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OESTRADIOL INDUCED RESPIRATORY AND ENZYMIC CHANGES IN RAT UTERUS

R. E. SHEPHERD and R. S. SNART
Department of Zoology, The University, Sheffield S10 2TN, England

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SUMMARY

We have studied the *in vitro* effect of oestradiol-17β on the oxygen consumption, succinate dehydrogenase and tyrosine amino transferase activity in rat uteri taken from female rats at the dioestrous stage of the oestrous cycle and from immature females. These results are compared with the enzyme activities measured in uteri taken from rats at various stages of the oestrous cycle. The results confirm a single receptor mechanism of action of oestradiol-17β in rat uterus and a comparison has been made with the two receptor control of the same enzyme systems by corticosterone in rat liver.

INTRODUCTION

A CONSIDERABLE amount of work has been carried out on oestradiol-17β binding in rat uterus [1, 2] which has demonstrated the process by which steroids enter target cells, how the hormone is transported to the nucleus and becomes attached to a non-histone protein of the chromatin [3]. It has been shown that in this tissue a single acidic receptor protein complex can be formed by addition of oestradiol-17β to uterine cytosol. This large cytoplasmic receptor was shown to readily dissociate into sub-units and appears to interact with the nucleus to form a nuclear receptor unit of intermediate size. The nuclear uptake of the hormone has been shown to be metabolically controlled [4]. It was clear that there was no receptor in the nucleus before hormone action and it has been shown that the cytosol binding disappears or loses its ability to bind following hormone treatment. In purified nuclei obtained from calf uterus not having been exposed to oestradiol-17β a specific protein binding system of high affinity for oestradiol has been found in the acidic protein fraction of a chromatin preparation [5]. An acidic receptor-hormone complex could serve as a derepressor in the transcriptional control of protein synthesis, though recent work by Barry and Gorski [6] and Mohla *et al.* [7] indicates that activation of RNA polymerase activity occurs as part of the mechanism of oestrogen action in the uterus.

Our studies on aldosterone binding in toad bladder [8] and corticosterone in rat liver [9] have shown the presence of two steroid receptors in contrast to the single receptor found for oestradiol-17β binding in rat uterus. Our protein characterization work [10] leads us to conclude that the role of two receptors in the mechanism of steroid hormone action may allow control of protein synthesis at the transcriptional and translational level. In the case of corticosterone binding in rat liver [11] two physiologically active receptor proteins identified in our studies of corticosterone binding in rat liver [9] are believed to be separately associated with hormonal effects on amino acid and fat metabolism during gluconeogenesis [11].

The physiological activity of these two binding sites was demonstrated by the dose-response characteristics of the hormone-stimulated oxygen consumption in the tissue[11] and by the maximum stimulation of succinate dehydrogenase activity with 10^{-9}M corticosterone and maximum stimulation of tyrosine transaminase activity with 10^{-7}M corticosterone.

In the present work we have examined the effect of oestradiol-17β on the respiratory and enzymic activities of rat uteri taken from rats at the di-oestrus stage of the oestrus cycle and from immature rats. These results are compared with the enzyme activities measured in uteri taken from rats at the di-oestrus, pro-oestrus and oestrus stage of the cycle.

EXPERIMENTAL

Adult Wistar rats shown by daily vaginal smears to have a regular oestrous cycle have been used for measurement of tyrosine amino transferase and succinate dehydrogenase activity in homogenates of uteri removed at different stages of the oestrous cycle. Before each experiment, rats were tested in order to determine the stage of the cycle. The rats were killed by a blow on the head and the uteri quickly excised. Excess fat and connective tissue was removed and the two uteri were homogenised in Krebs–Ringer solution (5 ml). Homogenates were then used for determination of tyrosine amino transferase[12] and succinate dehydrogenase[13] activity, the total protein being determined by the Lowry method and DNA using the method described by Burton[14]. The experiment was repeated six times for animals at oestrus, di-oestrus and pro-oestrous.

Oestradiol-17β stimulation of tyrosine amino transferase and succinate dehydrogenase activity was measured in uteri taken from immature females and animals taken at di-oestrous. Three female rats were killed one day after oestrous. The uteri were excised, the excess fat and connective tissue was removed and each uterus cut into three pieces. These were incubated separately in Krebs–Ringer solution (5 ml) or the same volume of Ringer containing 10^{-7}M or 10^{-9}M oestradiol-17β. The incubation was carried out for 4 h at 37°C. The tissue was removed, homogenised and the activity of tyrosine amino transferase and succinate dehydrogenase was measured and the amount of protein determined. This experiment has been repeated.

In a similar experiment nine immature female rats (4–5 weeks old) were killed and the uteri excised. Excess fat was removed and the uteri were divided equally into three bathing media as described previously and incubated for 4 h at 37°C. The tissue was removed and the enzymes assayed. This experiment has been repeated three times.

