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CHARACTERIZATION OF ESTROGEN RECEPTORS IN THE HAMSTER BRAIN[1]

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Summary—Although the hamster is frequently used as an experimental animal for studying reproductive neuroendocrinology and sex behavior, estrogen receptors (ER) in the central nervous system have not been fully characterized. Using Sephadex LH-20 gel filtration and DNA-cellulose affinity chromatography, estrogen binding macromolecules having the physicochemical properties of classical ER were identified in cytosolic and nuclear extracts of brain tissues. These receptors exhibited high affinity for estradiol ($K_d = 10^{-9}$ M), limited capacity (30–50 fmol/g tissue), and estrogen specificity; however, competition studies indicate that brain and uterine ER have different binding kinetics. The neuroanatomic distribution of ER was similar in males and females with highest levels in the limbic brain and consistently low levels in remaining forebrain and mid/hindbrain. No sex differences in receptor number or other binding parameters were evident. Sucrose gradient centrifugation showed that cytosolic ER sedimented in the 7–8S region of a 5–20% linear gradient (no salt), whereas nuclear ER had a sedimentation coefficient of 5S under high ionic strength. On DNA-cellulose affinity columns, these receptors had an elution maximum of 0.18 M NaCl. After a single injection of estradiol, nuclear ER increased and cytosolic ER were depleted. The lower estradiol binding affinity and receptor levels in hamster brain as compared to the rat are consistent with observed species differences in neural sensitivity to estrogen. We expect these data in hamsters, a markedly photosensitive species, to provide a basis for future studies examining the role of receptors in mediating the effects of day-length on steroid dependent feedback and behavioral responses.

INTRODUCTION

Activational and organizational actions of estrogen (E) on the brain of several species have been well-documented [1, 2], and there is good evidence that these actions are mediated by classical estrogen receptors (ER) [2]. ER have been described in the central nervous system [CNS] of the rat [3], mouse [4], gerbil [5], guinea pig [6] and more recently the freshwater turtle [7]. Despite the fact that the hamster uterus is frequently used as a model of E and progesterone action [8], ER have not been fully characterized in the hamster brain. In early studies, Ciaccio and Lisk [9] showed that [^{3}H]estradiol (E_2) was taken up and retained in the preoptic area (POA) and hypothalamus (HTH) of male and female hamsters. Subsequently, E-concentrating cells were localized in discrete limbic and other brain regions by autoradiographic methods [10]. An E-binding macromolecule having a sedimentation coefficient similar to that in the uterus was detected in cytosolic subfractions of brain [11]. More recently, an E-binding component of brain cytosol was shown to adhere to DNA-cellulose affinity columns and to exhibit saturation at 1–5 nM estradiol [12]. Although these preliminary reports do not provide critical information on steroid binding affinity or specificity, they suggest that the E-binding mechanism in hamster brain differs in some respects from that in the rat. For example, following injection of [^{3}H]E_2, radioactivity in the POA and HTH of hamsters is 3–9 times lower than that in rats [9]. This result is consistent with a reduced number and distribution of labeled cells and a lower intensity of labeling of individual cell nuclei as seen in autoradiograms [10]. It has been suggested that lower E-binding activity in the hamster's CNS may somehow be related to higher levels of circulating E and to the higher dose of E_2 required to induce estrous in hamsters as compared to rats [12]. Also, of the two rodent species, hamsters are characterized by somewhat higher brain aromatase activity, implying that higher local concentrations of estrogen are required for biological activiation of neural pathways [14].

The hamster has proven to be a useful alternative to the rat for studying steroid hormone effects on brain sex differentiation in neonates [15] and on sex behavior [16] and gonadotropin secretion [17] in adult animals. Moreover, the hamster is a species which continues to exhibit marked seasonal reproductive cyclicity in the laboratory when day length is manipulated. This is advantageous for examining the possible role of receptors in mediating light-induced behavioral and feedback responses to steroids. The present study was undertaken to further characterize ER in hamster brain and to provide biochemical evidence for E-binding to brain cell nuclei upon exposure to hormone *in vivo*. In a subsequent report we describe the effects of photoperiod on the E-binding mechanism of hamster CNS [18].

