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INDUCTION OF ORNITHINE DECARBOXYLASE IN RAT OVARY AFTER ADMINISTRATION OF LUTEINIZING HORMONE OR HUMAN CHORIONIC GONADOTROPHIN*

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Abstract—Ornithine decarboxylase in rat ovary is stimulated 5- to 10-fold by a subcutaneous injection of luteinizing hormone (LH) or human chorionic gonadotrophin (HCG). A peak is observed between 3 and 5 hr after which the enzyme activity declines rapidly. The pattern of enzyme response to hormonal administration is similar to that observed during late proestrus. *S*-adenosylmethionine decarboxylase is not altered during this period or after administration of LH or HCG. Cycloheximide administered *in vivo* markedly reduced ovarian ornithine decarboxylase within 15 min, suggesting that the turnover of this enzyme in the ovary is very rapid. When injected immediately prior to HCG, cycloheximide also prevented the rise in ornithine decarboxylase activity. High doses of actinomycin D were also effective in blocking the response to HCG. Ornithine decarboxylase responds to a single injection of HCG at all stages of the estrus cycle, but repeated injections do not prevent the decline in enzyme activity occurring between 5 and 7 hr. Anti-HCG injected immediately before the primary hormone completely inhbited the rise in ornithine decarboxylase, but when given 1 hr after the primary injection, it was ineffective. It is suggested that these characteristics of ornithine decarboxylase, i.e. high rate of turnover, rapid induction and the inability of repeated hormone injections to prevent the eventual decline of enzyme activity, ensure that the increase in the formation of putrescine during proestrus is restricted to a relatively narrow time period, thus supporting the view that putrescine may have a specific role in the regulation of protein and RNA synthesis involved in the early phase of LH action on the ovary.

ORNITHINE decarboxylase, the enzyme catalyzing the formation of putrescine, is a key factor in the biosynthesis of the polyamines spermidine and spermine.[1–3] The enzyme is widely distributed in tissues, and high levels are found in the regenerating rat liver,[1–3] in certain tumors[2] and in several tissues in response to hormonal administration.[4,5] Many of these studies have sought to establish a relationship between polyamines and nucleic acids but their precise role has not been established.

Earlier reports from our laboratory demonstrated that ornithine decarboxylase in the rat ovary was markedly raised during late proestrus and suggested that the enzyme in this tissue was regulated by luteinizing hormone,[6,7] The present experiments provide further evidence in support of this contention and highlight some of the interesting characteristics of this enzyme system.

MATERIALS AND METHODS

Animals. Female Sprague–Dawley rats (Charles River Co.) weighing between 150 and 180 g were used in all experiments. The animals were housed in light and temperature regulated quarters and were fed a diet of commercial rat chow and water *ad lib.*

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The rats were followed through three consecutive estrus cycles, and only those exhibiting a 4-day cycle were used. Drugs and hormones were injected subcutaneously unless otherwise indicated.

Chemicals and hormones. DL-(1-^{14}C)ornithine monohydrochloride (sp. act. 1·0 mCi/m-mole) and *S*-adenosyl-L-[1-^{14}C]methionine (0·54 mCi/m-mole) were purchased from the New England Nuclear Co. Ovine luteinizing hormone NIH-LH-B6 was a gift from NIAMD. Human chorionic gonadotrophin was a gift from Ayerst Laboratories, Montreal, and the anti-HCG from Dr. R. B. Snook of Cornell University. Actinomycin D and cycloheximide were obtained from CalBiochem.

Ornithine decarboxylase activity. Ornithine decarboxylase was assayed by measuring the liberation of $^{14}CO_2$ from carboxyl-labeled substrate as described by Russell and Snyder.[2] Minor modifications included carrying out the reaction in a Warburg flask and trapping the evolved carbon dioxide on Hyamine-impregnated filter paper as described previously for histidine decarboxylase.[8] The assay medium contained 100 mg homogenized tissue, 0·2 μmole pyridoxal phosphate, 0·1 M phosphate buffer, pH 7·2, in a final volume of 2 ml. Incubation was carried out in air at 37° for 30 min. The reaction was stopped with 0·2 ml of 1 M citric acid injected through a rubber septum, and the flasks were shaken for an additional 30 min. The filter paper was transferred to a counting vial containing 3 ml ethanol and 7 ml toluene (0·4 per cent of 2,4-diphenyloxazole and 0·01 per cent of *p*-bis-(*o*-methylstyryl)benzene and assayed for radioactivity in a liquid scintillation spectrometer. Enzyme activity is expressed as nmoles CO_2/g of tissue/hr.

