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The nuclear oestrogen receptor in the female rat

Effects of oestradiol administration during the oestrous cycle on the uterus and contrasting effects of progesterone on the uterus and hypothalamus

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Oestradiol administration to immature or ovariectomized rats has been reported to increase the uterine content of long-term nuclear oestrogen receptors. However, in the intact adult female rat, oestradiol administration did not increase the concentration of long-term nuclear oestrogen receptors at all phases of the oestrous cycle. Progesterone administration to rats in late dioestrus did not affect the concentration of uterine nuclear oestrogen receptors 24h later, although it did prevent the normal cyclic increase at pro-oestrus in the concentration of hypothalamic nuclear oestrogen receptors. Our results therefore show that in the intact adult rat, factors other than the concentration of progesterone or oestradiol determine the nuclear concentration of oestrogen receptors in the uterus. They also demonstrate differences between neural and non-neural tissues in the regulation of oestrogen–receptor interactions.

The administration of oestradiol to female rats promotes translocation of oestrogen receptors in the uterus from the cytosol to the nucleus, where they initiate the oestrogenic responses of the tissue (Brenner & West, 1975; Buller & O'Malley, 1976). Clark *et al.* (1973) have presented evidence that the uterotrophic response is mediated by translocated receptors that are retained within the nucleus for periods longer than 6h after oestradiol administration, and that these long-term nuclear receptors represent a saturable population. The concentration of these long-term nuclear receptors is thought to be determined by a fixed concentration of nuclear 'acceptor' sites for the translocated receptor (Clark & Peck, 1976).

We have sought to extend these studies, which were previously carried out on immature rats, to the more immediately relevant area of adult oestrogenic responses during the normal oestrous cycle. In the adult rat, the concentration of nuclear oestrogen receptors shows cyclic changes (Clark *et al.*, 1973; White *et al.*, 1978), which apparently parallel changes in the concentration of circulating oestrogens (Brenner & West, 1975). However, we have found that in uterine horns fitted with an intrauterine device the concentration of nuclear receptors is significantly lower than in control contralateral unaffected horns at pro-oestrus, despite the presence of normal concentrations of the cytosol receptor and exposure to the same circulating concentrations of oestrogens (Myatt *et al.*, 1978).

In experiments reported in the present paper we set out to determine whether, as in the immature rat, administration of oestradiol will increase the concentration of long-term uterine nuclear oestrogen receptors in intact adult rats. We have also examined the action of progesterone in the regulation of nuclear oestrogen receptors both in the uterus and in the hypothalamus for a direct comparison of the two tissues. The results show that the response of the adult rat uterus to oestradiol administration, throughout the cycle, is different from that of the immature rat and that there are differences between the response of the hypothalamus and the uterus to progesterone administration.

Experimental

Female Wistar rats were obtained from Charles River (Margate, Kent, U.K.) at 46 days of age, and maintained for 1 week in our laboratories, during which they were monitored for phase of oestrous cycle daily (including the day of killing) by means of vaginal smears. All animals showed a 4-day cycle: oestrus/metoestrus, early dioestrus/late dioestrus/pro-oestrus.

Oestradiol-17β and progesterone were administered intraperitoneally in 50% (v/v) ethanol in

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saline (0.9% NaCl). Controls received vehicle alone. In the first experiment oestradiol (2.5 μg) was administered to rats of each phase at 10:00h; and these rats, with a similar number of control rats in each phase, were killed between 17:00 and 18:00h. In other experiments a group of rats in late dioestrus was injected with 1 mg of progesterone each at 11:00h, and the rats were killed, together with a control group in the same phase, 24h later during pro-oestrus. In another set of experiments progesterone-injected rats in late dioestrus were killed, together with controls, 7h after the administration.

The uterus, and hypothalamic block when required (White *et al.*, 1978), were removed individually into 5 ml of ice-cold buffer [TSC buffer: 10mM-Tris/HCl (pH 7.4)/0.25 M-sucrose/1 mM-$CaCl_2$ in experiment 1; TEGD buffer: 10mM-Tris/HCl (pH 7.4)/1 mM-EDTA/1 mM-dithiothreitol/10% (v/v) glycerol in subsequent experiments]. The uteri were chopped finely, and both tissues were homogenized using a type X1020 homogenizer (The Scientific Instrument Centre, London WC1, U.K.) (at lowest speed for the hypothalamic tissue).