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EXPERIMENTAL

Animals and steroid treatment

Adult male and female hamsters [LVG], weighing 100–110 g were purchased from Charles River Breeding Laboratories, Wilmington, MA. Animals were housed individually with food and water *ad libitum* under long days [16:8 LD]. Where noted, castration was performed surgically under light ether anesthesia. Prior to sacrifice, animals were lightly anesthetized with ether and killed by exsanguination. Whole brain was divided into a limbic block (containing POA, HTH, amygdala, and septum), remaining forebrain and mid/hindbrain as previously described [14]. Uterus was used as a control tissue. To determine effects of E treatment on the subfractional distribution of receptors and to obtain sufficient nuclear receptors for characterization, hamsters were injected (i.p.) with E_2 (150 μg) dissolved in propylene glycol 3 h prior to sacrifice.

Chemicals and buffers

[2,4,6,7-^{3}H]$E_2$17β (Sp. act. = 95–115 Ci/mmol) and [^{14}C]bovine serum albumin (Sp. act. = 20 μ Ci/mg) were purchased from New England Nuclear Corporation. Radioinert steroids were obtained from Sigma Chemicals. Bovine serum albumin (BSA, Pentex) was purchased from Miles Laboratory, calf thymus DNA from Worthington Biochemicals, Munktell 410-cellulose from Bio-Rad, Sephadex LH-20 gel from Pharmacia Fine Chemicals, PPO (2,5-diphenyloxazolyl) and POPOP [*p-bis*-(2,5-phenyloxolzolyl)benzene] from Research Products International. Other chemicals were reagent grade. [^{3}H]E_2 was further purified by thin layer chromatography using ether–hexane (3:1, v/v).

The buffers used were as follows: TEMG buffer (10 mM Tris–HCl, 1 mM EDTA, 1 mm 2-mercaptoethanol, 10% glycerol, pH 7.5); homogenization buffer (B_H, 50 mM Tris HCl, 1 mM EDTA, 12 mM monothioglycerol and 30% glycerol, pH 7.5); washing buffer for nuclear pellets (B_W, 10 mM Tris–HCl, 3 mM $MgCl_2$, 2 mM monothioglycerol and 0.25 M sucrose, pH 7.5); extraction buffer for nuclear pellets (B_{EX}, 0.7 M KCl in B_H, pH 7.5); elution buffers for DNA-cellulose affinity columns (0.05 M or 0.4 M NaCl in TEMG, each with 0.2 mg BSA per ml); elution buffers for Sephadex LH-20 columns (TEMG for cytosol; TEMG with 0.5 M KCl for nuclear extract). Dextran coated charcoal suspension contained Norit A, 0.5% (w/v) and Dextran T-70, 0.05% (w/v) in TEMG buffer.

Preparation of cytosolic and crude nuclear extracts

Dissected brain regions from animals with similar treatments were washed twice with ice cold TEMG buffer and homogenized in 3 vol of B_H by three 5-s bursts with a polytron PT-10/35 homogenizer (Brinkmann). These and all subsequent procedures were carried out at 4°C. The homogenate was centrifuged at 1,000 *g* for 15 min. The resulting supernatant was centrifuged at 100,000 *g* for 1 h (37,000 rpm, Rotor TV-865, Sorvall OTD-65) to obtain crude cytosol. The remaining crude nuclear pellets were washed three times with 10 vol of washing buffer and incubated with 0.7 M KCl extraction buffer for 1 h at 4°C with mixing every 15 min. The final nuclear suspension was centrifuged at 100,000 *g* for 1 h and the clear supernatant designated nuclear extract. Uterine tissues were processed in the same way as brain tissues. All samples were stored at −70°C and analyzed within 2–3 weeks.

Determination of E-binding activity

Cytosolic and nuclear E-binding activities were assayed using a protocol devised for measuring free and occupied ER in hamster uterus [19]. Briefly, aliquots (400 μl) of cytosol were incubated with [^{3}H]E_2 (5 or 10 nM) ± 100-fold excess radioinert competitors at 4°C for 15–18 h (non-exchange condition to measure unoccupied ER). To determine total nuclear E-binding, crude nuclear extracts (400 μl) were incubated with [^{3}H]E_2 (5 or 10 nM) ± 100-fold excess radioinert competitors at 30°C for 1 h followed at 15 min at 4°C (complete exchange conditions for free and occupied ER). Bound and free steroid were separated either by Sephadex LH-20 gel filtration or DNA-cellulose affinity chromatography using procedures described in detail elsewhere [20, 21]. Preliminary data indicated that a conventional charcoal adsorption assay was not suitable for measuring E-binding in brain tissues due to the low levels of specific binding and apparently high non-specific binding. For saturation analysis, cytosolic or crude nuclear extracts (400 μl) were incubated with increasing concentrations of [^{3}H]E_2 (0.5–10.0 nM) in the absence or presence of 100-fold excess radioinert E_2 to determine total binding and non-specific bidning, respectively. Data were analyzed according to Scatchard[22] to estimate the equilibrium dissociation constant (K_d).

