism leading to this change is also unknown. The possibility that the free radical of ascorbate formed by the action of peroxidase may trigger the oxidation of pregnenolone to progesterone in corpora lutea (Fig. 3) is strongly indicated, although it is still an open question. It has been shown, however, by McNabb *et al.* (1975) that the presence of phenolic compounds activates uterine peroxidase-dependent oxidation of oestradiol and converts the latter into soluble products. The suggestion has also been made that the free radical of oestradiol may be an intermediate in the reaction. Linked oxidation of ascorbate in peroxidase-mediated reactions have been shown earlier (Laloraya *et al.*, 1972; Yamazaki & Souzu, 1960), and whether or not this is involved in the oxidation of pregnenolone to progesterone requires study.

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References

Deane, H. W. (1952) *Am. J. Anat.* **91**, 363–413
Datta, S. & Sanyal, S. (1975) *Abstr. All India Symp. Comp. Anim. Physiol.* p. 76
De Feo, V. J. (1966) *Endocrinology* **79**, 440–442
Foreman, D. (1963) *Endocrinology* **72**, 693–700
Goldstein, D. P. & Sturgis, S. H. (1961) *Am. J. Physiol.* **201**, 1053–1056
Laloraya, M. M., Shrivastav, H. N., Bakshi, N., Shah, N., Pandya, K. J. & Singh, Y. D. (1972) *Biol. Land Plants* 143–159
Maehly, A. C. & Chance, B. (1954) *Methods Biochem. Anal.* **1**, 357–424
McNabb, T., Sporoul, J. & Jellinck, P. H. (1975) *Can. J. Biochem.* **53**, 855–860
Mindlin, R. L. & Butler, A. M. (1938) *J. Biol. Chem.* **122**, 673–686
Parlow, A. F. (1958) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **17**, 402
Parlow, A. F. (1961) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **20**, 187
Yamazaki, I. & Souzu, H. (1960) *Arch. Biochem. Biophys.* **86**, 294–301