Assay of S-adenosyl-L-methionine decarboxylase. The activity of this enzyme was measured by the rate of $^{14}CO_2$ evolved from carboxyl-labeled *S*-adenosyl-L-methionine. The procedure was identical to that for the assay of ornithine decarboxylase except that the incubation mixture contained 0·2 μmole *S*-adenosyl-L-(1-^{14}C)-methionine in place of the labeled ornithine and 2·5 μmoles putrescine was added.

RESULTS

Rat ovarian ornithine decarboxylase activity between 5:00 and 10:00 p.m. on each day of the estrus cycle is illustrated in Fig. 1. During late proestrus, there is a sharp rise in ornithine decarboxylase activity with a peak usually occurring between 6:00 and 9:00 p.m. By 10:00 p.m., the activity is returning to control levels. Previously, we have shown that ornithine decarboxylase in the ovary responds to luteinizing hormone (LH) or human chorionic gonadotrophin (HCG), and not to a variety of other hormones such as estradiol or progesterone.[7] The time course of response to 100 μg/kg of LH and 50 units/kg of HCG injected at 9:00 a.m. on proestrus is shown in Fig. 2. Both these doses produced maximal stimulation of ornithine decarboxylase. The salient features of the response are the rapid rise in enzyme activity reaching about five times the basal levels in 4 hr followed by a sharp decline. In most of these experiments, the peak in enzyme activity was reached between 3 and 5 hr and by 6–8 hr the enzyme activity was returning rapidly to basal levels. The pattern of enzyme response after hormone administration is, therefore, similar to that observed during late proestrus. *S*-adenosylmethionine decarboxylase was also determined in these animals. The activity of this enzyme in the ovary is very much less than that of ornithine decarboxylase. In control animals, the activity was $0{\cdot}75 \pm 0{\cdot}17$ nmole g^{-1} hr^{-1}

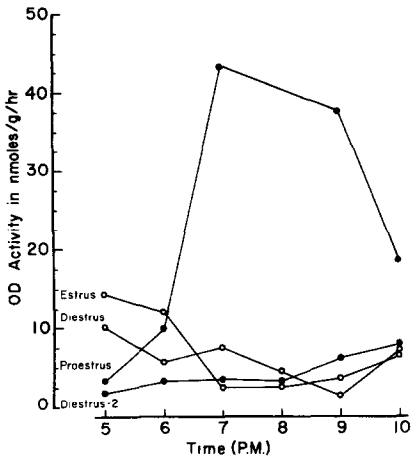

FIG. 1. Ornithine decarboxylase activity in the ovary at different stages of the estrus cycle. Each point is the mean of four rats.

(n = 5) and 4 hr after HCG the values were 0·70 ± 0·23 nmole g^{-1} hr^{-1}. Similar values were obtained 2 or 6 hr after HCG administration.

To ascertain whether the decline in ornithine decarboxylase occurring between 5 and 7 hr after hormone administration was due to the decay of the stimulus *in vivo*, repeated injections of HCG were given at 2-hr intervals. Groups of animals were killed 1 hr after the second injection or 1 hr after the third injection, and the decline in ornithine decarboxylase between these two time periods was compared with that observed in animals given a single dose of HCG. As shown in Table 1, repeated injections of HCG do not prevent the decline in enzyme activity.

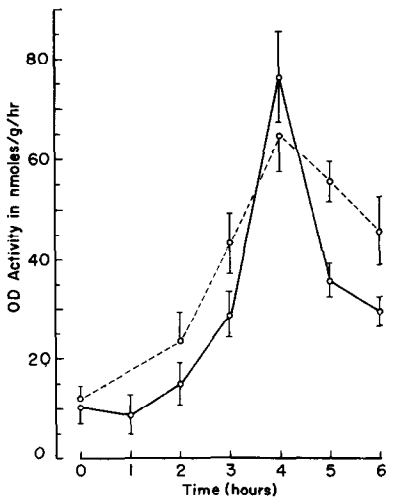

FIG. 2. Stimulation of ornithine decarboxylase in rat ovary by LH(○—○) and by HCG (○---○). Each point is the mean of four rats.

TABLE 1. EFFECT OF REPEATED DOSES OF HCG ON OVARIAN ORNITHINE DECARBOXYLASE*

Treatment	Ornithine decarboxylase activity (nmoles g^{-1} hr^{-1} ± S. E. M.)	
	4 hr	7 hr
Controls, no hormone	7·10 ± 1·21	4·90 ± 1·27
Single injection HCG	78·05 ± 7·83	51·50 ± 4·95
Repeated injections	63·15 ± 4·25	42·05 ± 2·30

* Rats at 9:00 a.m. on the morning of proestrus were injected with 50 units/kg of HCG. One group was given repeated doses every 2 hr. There were five animals per group.