Sedimentation analysis

The Sephadex LH-20 column eluates (400 μl) which contained the [^{3}H]E_2-bound fractions from cytosolic and crude nuclear extracts were mixed with [^{14}C]BSA (2 μl) and layered on 5–20% linear sucrose gradients (4 ml) prepared in TEMG buffer (cytosol) or TEMG buffer containing 0.5 M NaCl (crude nuclear extract). Gradients were centrifuged at 219,000 *g* for 4.5 h (50,000 rpm in a Sorvall OTD-65 ultracentrifuge equipped with a w^2dt integrator with a Sorvall TV 865B vertical rotor). Fractions (0.22 ml) were collected from the top of the gradient using an Auto Densi-Flow Unit (Buchler) and radioactivity estimated.

Scintillation counting

Samples (0.2–0.5 ml) were added to scintillation cocktail (4.5 ml) comprised of 9.0 g PPO, 0.75 g POPOP, 750 ml Triton X-100 and 2.25 l xylene. Radioactivity was measured using a Tracor scintillation counter with a counting efficiency of 45% for tritium.

Protein determination

Cytosolic protein was determined by the procedure of Lowry *et al.*[22]. BSA was used as the standard.

RESULTS

[^{3}H]E_2 binding activity in cytosolic versus crude nuclear extracts

When cytosolic extracts were incubated with [^{3}H]E_2 (10 nM) ± 100-fold excess radioinert E_2 and chromatographed onto DNA-cellulose (Fig. 1a) or Sephadex LH-20 columns (Fig. 1b), a [^{3}H]E_2 binding component displaceable by excess radioinert E_2 was detected. As shown in Fig. 1a, this E binding moiety had an elution maximum of 0.18 M NaCl on a DNA-cellulose affinity column, although for routine analysis a step gradient (0.05 M, 0.4 NaCl) was used. Cytosolic ER were high in castrated male hamsters; however, 3 h following an injection of E_2, cytosolic ER were depleted (42%) and a concomitant increase in nuclear ER was seen (Fig. 2).

Saturation analysis of cycosolic and nuclear E binding

When E binding affinity was examined by Sephadex LH-20 column chromatography, the binding components in both cytosolic and crude nuclear extracts had the same K_d values: 1.1×10^{-9} M (Fig. 3a) and 1.2×10^{-9} M (Fig. 3b) with maximum binding capacity of 48 and 22 fmol/g tissue, respectively. A similar binding affinity ($K_d = 3.3 \times 10^{-9}$ M) was observed when cytosolic extracts were analyzed by DNA-cellulose affinity chromatography (not shown).

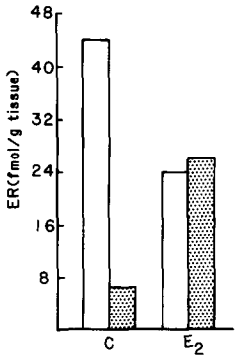

Fig. 2. Cytosolic versus nuclear estrogen receptor distribution in male hamster brain after *in vivo* estradiol treatment. Animals castrated for 2 weeks were injected (i.p.) with oil (C) or estradiol (150 μg) (E_2) and sacrificed 3 h later. Cytosolic (□) and nuclear (▩) receptors in limbic brain were measured by Sephadex LH-20 column chromatography. Values represent the mean of pooled tissues from 4–5 animals in 2 experiments.