After a low-speed (1400 ***g*** for 10min) centrifugation of the homogenates, the supernatants were spun at full speed for 15min in an Eppendorf bench centrifuge, and portions of the clear supernatants made 5nM (uterus) or 1nM (hypothalamus) with respect to [^{3}H]oestradiol {[2,4,6,7(n)-^{3}H]oestradiol-17β] (sp. radioactivity 80–100Ci/mmol), from The Radiochemical Centre, Amersham, Bucks., U.K., with and without 1 μM-diethylstilboestrol added. After about 5h incubation at 4°C, the macromolecular-bound [^{3}H]oestradiol in matched samples with and without added diethylstilboestrol was estimated by exclusion chromatography on Sephadex LH-20 (White *et al.*, 1978). The amount of cytosol oestrogen receptor present was calculated from the difference in bound radioactivity between matched samples.

The low-speed pellets, or crude nuclear preparations, were washed with 5 ml of buffer (×4), and resuspended in buffer containing 5nM-[^{3}H]oestradiol (uterine tissue) or 1nM-[^{3}H]oestradiol (hypothalamic tissue). Four portions of each suspension, two of which were made 1 μM with respect to diethylstilboestrol, were incubated for 30min at 4°C, then for 30min at 37°C. Then 1 ml of ice-cold buffer [containing 1% (w/v) bovine serum albumin] was added, and after 30min at 4°C the samples were washed (×3) with 1 ml of buffer, then washed into scintillation vials and counted for radioactivity with toluene/5-(biphenyl-4-yl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole as scintillant. This modified assay and washing procedure for hypothalamic nuclei resulted in a lower non-specific background of bound [^{3}H]oestradiol, and a 2-fold higher recovery of oestrogen receptor compared with our previous procedure (White *et al.*, 1978).

The techniques employed are based on those routinely used to measure high-affinity oestrogen receptors (K_d in nM range) both in the cytosol and in the nucleus, where the exchange assay (at 37°C for 30min) has been shown to result in complete exchange of bound endogenous oestradiol with [^{3}H]oestradiol in the assay medium (Anderson *et al.*, 1972; White *et al.*, 1978; Myatt *et al.*, 1978).

Results and discussion

Changes in the concentration of uterine nuclear oestrogen receptor during the oestrus cycle: effect of oestradiol administration

In a previous publication (Thrower *et al.*, 1981) we have demonstrated that the administration of 2.5 μg of oestradiol-17β to immature (29-day-old) rats resulted in substantial increases in the concentration of long-term uterine nuclear oestrogen receptors, i.e. those present 8h after the administration. In these present experiments, a similar administration of oestradiol did not alter the concentration of 'long-term' uterine nuclear receptors in adult females. Fig. 1(*a*) shows that the concentration of uterine nuclear oestrogen receptor, measured at 17:00–18:00h on the afternoon of each day of the cycle, was unaffected by administration of 2.5 μg of oestradiol 7–8h earlier, whatever the stage of the cycle. The content of receptor after oestrogen injection was still significantly lower in metoestrus than in late dioestrus and pro-oestrus.

We have previously also demonstrated (Thrower *et al.*, 1977) that the administration of 2.5 μg of oestradiol led to increases of the order of 50–100% in the concentration of nuclear oestrogen receptors at each phase, 1.3h after the administration (i.e. 'short-term' receptors). Fig. 1(*b*) shows that 7–8h after oestradiol administration at each phase, receptor concentration had recovered to approximately control values from an average of less than 25% of control, at 1.3h after administration (Thrower *et al.*, 1977). In a group of rats, pooled regardless of phase, the cytosol receptor content in controls was 12.19 ± 1.36 (3) compared with 1.43 ± 1.01 (3) (pmol/g of uterus) at 1h after oestradiol administration, showing that in these present experiments translocation had also occurred.

Effect of administration of progesterone on uterine and hypothalamic oestrogen receptor content

Since progesterone concentrations also fluctuate during the oestrous cycle (Brenner & West, 1975), we administered progesterone to rats at late dioestrus to determine whether this hormone directly influenced the concentration of nuclear oestrogen receptors. Fig. 2(*a*) shows that administration of