Several female rats taken one day after oestrous were killed and the uteri excised, cut open and incubated in separate Warburg flasks pre-equilibrated at 37° in a Gilson respirometer. The flasks contained either 3 ml of Krebs–Ringer solution or 3 ml Krebs–Ringers solution containing 10^{-9}M or 10^{-7}M oestradiol-17β. The oxygen consumption was measured over a six hour period. The tissue in each flask was then dried overnight in order to obtain a dry weight. This experiment has been repeated.

RESULTS

The effect of 10^{-9}M and 10^{-7}M oestradiol-17β on the respiration of rat uterus increased after a lag period of about 90 min. and reached a maximum after 4 h (Table 1). The doses of hormone were chosen as suitable for saturation of the

Table 1. Shows the oxygen consumption ($Q_{O_2} = \mu l O_2$/mg protein/h) measured in rat uteri, given as the mean of 8–10 determinations ±S.D. in the presence and absence of 10^{-9}M or 10^{-7}M oestradiol

Treatment	1st hour	2nd hour	3rd hour	4th hour	5th hour	6th hour
Control	0·99 ± 0·10	0·97 ± 0·10	0·96 ± 0·09	1·08 ± 0·06	1·49 ± 0·08	1·95 ± 0·19
(10^{-9}M oestradiol)	1·04 ± 0·11	1·10 ± 0·08	1·57 ± 0·08	3·14 ± 0·14	5·33 ± 0·19	4·67 ± 0·45
(10^{-7}M oestradiol)	0·81 ± 0·05	0·91 ± 0·09	1·56 ± 0·08	2·96 ± 0·08	5·66 ± 0·16	5·51 ± 0·20

physiological receptor (K = 10^{10} 1/mole) identified in binding studies but sufficiently different to distinguish the possibility of two receptors similar to those characterised in toad bladder or rat liver[9, 10]. There is no significant difference between the maximum oxygen consumption stimulated by 10^{-9}M and 10^{-7}M oestradiol.

The oestradiol-17β stimulation of succinate dehydrogenase measured in rat uteri homogenates prepared from immature and di-oestrous female rats (Table 2) has similarly been studied at 10^{-9}M and 10^{-7}M oestradiol. The results show an increase in enzyme activity measured 4 h after addition of the hormone. There was no significant difference between the maximum enzyme stimulated by 10^{-9}M and 10^{-7}M oestradiol-17β. A similar observation is noted for tyrosine amino transferase activity in the same homogenates (Table 3). In a final experiment the succinate dehydrogenase activity and tyrosine amino transferase activities in homogenates of rat uteri taken from animals at the di-oestrous, pro-oestrous and oestrous stage of the reproductive cycle have been compared (Fig. 1). The results show that the succinate dehydrogenase activity corresponds to peak concentration of circulating oestradiol[15]. However, the tyrosine amino transferase does not show the same *in vivo* correlation.

Table 2. Succinate dehydrogenase activity (E_{490}/mg protein/15 min) in uteri taken from immature and di-oestrus rats, measused 4–5 h after incubation in Krebs–Ringer solution in the absence and presence of 10^{-9}M and 10^{-7}M oestradiol

Immature	Control	10^{-9}M oestradiol	10^{-7}M oestradiol
Expt. 1 (4 h)	0·87 ± 0·03	1·06 ± 0·05	1·09 ± 0·05
Expt. 3 (4 h)	0·92 ± 0·05	1·06 ± 0·05	1·17 ± 0·05
Expt. 2 (5 h)	0·96 ± 0·02	1·21 ± 0·01	1·38 ± 0·07
Mean	0·92	1·11	1·21
Di-oestrus			
Expt. 1 (4 h)	0·75 ± 0·03	0·89 ± 0·04	0·92 ± 0·02
Expt. 2 (5 h)	0·79 ± 0·02	0·98 ± 0·02	1·01 ± 0·01
Mean	0·77	0·94	0·96

Each experiment represents the mean ± S.D. for the enzyme assay on 6 samples of homogenate from each incubation using uteri from nine immature animals or from 3 di-oestrus animals.

