Developmental Changes in the Content of Oestrogen Receptors in the Hypothalamus of the Female Rat

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Hypothalamic cytosol and nuclear oestrogen receptors are present at birth. A 2-fold increase in cytoplasmic receptor content occurs by the second week, whereas the first significant and equivalent increase in nuclear receptor occurs in the fourth week. The latter reflects reported increases in oestradiol availability thought to lead to complete feminine sexual differentiation. The presence of nuclear receptors in the newborn suggests a requirement for oestrogenic stimulation in early development.

Sexual differentiation of the rat hypothalamus occurs post-natally. During the first 10 days after birth the administration of androgens or oestrogens to the female results in 'masculinization' of the hypothalamus, with attendant abnormalities in the pattern of gonadotropin release and sexual behaviour as adults (Gorski, 1971). Because of this effect, sexual differentiation of the hypothalamus of the female has been considered to be independent of oestrogen, which is prevented from interacting with the hypothalamus by the oestrogen-binding α -foetoprotein present in plasma (McEwen *et al.*, 1975). However, recent evidence has suggested that neonatal exposure to testosterone, which may be converted into oestrogen in the hypothalamus, is actually required for normal development of female sexual behaviour (Christensen & Gorski, 1978). The occurrence of cytosol oestrogen receptors in the hypothalamus of the female neonatal rat has been reported (Plapinger & McEwen, 1973; Barley *et al.*, 1974; Westley *et al.*, 1976). However, their role remains ill-defined. In this paper we report on the nucleocytoplasmic relationships of the oestrogen receptor in the first 4 weeks *post partum* and on the presence of nuclear receptors in the hypothalamus of the neonatal female rat. The latter finding suggests the occurrence of oestrogen-stimulated translocation of receptor in the newborn. In addition, we have observed binding of the cytosol receptor to oligo(dT)-cellulose, which in the adult has been proposed to serve as an index for nuclear binding of receptor *in vivo* (Thrower *et al.*, 1976; White *et al.*, 1978). This binding demonstrates that the potential for nuclear binding of receptor exists in these neonatal animals.

Experimental

Female Wistar rats from our animal colony were used. In experiments to study the effect of oestradiol

on nuclear receptor content, 2.5 μ g of oestradiol-17 β was administered intraperitoneally in 0.1 ml of 25% (v/v) ethanol in saline (0.9% NaCl) and animals were killed 6 h later.

Oestradiol-receptor content was measured in nuclear and cytosol preparations obtained from hypothalamic tissue as previously described (White, 1978; White *et al.*, 1978). Briefly, nuclear receptor measurements involved incubation of nuclear fractions at 37°C for 1 h with saturating concentrations of [³H]oestradiol (Anderson *et al.*, 1973).

The nuclear content of receptor per hypothalamic block was independent of the weight of the block ($r = 0.35$; $n = 22$) over the weight range 0.1–0.24 g (White, 1978). Expression of nuclear content in terms of DNA would therefore lead to underestimation as the size of the block increases. In this particular tissue, high concentrations of the oestrogen receptors are confined to specific neurons within the hypothalamic block. Results are therefore expressed as fmol/brain. Cytosol receptor content was measured by exclusion chromatography (on Sephadex LH-20) after incubation at 4°C for 18–24 h with saturating concentrations of [³H]oestradiol. In each case the binding of radioactive oestradiol to specific receptor was assessed on the basis of parallel incubations in the presence of an excess of unlabelled diethylstilboestrol (Raynaud *et al.*, 1971). The ability of cytosol receptor, preincubated with [³H]oestradiol, to bind to 100 mg of oligo(dT)-cellulose was determined by using a batchwise centrifugation technique, as previously described (White *et al.*, 1978).