These experiments were carried out on rats at 9:00 a.m. in the morning of proestrus. The failure of HCG (and also LH) to maintain ornithine decarboxylase raised the question whether the enzyme would respond to stimulation only when animals were in proestrus. Animals at other stages of the cycle were injected with HCG and, as shown in Table 2, there is, in all cases, a substantial increase in ornithine decarboxylase 4 hr later. The ability of the enzyme to respond to hormonal treatment is not, therefore, dependent upon the stage of the estrus cycle.

When anti-HCG (0·2 ml) was given 5 min before a maximum dose of HCG, the rise in ornithine decarboxylase was prevented. Graded inhibition was obtained when anti-HCG was given 20 or 40 min after hormonal treatment. When anti-HCG was given 60 min after HCG, the response of ornithine decarboxylase was indistinguishable from that obtained when HCG was given alone (Table 3). These results yield a half-life for HCG stimulation of ornithine decarboxylase of approximately 30 min.

Intraperitoneal injection of cycloheximide (10 mg/kg) or actinomycin D (1mg/kg) 5 min before injecting HCG markedly impaired the response to the stimulant hormone (Table 4). When the inhibitors were given 90 min after HCG administration, cycloheximide was still effective in preventing the rise in ornithine decarboxylase, whereas actinomycin D was without effect (Table 4). Cycloheximide alone produced a sharp reduction in ornithine decarboxylase within a few min, suggesting a rapid

TABLE 2. STIMULATION OF ORNITHINE DECARBOXYLASE BY HCG AT DIFFERENT STAGES OF THE ESTRUS CYCLE*

Stage of cycle	Ornithine decarboxylase activity (nmoles g^{-1} hr^{-1} ± S. E. M.)	
	Control	HCG
Proestrus	5·43 ± 1·35	31·63 ± 12·23
Estrus	11·28 ± 0·75	33·9 ± 7·63
Diestrus	7·75 ± 2·18	26·9 ± 3·78
Diestrus	3·30 ± 0·53	20·8 ± 0·80

* Rats were injected at 9:00 a.m. with HCG (50 units/kg) and ovarian ornithine decarboxylase was determined 4 hr later. There were five animals per group.

TABLE 3. INHIBITION OF HCG (50 units/kg)-STIMULATED ORNITHINE DECARBOXYLASE BY ANTISERUM (0·2 ml)*

Treatment	Ornithine decarboxylase activity (nmoles g^{-1} hr^{-1} ± S. E. M.)
Control	13·5 ± 2·58
HCG	95·53 ± 17·65
Anti-HCG given 5 min before HCG	10·08 ± 2·48
Anti-HCG give 20 min after HCG	28·40 ± 10·33
Anti-HCG given 40 min after HCG	52·73 ± 7·00
Anti-HCG given 60 min after HCG	93·98 ± 20·08

* Rats were injected at 9:00 a.m. on the morning of proestrus. Animals were killed 4 hr after administration of HCG. Each value is the mean of five animals.

turnover of the enzyme in this tissue (Table 5). Since the objective of this experiment was to monitor a decrease in enzyme activity, estrus rats were used because the activity at 9:00 a.m. is highest at this stage of the cycle.

DISCUSSION

The increase in ovarian ornithine decarboxylase with maximum doses of HCG (or LH) was similar both qualitatively and quantitatively to that observed during late proestrus, suggesting that the physiological response of the enzyme is also maximal. The enzyme is rapidly inducible with a high rate of turnover and, in this regard, is similar to that previously described in regenerating rat liver.[9] The functional half-life of HCG in relation to stimulation of ornithine decarboxylase in the ovary is about 30 min, which corresponds approximately to the plasma $T_{1/2}$ of LH.[10] Since LH is released primarily between 3:00 and 6:00 p.m.[10–13] and ornithine decarboxylase is maximal between 6:00 and 9:00 p.m., it is probable that the enzyme responds to the early phase of LH released from the pituitary. It is also clear that, during late proestrus, LH is still present in the plasma in substantial concentrations, when ornithine decarboxylase activity is declining rapidly. After the initial stimulation, therefore, there is a refractory period, and this is supported by the fact that repeated doses of HCG did not prevent the decline in enzyme activity occurring between 5 and 7 hr

TABLE 4. EFFECT OF ACTINOMYCIN D (1 mg/kg, INTRAPERITONEAL) AND CYCLOHEXIMIDE (10 mg/kg, INTRAPERITONEAL) ON THE STIMULATION OF ORNITHINE DECARBOXYLASE IN RAT OVARY BY HCG (50 units/kg, SUBCUTANEOUSLY)

Treatment	Ornithine decarboxylase activity (nmoles g^{-1} hr^{-1} ± S. E. M.)	
Control, saline	6·45 ± 1·33	(9)
HCG	46·45 ± 6·30	(6)
HCG + actinomycin D	16·08 ± 3·68	(5)
HCG + cycloheximide	0·73 ± 0·38	(6)
HCG + actinomycin D*	41·70 ± 14·10	(3)
HCG + cylcoheximide*	3·00 ± 1·50	(3)

* In these experiments, the inhibitors were given 90 min after HCG. All animals were killed 4 hr after injection.