Steroid binding specificity

When cytosolic extracts derived from limbic brain were examined for steroid binding specificity using [^{3}H]E_2 concentrations of 5 nM ± 100-fold excess

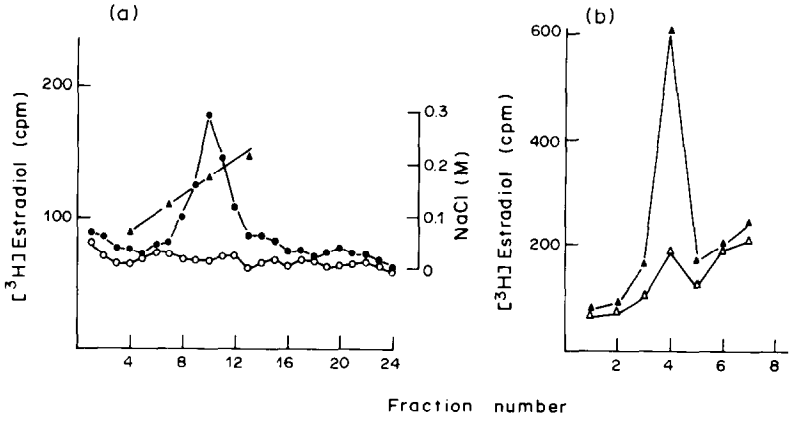

Fig. 1a. DNA-cellulose affinity chromatography of estradiol binding in limbic brain of intact male hamsters. Aliquots of cytosol (3.2 mg protein) were incubated with [^{3}H]E_2 (10 nM) in the absence (●——●) or presence (○——○) of 100-fold excess radioinert E_2. Labeled cytosolic extracts were chromatographed onto DNA-cellulose columns which had been equilibrated with 0.05 M elution buffer at 4°C and allowed to incubate with DNA-cellulose for 1 h at 22°C followed by 15 min at 4°C. Columns were then washed with 0.05 M elution buffer (8–10 bed vol) to remove free steroid and non-DNA-adhering components. [^{3}H]E_2 DNA-adhering components were eluted with a linear NaCl gradient (0.05–0.4 M, ▲——▲) and 0.5 ml fractions collected (two determinations.)

Fig. 1b. Sephadex LH-20 column chromatography of estradiol binding in limbic brain cytosol of intact male hamsters. Aliquots (400 μl) of cytosol were incubated with [^{3}H]E_2 (10 nM) in the absence (▲——▲) or presence (△——△) of 100-fold excess radioinert E_2. Samples were chromatographed onto Sephadex LH-20 columns, which had been equilibrated with TEMG buffer for 4–5 h and 0.2 ml fractions collected.

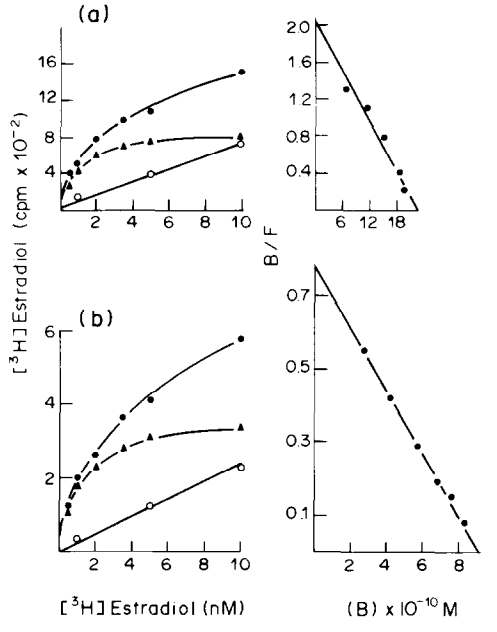

Fig. 3. Saturation analysis of cytosolic and nuclear estrogen binding in male hamster brain. Cytosolic or nuclear extracts from limbic brains were incubated with increasing concentrations of [^{3}H]E_2 (0.5–10 nM) in the absence or presence of 100-fold excess radioinert E_2. Bound and free steroid were separated on Sephadex LH-20 columns and specific binding (▲——▲) calculated as the difference between total (●——●) and non-specific (○——○) binding. Saturation curves are shown on the left and Scatchard analysis of the same data on the right. $K_d = 1.1 \times 10^{-9}$ M for cytosol (a) 1.2×10^{-9} M for nuclear extract (b). Data shown are representative of 2 determinations.