Table 3. Tyrosine transaminase activity (μmoles p-hydroxyphenyl-pyruvate/mg protein/h) measured in uterine homogenates obtained under conditions corresponding to those described in Table 1

Immature	Control	10^{-9}M oestradiol	10^{-7}M oestradiol
Expt. 1 (4 h)	0·333 ± 0·003	0·056 ± 0·002	0·052 ± 0·003
Expt. 3 (4 h)	0·034 ± 0·003	0·052 ± 0·003	0·056 ± 0·002
Expt. 2 (5 h)	0·037 ± 0·004	0·066 ± 0·003	0·064 ± 0·003
Mean	0·035	0·058	0·057
Di-oestrus			
Expt. 1 (4 h)	0·032 ± 0·003	0·038 ± 0·003	0·039 ± 0·002
Expt. 2 (5 h)	0·021 ± 0·002	0·036 ± 0·003	0·038 ± 0·002
Mean	0·026	0·037	0·038

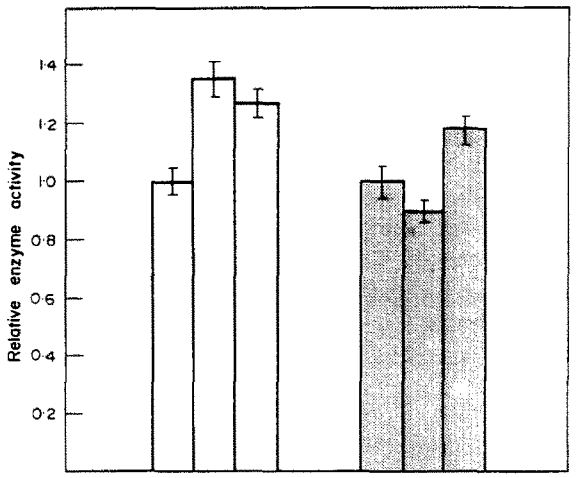

Fig. 1. Relative succinate dehydrogenase activity ±S.E.M. (unhatched) measured in homogenates of rat uteri taken in the order di-oestrous, pro-oestrous and oestrous and the relative tyrosine amino transferase ±S.E.M. (cross hatched) measured in the same homogenates. The mean values ±S.E.M. for the dehydrogenase activity in di-oestrous was 0·90 ± 0·04 (E490/mg protein/15 min) and for the tyrosine amino transferase was 0·039 ± 0·003 (μMp. hydroxyphenyl pyruvate/mg protein/h), these values on the relative scale are taken as unity.

DISCUSSION

The present work indicates a single receptor mechanism for stimulation of two key metabolic enzymes. We believe that a target tissue may be defined in terms of the specific binding and metabolic effects of the hormone. On this basis we take our respiratory and enzyme changes in rat uterus to confirm a single receptor mechanism for oestradiol, in contrast to the two receptor mechanisms outlined in our previous work on aldosterone stimulation of Na^+ transport across toad bladder and corticosterone activation of gluconeogenesis in rat liver [16]. In both of these tissues we have characterized the presence of a basic receptor protein [8, 10] which has a Kass value of the order 10^8 l/mole and may act as a repressor

in the control of post-transcriptional inhibitors. Corticosteroid binding to an acidic protein prepared from rat liver chromatin has been characterized[17] and Edelman [18] has described some characteristics of the aldosterone binding proteins isolated from rat kidney chromatin. It is tempting to suggest that an acidic receptor protein ($K = 10^{10}$ l/mole) may be common to the mechanism of steroid hormone action in toad bladder, rat kidney, uterus and liver and acts at the transcriptional level in the hormonal control of protein synthesis.

An important question arises as to the significance of one or two receptors in the mechanism of steroid hormone action. The single receptor mechanism providing control of protein synthesis at the transcriptional level could allow indution of all proteins associated with any physiological/biochemical effects of the hormones. However, control of protein synthesis at the translational level may be of importance when the physiological/biochemical process involves a rapid turnover of protein as part of the normal activity of the cell.

We have found two receptors for aldosterone binding in toad bladder and for corticosterone in rat liver[8, 10]. The role of two receptors in the mechanism of corticosterone action in rat liver may be associated with transcriptional and translational control of protein synthesis[19, 20]. It would therefore seem reasonable to suppose that the mechanism of action of aldosterone similarly involves these two levels of protein synthesis control. The stimulation of sodium transport across toad bladder might then be expected to show a differential sensitivity to inhibitors of protein synthesis at the transcriptional and translational level. It has been reported by Edelman *et al.*[21] that both actinomycin D and puromycin inhibit the aldosterone response. However, the results shown in this paper indicate that the effect of puromycin is considerably greater than the effect of actinomycin D which only partially inhibits the aldosterone response. Such differential effects of actinomycin D and puromycin favour the idea of separate effects of the hormone that contribute to the stimulation of Na+ transport and would be in keeping with the two stage nature of the aldosterone response described by Snart[22]. Further evidence in support of a possible post transcriptional control of protein synthesis following aldosterone treatment is suggested by the work described by Williamson[23] in which the aldosterone effect on K+ excretion is completely unaffected by actinomycin D.

Analysis of these systems is taken to suggest that the role of two receptors in the mechanism of steroid hormone action may be to allow control of protein synthesis at the transcriptional and translational level.

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