Results and Discussion

Hypothalamic cytosol and nuclear receptor contents during the developmental period

Hypothalamic cytosol oestrogen receptors were present in each age group studied (see Table 1). The concentration of cytosol receptor was lowest during

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Table 1. *Changes in the concentration of hypothalamic oestrogen receptors during the developmental period*

Female rats of the exact same age (five animals per age) were used in individual experiments; data were subsequently grouped according to week number as shown (ages in days are given in parentheses). Hypothalamic tissue was dissected and weighed before homogenization in 4 vol. of TED buffer [10 mM-Tris/HCl (pH 7.6)/1.5 mM-EDTA/1 mM-dithiothreitol]. A crude nuclear pellet was obtained by centrifugation of the homogenate at 1400g for 10 min at 4°C, followed by at least four similar resuspension and centrifugation procedures. The initial supernatant was centrifuged at 200000g for 1–1.5 h at 4°C to prepare the cytosol fraction. Nuclear receptors were measured by using an 'exchange assay' in the presence of 2 nM-[³H]oestradiol; for cytosol receptor measurement the final concentration of [³H]oestradiol used was 5 nM. Results shown are means $\pm$ S.D. Significance of differences was assessed by Student's *t* test: †week 4 significantly higher than week 3 ($P < 0.05$); ††week 5 significantly higher than week 3 ($P < 0.01$); *week 2 significantly higher than week 1 ($P < 0.001$); **week 4 significantly different from weeks 3 and 5 ($P < 0.05$ and $P < 0.01$ respectively).

	Age (week no.)				
	1 (0–7)	2 (8–14)	3 (15–22)	4 (23–28)	5 (29–35)
Wet wt. (g)	0.11 $\pm$ 0.02 (9)	0.19 $\pm$ 0.02 (7)	0.20 $\pm$ 0.02 (10)	0.21 $\pm$ 0.06 (7)	0.23 $\pm$ 0.04 (5)
Nuclear receptor (fmol/ brain)	0.93 $\pm$ 0.22 (4)	1.43 $\pm$ 0.31 (7)	1.20 $\pm$ 0.31 (10)	1.93 $\pm$ 0.94† (7)	2.66 $\pm$ 1.17†† (5)
Cytosol receptor (fmol/ brain)	17.00 $\pm$ 5.81 (9)	34.12* $\pm$ 7.90 (5)	44.30 $\pm$ 7.68 (9)	46.36 $\pm$ 0.65 (3)	48.43 $\pm$ 6.16 (5)
Cytosol receptor binding to oligo(dT)– cellulose (fmol/brain)	1.98 $\pm$ 0.67 (6)	3.70 $\pm$ 1.21 (7)	3.47 $\pm$ 0.60 (6)	2.36 $\pm$ 0.35** (3)	4.00 $\pm$ 0.57 (5)

the first week after birth, increasing approximately 2-fold by the second week. Thereafter there was a progressive increase in cytosol receptor content until adult concentrations were reached in the fifth week after birth. Nuclear receptors, although present in lower concentrations than cytosol receptors, were detectable in each age group studied. The change in the nuclear receptor concentration with age differed from that of the cytosol receptor. Nuclear receptor concentrations were lowest in the first week after birth and increased gradually in the second and third weeks. The most significant increase in nuclear receptor content occurred during the fourth and fifth weeks after birth. This increase coincided with the reported disappearance of the plasma oestrogen-binding α -foetoprotein (Plapinger *et al.*, 1973) and presumably reflects the resultant increased intracellular exposure to circulating oestrogens.

Cytosol receptor binding to oligo(dT)–cellulose

The oestrogen receptor in cytosol preparations obtained from the hypothalamus of adult female rats binds to oligo(dT)–cellulose. An inverse relationship exists between this binding ability and the nuclear content of receptor, suggesting the presence of limiting amounts of a common factor regulating these two processes (White *et al.*, 1978). In hypothalamic cytosol obtained from developing females the oestrogen receptor was able to bind to oligo(dT)–cellulose. The amount bound was lowest during the first week; in the second and third weeks the values were higher and approximately paralleled the increase in cytosol receptor content. During the fourth

week after birth, despite the maintenance of cytosol receptor content, the binding capacity appeared to be decreased. Interestingly, this was also the period when the most marked increase of nuclear receptor content occurred, further indicating an inverse relationship between receptor binding *in vivo* and binding to oligo(dT)–cellulose *in vitro*. Further work, however, is required to establish this putative relationship. In the fifth week after birth, binding of receptor to oligo(dT)–cellulose was similar to that of the first 3 weeks; the sum of this binding together with nuclear receptor content was highest during this period.