radioinert competitors, either androgens (testosterone or dihydrotestosterone) or progesterone could displace total [^{3}H]E_2 binding equally well (74–92% inhibition) and this was true regardless of sex (Table 1). This apparent lack of specificity was also seen using crude nuclear extracts which were analyzed by Sephadex LH-20 column chromatography. By contrast, when cytosolic or crude nuclear extracts prepared from uterine tissues were assayed under the same conditions, androgens or progesterone were poor competitors (Table 1). E specificity was exhibited, however, when cytosolic or crude nuclear extracts prepared from male limbic brains were incubated with 10 nM [^{3}H]E_2 ± 100-fold excess radioinert competitors (Table 2). At this ligand concentration, the [^{3}H]E_2 binding components eluting from LH-20 columns were displaced by natural and synthetic E (E_2 > diethylstilbestrol > estrone) but not by testosterone, 5α-dihydrotestosterone (DHT), progesterone or 5α-androstane-3α,17β-diol. By contrast, 5α-androstane-3β,17β-diol was a relatively good competitor.

Table 2. Binding specificity of brain extracts at 10 nM [^{3}H]estradiol ± 100-fold excess radioinert competitors

	(% Inhibition)	
Competitors	Cytosol	Nuclear extract
Estradiol-17β	100	100
Diethylstilbestrol	90	92
3β-Androstanediol	65	70
Estrone	37	55
3α-Androstanediol	12	10
Testosterone	22	3
5α-Dihydrotestosterone	6	0
Progesterone	6	0

Cytosolic or crude nuclear extracts prepared from male hamster limbic brains as described in Table 1 were incubated with 10 nM [^{3}H]E_2 ± 100-fold excess radioinert competitors as described in legend to Table 1. Bound and free steroid were separated on Sephadex LH-2 columns. Values represent means of 2–3 experiments.

Sedimentation analysis

The bound [^{3}H]E_2 in crude cytosol sedimented in the 7-8S region of a 5–20% linear gradient containing no NaCl (Fig. 4a); however, the [^{3}H]E_2 binding moiety of the nuclear extract had a sedimentation coefficient of 5S in a high salt (0.4 M NaCl) gradient (Fig. 4b). Both of the [^{3}H]E_2 binding components could be displaced by 100-fold excess radioinert E_2.

Neuroanatomical distribution of cytosolic and nuclear ER in intact and castrated hamsters

The neuroanatomical distribution of cytosolic ER in castrated hamsters when measured by Sephadex LH-20 column chromatography was as follows: limbic brain, remaining forebrain, mid/hindbrain (Fig. 5). Nuclear ER levels in all brain regions were close to the limits of detection. DNA-cellulose affinity chromatography revealed a similar distribution of cytosolic ER in intact male and female hamsters with receptors concentrated in the limbic block (Table 3).

Table 1. Binding specificity of brain versus uterine extracts at 5 nM [^{3}H]estradiol ± 100-fold excess radioinert competitors

	(% Inhibition)					
	Cytosol			Nuclear extract		
Competitors	Male brain	Female brain	Uterus	Male brain	Female brain	Uterus
Estradiol-17β	100	100	100	100	100	100
Diethylstilbestrol	92	98	100	95	100	100
Testosterone	84	82	0	76	60	2
5α-Dihydrotestosterone	82	82	0	92	56	5
Progesterone	74	80	0	79	58	0

Limbic brain cytosolic extracts prepared from intact animals or crude nuclear extracts prepared from estrogen-treated animals were incubated with 5 nM [^{3}H]E_2 ± 100-fold excess radioinert competitors as described in Experimental. [^{3}H]E_2 bound fractions in cytosolic and crude nuclear extracts were obtained by DNA-cellulose affinity column and Sephadex LH-20 gel filtration, respectively. The percent inhibition of total [^{3}H]E_2 binding by 100-fold excess radioinert E_2 was set at 100. Values represent means of 2–3 experiments.