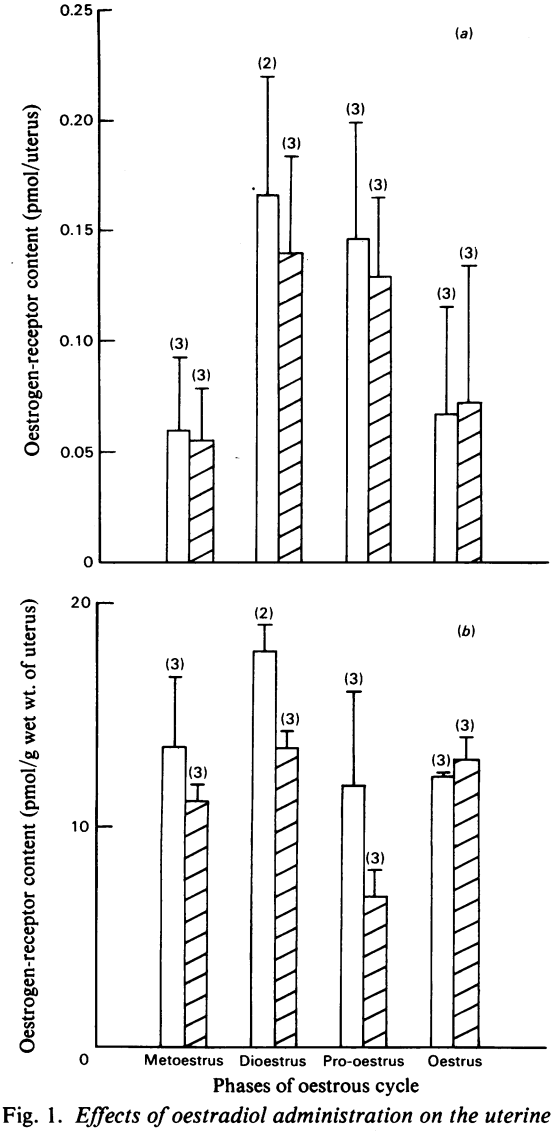

Fig. 1. *Effects of oestradiol administration on the uterine content of (a) long-term nuclear and (b) cytosol oestrogen receptors in rats throughout the oestrous cycle*

Female rats were grouped according to phase of the cycle. Half of the animals in each group were administered 2.5 μg of oestradiol/rat. These animals along with the controls were killed 7–8 h later and the nuclear and cytosol oestrogen receptor content of the hypothalamus and uterus of each individual animal was determined as described in the Experimental section. In this and subsequent Figures numbers in parentheses refer to numbers of separate determinations made. The difference between the nuclear receptor content in the oestradiol-injected rats at metoestrus and either at late dioestrus or at pro-oestrus was significant ($P < 0.025$). Statistical evaluation of differences in this and subsequent Figures was by the Student's *t* test. □, Control; ▨, oestradiol-injected. Results are means and the bars represent S.D.

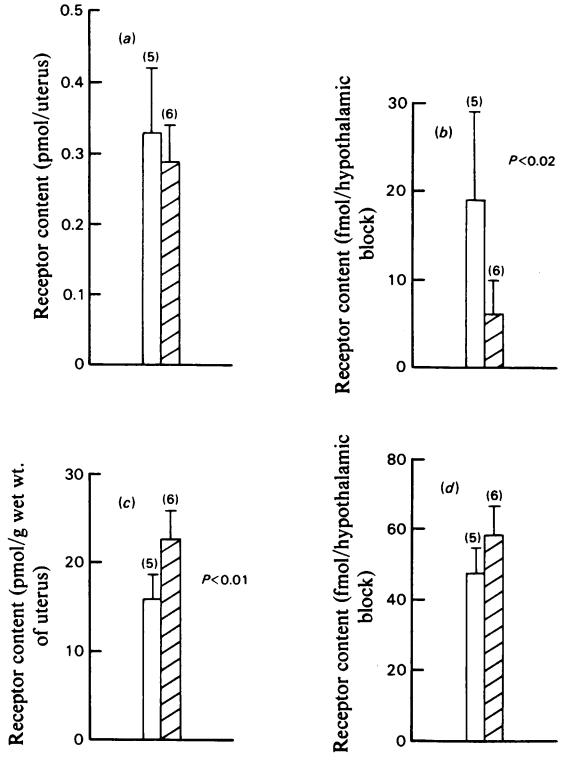

Fig. 2. *Effect of progesterone administration to rats in late dioestrus on the content of nuclear (a and b) and cytosol (c and d) oestrogen receptors in the uterus and the hypothalamus after 24 h*

Progesterone (1 mg) was administered intraperitoneally to rats in late dioestrus. These were killed, together with control rats, 24 h later, i.e. at the pro-oestrous phase, and the nuclear and cytosol oestrogen receptor content of the uterus (*a* and *c*) and hypothalamus (*b* and *d*) was determined as described in the Experimental section. □, Control; ▨, progesterone-injected. Results are means and the bars represent S.D.

1 mg of progesterone at 11:00 h on late dioestrus had no significant effect on uterine nuclear oestrogen receptor content 24 h later, on the morning of pro-oestrus. However, there was a significant decrease in the content of hypothalamic nuclear oestrogen receptor (Fig. 2*b*).