TABLE 5. INHIBITION OF ORNITHINE DECARBOXYLASE IN RAT OVARY BY CYCLOHEXIMIDE*

Time	Ornithine decarboxylase activity (nmoles g^{-1} hr^{-1} ± S. E. M.)
Control	13·63 ± 2·43
15 min	6·45 ± 1·80
30 min	4·63 ± 1·53
4 hr	0·83 ± 0·38

* Estrus rats were injected intraperitoneally with 10 mg/kg of cycloheximide. Each value is the mean of four animals.

after the initial stimulation. Preliminary evidence indicates that this refractory period during late proestrus extends up to the time of ovulation (in our animals some 12–18 hr after LH release), and while the responsiveness to HCG is restored by 9:00 a.m. on the morning of estrus, we have not yet established the earliest time after ovulation at which the response of ornithine decarboxylase to hormonal stimulation is recovered.

The difference in the levels of activity between *S*-adenosylmethionine decarboxylase and ornithine decarboxylase suggests that the ovary represents another example of a tissue where the putrescine-forming capacity of the hormonally stimulated tissue is greatly in excess of the capacity of the tissue to convert it to spermidine. Williams-Ashman *et al.*[14] have shown that spermidine levels in the ovary are not altered at the time that ornithine decarboxylase is markedly enhanced. While the differences in *S*-adenosylmethionine and ornithine decarboxylase activities are not as marked as in the placenta,* the possibility must be raised that putrescine may have additional roles to being simply a precursor for spermidine. That is, putrescine may itself be involved, in some tissues, in the regulation of cellular activities.

The characteristics of ornithine decarboxylase described above, i.e. rapid inducibility, maximum response and refractiveness, ensure that the increased formation of putrescine occurring during proestrus is restricted to a relatively narrow time period. This temporal element is undoubtedly important in determining the biological role of the polyamines. As Cohen[15] has pointed out, these amines could play a role in almost every step in the synthesis of RNA and protein, and the dilemma is which, if any, of the many possible actions are actually manifest in the intact cell. Specificity within the intact cell is only partially determined by the molecular configuration and ionic deposition. Other restraints must apply *in vivo* of which the temporal and spatial factors are pre-eminent, i.e. when and where these substances are made in relation to other cellular events. In the case of LH stimulation of the ovary, the pattern of ornithine decarboxylase broadly correlates with increased RNA and protein synthesis involved in the early phase of LH action.[16] We have not yet established, however, that the changes in ornithine decarboxylase rigorously correlate with changes in putrescine formation in the intact cell.

Studies on the molecular mechanisms of hormone action have indicated two general patterns. One applies to the polypeptide hormones which act primarily through activation of adenyl cyclase with the subsequent formation of cyclic AMP,[17] and the other applies to the steroid hormones which act primarily at the gene locus

* D. V. Maudsley and Y. Kobayashi, manuscript submitted for publication.

to initiate the synthesis of specific species of RNA.[18] The hormonal stimulation of ornithine decarboxylase or *S*-adenosylmethionine decarboxylase is not so easily delineated, since each enzyme is known to be modified by both types of hormone. Ornithine decarboxylase is stimulated by growth hormone in the liver,[2] LH in the ovary,[7] estradiol in the rat uterus and chick oviduct,[5] testosterone in the prostate[19] and ACTH in the adrenal.[20,21] *S*-adenosylmethionine decarboxylase is elevated by growth hormone in liver,[22] by estradiol in the uterus,[23] and by androgen treatment in the prostate.[19] The pattern of response is also similar for the two types of hormones; for example, our results on the LH stimulation of ornithine decarboxylase in the ovary are very similar to those of Cohen *et al.*[5] on the estrogenic stimulation of the same enzyme in the uterus. This similarity has also been pointed out by Kaye *et al.*,[24] and these workers also showed that actinomycin D and cycloheximide prevented the response of ornithine decarboxylase to LH administration. However, caution in interpreting the results with actinomycin D has been advocated by several workers,[25,26] in part, on the grounds that the doses required to inhibit ornithine decarboxylase are in excess of those required to inhibit RNA synthesis. In some systems, therefore, ornithine decarboxylase may be regulated primarily at the translational level. It is tempting to speculate, therefore, that in the link between the initial stimulation, i.e. hormone–receptor interaction, and the final response of hormonally sensitive systems part of the coupling process is similar for both polypeptide and steroid hormones, and further that this sequence is manifest at the translational level and may be independent of effects upon transcription.

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