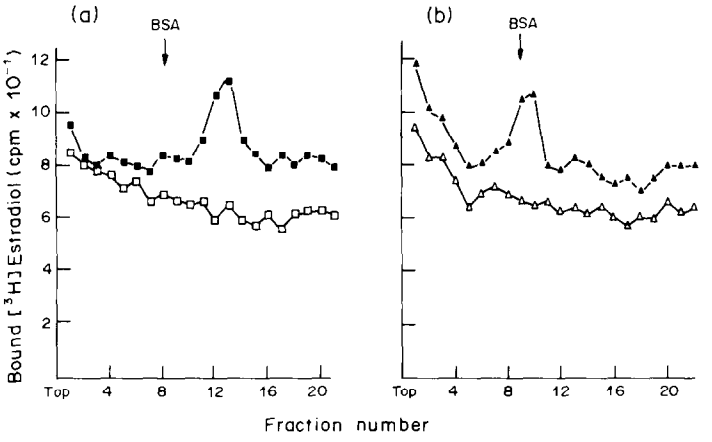

Fig. 4. Sedimentation analysis of cytosolic and nuclear estrogen receptors in limbic brain of male hamsters. After incubation with 10 nM [^{3}H]E_2 ± 100-fold excess radioinert E_2, [^{3}H]E_2-bound fractions from cytosol (a) or crude nuclear extract (b) were sedimented on 5–20% linear sucrose gradients containing no salt and 0.5 M NaCl, respectively. The arrow indicates the position of [^{14}C]BSA (4.7S). Total and non-specific binding are represented by closed and open symbols, respectively. (Two determinations.)

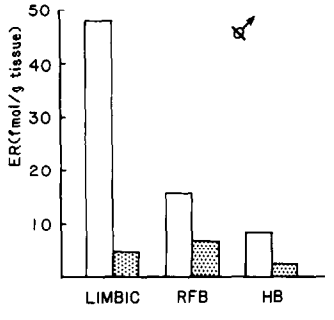

Fig. 5. Neuroanatomical distribution of estrogen receptors in castrated male hamsters. Cytosolic (□) and nuclear (▩) receptors were measured in limbic brain, remaining forebrain (RFB) and mid/hindbrain (HB) using Sephadex LH-20 column chromatography. Values represent the average of 2 separate experiments using pooled tissues from 3–4 animals.

DISCUSSION

These results demonstrate the presence in hamster brain of cytosolic and nuclear E-binding macromolecules having all the physicochemical characteristics of classical ER (high affinity, low capacity, estrogen and tissue specificity). Their sedimentation properties are similar to those reported for ER of non-neural target tissues: 7S for the cytosolic form in low salt buffers versus 5S for the nuclear form under conditions of high salt. Both Sephadex LH-20 gel filtration and DNA-cellulose chromatography are effective in separating free and bound steroid in this system where receptor levels are low, and the two methods give comparable results with respect to affinity constants and receptor number.

Although the elution profile of these ER on DNA-cellulose affinity columns (elution maximum: 0.18 M NaCl) is similar to recent observations by Vito *et al.* in the same species [12], our saturation analysis indicates that specific estrogen binding approaches saturation at 4–5 nM [^{3}H]E_2, whereas the earlier study utilizing the same methodology reported that [^{3}H]E_2 binding saturated at 1–5 nM. These experimental differences require further investigation since receptor binding sites will be underestimated if a concentration of [^{3}H]E_2 below saturation is used for measuring receptor levels. Furthermore, the cytosolic concentrations observed in castrated animals in the present study are higher than those reported by Vito *et al.*[12] although their tissue samples (HTH/POA) are more likely to be receptor-rich than the larger limbic block used here. We attribute these differences in part to the addition of 30% glycerol to our homogenization buffers, since this reagent has been shown to enhance E-binding activity in hamster uterus [19].

Table 3. Neuroanatomical distribution of cytosolic estrogen receptor (fmol/g tissue) in male and female hamster brain

	Limbic brain		Remaining forebrain		Mid/hindbrain	
	Male	Female	Male	Female	Male	Female
Experiment 1	50	75	37	40	13	15
Experiment 2	45	68	24	38	9	10

Intact male and female hamster brains were dissected into limbic blocks, remaining forebrains and mid/hindbrains as described in Experimental. Cytosolic ER in pooled brain regions were measured by DNA-cellulose affinity chromatography after incubation with [^{3}H]E_2 (10 nM) ± 100-fold excess radioinert E_2.

The gross neuroanatomic distribution of ER within the hamster CNS is like that previously described for other vertebrates [3–7] with greatest concentrations in limbic regions. Nonetheless, low levels of specific E-binding activity are consistently detectable in remaining forebrain and mid/hindbrain tissues, presumably reflecting the presence of scattered target cells seen in these regions by autoradiography [10].