To determine whether this hypothalamic effect was merely a result of a progestogenic advancement of the peak of nuclear receptor content, we examined animals at 18:00 h at late dioestrus, 7 h after progesterone administration. Fig. 3 shows that in the hypothalamus and uterus there was no significant difference between control and progesterone-administered animals in the content of nuclear receptors.

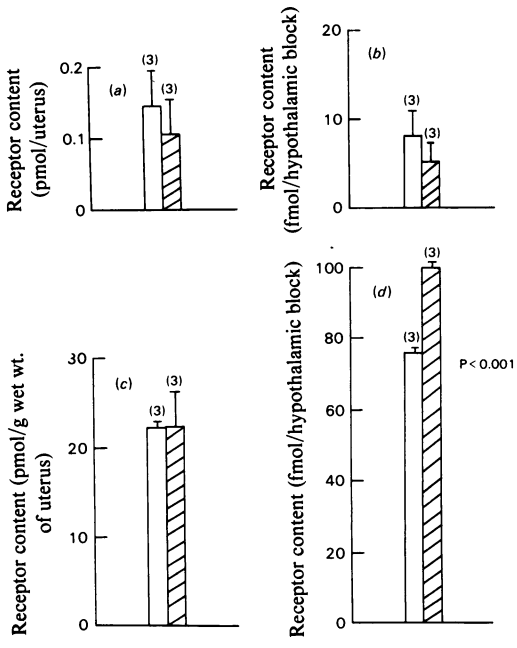

Fig. 3. *Effects of progesterone administration to rats in late dioestrus on the content of nuclear (a and b) and cytosol (c and d) oestrogen receptors in the uterus (a and c) and the hypothalamus (b and d) after 7 h*

Progesterone (1 mg) was administered to rats in late dioestrus at 11:00 h. These were killed, together with controls, 7 h later and receptor content was determined as before. □, Control; ▨, progesterone-injected. Results are means and the bars represent S.D.

The decrease in the hypothalamic concentration of nuclear oestrogen receptors resulting from progesterone administration 24 h previously could not be attributed to an unavailability of cytosol receptors (Hsueh *et al.*, 1976) since there was no significant effect at this time on the content of cytosol receptors (Fig. 2*b*). An interesting finding was that this progesterone administration resulted in an increase in uterine cytosol receptor content (Fig. 2*a*). A similar increase in the hypothalamus occurred earlier, 7 h after the administration (Fig. 3*b*).

Conclusions

The results reported here demonstrate that in the intact female rat, the concentration of long-term nuclear oestrogen receptors in the uterus at each phase was not altered by exogenous oestrogen. They suggest that the nuclear 'acceptor' sites involved in the retention of oestrogen receptors [as defined by Clark *et al.* (1973); Clark & Peck, 1976)] were saturated at all stages of the oestrous cycle by prevailing concentrations of oestrogen. The ability of administered oestradiol to promote increases in long-term oestrogen nuclear receptors in the uteri of immature (Clark *et al.*, 1973; Thrower *et al.*, 1981) or ovariectomized rats (Zava *et al.*, 1976) probably reflects low concentration of circulating oestrogen in uninjected rats and thus an under-saturation of existing nuclear 'acceptor' sites for the receptor. There is evidence that the concentration of these 'acceptor' sites may be regulated by other hormonal agents e.g. thyroid hormones (Gardner *et al.*, 1978). Since the secretion of pituitary thyrotropin is itself modulated by oestrogens (DeLean *et al.*, 1977), it is clear that the oestrogenic/thyroid status of the female rat needs to be considered when interpreting the effects of sex steroid administration on uterine nuclear oestrogen receptors.

The differences between the effects of oestrogen administration on the nuclear oestrogen receptor in the uterus of adult cyclic animals and in immature uteri (Clark *et al.*, 1973; Thrower *et al.*, 1981) suggest that findings derived from experiments on immature or ovariectomized rats are not applicable to the intact mature rat. The differences probably arise from differences in the overall hormonal status, in which the extent of oestrogenic exposure itself plays a large part.

In the adult hypothalamus, as distinct from the uterus, the ability of administered progesterone to interfere in the long term with nuclear oestrogen receptors (Fig. 2) may explain some of its known inhibitory effects on sexual functions that are dependent on oestrogen priming (Feder & Marrone, 1977) and presumably requiring the mediation of nuclear receptors. These inhibitory effects of progesterone require a long duration of exposure, which is consistent with our observations that the effects on nuclear receptor content were only significant 24 h after administration.

The present results also further emphasize dissimilarities in the operations of the oestrogen receptor in neural and non-neural tissues, in keeping with our previous reports on both the oestrogen receptor (White *et al.*, 1978; Thrower *et al.*, 1981) and on the progestin receptor (Thrower & Lim, 1980*a*,*b*).

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