Oestrogen-stimulated receptor translocation

The occurrence of nuclear oestrogen receptors, from the first week after birth, even at a low concentration, would of necessity require intracellular oestrogen. Plasma α -foetoprotein binds oestradiol with a relatively low affinity, but with a high capacity, and material immunochemically identical with this protein has been identified in hypothalamic preparations (McEwen *et al.*, 1975). Dissociation of oestrogen from such an intracellular component could result in the interaction of low concentrations of released steroid with the oestrogen receptor and its consequent translocation to the nucleus. Alternatively, intracellular oestrogens may be derived from aromatizable compounds, which evade the α -foetoprotein 'protective' mechanism; aromatizing enzymes capable of such conversions have been reported to be present in the hypothalamus of newborns (Reddy *et al.*, 1974).

Table 2. *Content of hypothalamic nuclear oestrogen receptor 6 h after oestradiol administration ('long-term' nuclear receptors)*
Groups of 5-, 21- and 30-day-old female rats were each injected intraperitoneally with 2.5 µg of oestradiol and hypothalamic nuclear oestrogen receptor content was determined 6 h later. The measurements (fmol/brain) are also expressed as a percentage of the total receptor (nuclear + cytosol) normally present in control animals of the exact same age.

	Age (days)		
	5	21	30
Nuclear receptor (fmol/brain)	4.63 ± 2.15 (7)	6.3 ± 2.37 (4)	4.57 ± 2.45 (6)
Proportion of total receptor retained in the nucleus (%)	25.8	13.8	8.9

The administration of high doses of oestradiol or of the synthetic compound diethylstilboestrol to neonatal female rats overcomes the 'protective' mechanism, resulting in translocation of receptor into the nucleus, as measured 1 h later (Westley & Salaman, 1977; Hall, 1978). However, the nuclear receptors present 1 h after stimulation are considered to comprise 'short-term' and 'long-term' receptors (Clark & Peck, 1976); those 'long-term' receptors present 6 h after stimulation have been implicated in the physiological response to oestrogens. We therefore measured the concentration of nuclear oestrogen receptors 6 h after oestradiol administration to determine if the hypothalamus of the newborn was capable of retaining 'long-term' nuclear receptors. Older rats (21 and 30 days old) were used for comparison.

At each age, the concentration of 'long-term' nuclear receptors was increased after the administration of 2.5 µg of oestradiol (Table 2). In the neonatal female, a greater proportion of the total receptor was retained, indicating that there was an extensive capability to produce 'long-term', presumably physiologically active, nuclear receptors (provided that oestradiol was available). Under normal physiological conditions, oestradiol is most probably limiting. However, in neonatal males (where testosterone serves as a source of intracellular oestrogen) nuclear oestrogen receptors have been readily detected by others (Westley & Salaman, 1977).

Conclusion

To our knowledge, the detection of nuclear oestrogen receptors in the hypothalamus of the neonatal female rat under normal physiological conditions has not previously been reported. The presence of nuclear oestrogen receptors indicates that oestrogenic stimulation occurs in the hypothalamus of the newborn, possibly related to its development. Two lines of evidence lend support to such a relationship. Hypothalamic tissue, from neonatal female rats, in culture shows increased neurite outgrowth after oestrogen treatment (Toran-Allerand, 1976). Oestrogen-treated cultures also have a higher content of luteinizing hormone-releasing factor,

LH-RH), and it has been suggested that luteinizing hormone-releasing factor-containing neurons may be targets for oestrogen during development (Toran-Allerand, 1978). Secondly, neonatal exposure to low doses of testosterone propionate increases adult behavioural responses to oestrogen stimulation (Clemens *et al.*, 1969). The effects of such treatment have been localized to the preoptic area (Christensen & Gorski, 1978), and it was suggested that partial masculinization was obligatory for the normal development of female behavioural responses.

The results of the present study support the proposition that in the neonatal female sexual differentiation of the hypothalamus may not occur independently of sex steroids. In the newborn the oestrogenic stimulation is a limited one (Christensen & Gorski, 1978) and is reflected by the presence of low concentrations of nuclear oestrogen receptor. A further oestrogenic stimulation, which occurs at the fourth week (McEwen *et al.*, 1975), is associated with increases in nuclear oestrogen receptors, which is reflected by changes in RNA metabolism (Hall & Lim, 1978). If oestradiol is involved in the regulation of sexual differentiation, then the presence of nuclear oestrogen receptors is consistent with their participation in the cellular response to the hormone. The exact nature of the role of these receptors remains to be established.

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