Although species comparisons are not strictly accurate due to differences in assay methods and in the exact brain regions used for testing, our data in general confirm early biochemical and autoradiographic studies [10, 11] by demonstrating that ER levels in hamster brain (4–7 fmol/mg protein) are lower than those of adult rat and mouse (15–20 fmol/mg) [3,4]. They are similar, however, to those of the gerbil (6–8 fmol/mg) [5], guinea pig (7–9 fmol/mg) [6], perinatal rat (2–4 fmol/mg) [24] and freshwater turtle (1–5 fmol/mg) [7]. The dissociation constant reported here for hamster brain ER ($K_d = 10^{-1}$ M) is 10-fold higher than that of the rat [24], mouse [4], and guinea pig [6] and is 100-fold higher than that of the hamster uterine ER [19]. It is this property rather than receptor number *per se* which is more likely to account for the lower uptake of injected [^{3}H]E$_2$ [10] and the relative insensitivity of E-dependent behavioral and neuroendocrine responses in hamsters vs rats [13].

When we examined the binding specificity of the cytosolic and nuclear E-binding components in hamster brain using a ^{3}H-ligand concentration of 5 nM, E-binding was inhibited substantially by androgens and progesterone. This apparent lack of specificity was not apparent, however, when ligand concentration was increased to 10 nM or when hamster uterine receptor was tested under the same assay conditions at either 5 or 10 nM tracer concentrations. We infer from this that the different binding kinetics of receptor in hamster brain and uterus are due to differences in tissue receptor concentrations and binding affinities. For example, in tissues like brain with exceedingly low receptor levels, effective ligand concentrations may be lower than calculated due to large amounts of non-specific binding components in the sample. This, in turn, would affect tracer/competitor ratios. It has been shown that [^{3}H]E$_2$ binding in mouse brain cytosol was partially blocked by androgen at a concentration below saturation (2 nM) but not at 20 nM [25]. Also, androgens appear to inhibit the initial rate of ER complex formation in rat pituitary cytosol although no inhibition is seen under equilibrium conditions [26].

The ability of 5α-androstan-3β,17β-diol to compete for [^{3}H]E$_2$ binding in hamster brain is consistent with the observation that this metabolite can bind to and translocate estrogen receptors in the brain and pituitary of the rat [27]. It is interesting to note that DHT mimics certain E actions on copulatory behavior and brain sex differentiation in hamsters [14, 16]. Indeed, these observations have been used to argue against the role of aromatization in mediating certain androgen actions but do not take into account the possibility that DHT or its metabolites act via the ER system. Although both 5α and 5β-reductase activities are high in hamster brain [14], we have shown that the effects of testosterone on ER accumulation in nuclear subfractions can be blocked by an aromatase inhibitor [18]. This implies that 5α-reduced metabolites formed *in situ* from added testosterone have little or no effect *in vitro* but does not rule out the possibility that nuclear ER can be occupied when animals are treated directly with DHT or 5α-androstan-3β,17β-diol.

Although we found no sex differences in cytosolic receptor number in grossly dissected limbic brain regions, in an earlier study it was reported that male HTH accumulated more radioactivity than female HTH following [^{3}H]E$_2$ injection [9]. In these same experiments, radioactivity was equivalent in the POA regardless of sex. Sex differences in the number of steroid receptors in discrete locations within the limbic brain have also been described in rats [28]. No obvious male–female differences are observed in hamsters when steroid binding affinity, specificity, or gross neuroanatomic distribution of ER were examined in either cellular compartment.

After a single injection of E, there was an increase of ER in nuclear subfractions and a quantitative decrease in cytosolic ER; however, cytosol was only partially depleted of receptor. This contrasts with the situation in hamster uterus in which essentially all cytosolic ER can be translocated by the same dose of E$_2$ [8, 19]. A translocation-resistant component has been identified in brain cytosols of the rat [2] and turtle [29] regardless of E dosage, and is a feature of progesterone and glucocorticoid receptors in brain also [30, 31]. The reason for this partial translocation upon hormone administration is not presently understood but may be related to receptor turnover rates or separate receptor pools in the CNS when compared to non-neural targets.

This report which characterizes the ER system in hamster brain provides a basis for further studies of receptors in the neuroendocrine control of seasonal breeding.

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