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Estradiol Selectively Regulates Agonist Binding Sites on the *N*-Methyl-D-Aspartate Receptor Complex in the CA1 Region of the Hippocampus

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ABSTRACT

Estradiol alters cognitive function and lowers the threshold for seizures in women and laboratory animals. Both of these activities are modulated by the excitatory neurotransmitter glutamate in the hippocampus. To assess the hypothesis that estradiol increases the sensitivity of the hippocampus to glutamate activation by increasing glutamate binding sites, the densities of *N*-methyl-D-aspartate (NMDA) agonist sites (determined by NMDA displaced glutamate), competitive antagonist sites (CGP 39653), noncompetitive antagonist sites (MK801) as well as the non-NMDA glutamate receptors for kainate and AMPA (using kainate and CNQX, respectively) were measured using autoradiographic procedures. Two days of estradiol treatment increased the density of NMDA agonist, but not of competitive nor noncompetitive NMDA antagonist binding sites exclusively in the CA1 region of the hippocampus. The density of noncompetitive NMDA antagonist sites, however, was decreased in the dentate gyrus by estradiol treatment. Ovarian steroids had no effect on the density of kainate or AMPA receptors in any region of the hippocampus examined. These data indicate that the agonist and antagonist binding sites on the NMDA receptor/ion channel complex are regulated independently by an as yet unidentified mechanism, and that this regulation exhibits regional specificity in the hippocampus. The increase in NMDA agonist sites with ovarian hormone treatment should result in an increase in the sensitivity of the hippocampus to glutamate activation which may mediate some of the effects of estradiol on learning and epileptic seizure activity. (*Endocrinology* **131:** 662–668, 1992)

ESTRADIOL exerts significant effects on certain aspects of cognitive function in humans (1, 2) and laboratory animals (3, 4). Estradiol also depresses the threshold for seizure activity in women (5) and kindled animals, an experimental model for epilepsy (6–8). The hippocampus is the primary brain region which mediates these activities. It is a major component of the limbic system and plays a role in reproductive and nonreproductive behaviors associated with some forms of learning and memory. The hippocampus is also highly susceptible to ischemic damage, and injury to this region is associated with increased epileptiform activity and seizures (9).

The mechanisms through which estradiol exerts its effects on hippocampal functions have not been established. In other brain regions, the actions of estradiol on gonadotropin release and sexual behavior are mediated by alterations in the activity of several neurotransmitters or the density of their receptors including norepinephrine (10–12), serotonin (13), β-endorphin (14–16), and/or oxytocin (17–19). In the hippocampus, the major excitatory neurotransmitter is the glutamate system. The activation of glutamate receptors mediates processes involved in the synaptic plasticity associated with learning and memory (20, 21) and epileptogenesis (22–24). Treatment with estradiol alters the sensitivity of neurons to glutamate and *N*-methyl-D-aspartate (NMDA) (25–27) which correlates in the cerebellum with changes in sensorimotor actions (28). If a parallel situation exists in the hippocampus, estradiol may influence learning behaviors and seizure activity by altering the sensitivity of hippocampal neurons to glutamate activation.

A change in the density of neurotransmitter receptors is one mechanism through which the sensitivity of neurons may be altered. Glutamate activates at least three distinct classes of receptors: the NMDA iontropic receptors, the non-NMDA kainate/AMPA (α-amino-3-hydroxy-5-methyl-4-isoxasole propionic acid) iontropic receptors, and the glutamate metabotropic receptor (29). The NMDA receptor/ion channel complex is comprised of distinct binding domains through which the function of the channel can be modulated by estradiol. These sites include: 1) the transmitter recognition site which binds agonists such as NMDA and glutamate, 2) competitive antagonist site which binds 3-((±)-2-carboxypiperazine-4-yl)-propyl-1-phosphonate (CPP) (30), and CGP 39653 (31), 3) allosteric sites that bind glycine and polyamines which function to facilitate agonist-induced channel openings (32, 33), and 4) sites within the receptor-associated ion channel that bind noncompetitive antagonists such as MK801 (34) and PCP and for Mg^{2+} which inhibits current flow. Ovarian steroids could modulate the activity of the receptor complex by altering one or more of the binding sites. To address this issue, I have measured the effect of estradiol and progesterone treatment on the density of binding sites for agonists and antagonists of NMDA receptors and non-NMDA glutamate receptors in the hippocampus.

Materials and Methods

Animals

Sprague-Dawley (Zivic Miller, Allison Park, PA) female rats (3–4 months of age) were maintained in a temperature-regulated environment

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on a 14–10 light-dark cycle (lights on at 0400 h) for 1–2 weeks before being ovariectomized (n = 30) under ether anesthesia. One week after ovariectomy, 20 of the rats were implanted with Silastic capsules containing 180 μg 17β-estradiol/ml sesame oil (2 cm × 0.625 mm id). Two days later, 10 of the estradiol-treated rats received sc injections of progesterone (10 mg/kg BW) at 1030 h. This treatment paradigm was selected to produce hormone levels in serum within the physiological range to induce gonadotropin release simulating preovulatory surges (16). At 1500 h, all the animals were killed, the brains were removed and frozen on dry ice. Trunk blood was collected for determination of the concentration of estrogen, progesterone, and LH in serum. The brains were sectioned at 8 μm on a cryostat, thaw-mounted on gelatin-coated slides, and stored at −70 C until used for quantitative autoradiography of receptor densities.

Hormone assays

RIA of estradiol and progesterone was performed with kits purchased from Radioassay Systems (ICN Biomedicals, Inc., Costa Mesa, CA) using ^{125}I-labeled estradiol and progesterone with modifications as described previously (12). For the measurement of estradiol, 0.5 ml serum was extracted with ether before assay. If the level of estradiol was below the level of sensitivity of the assay, 5 pg/ml, this value was used in the statistical analysis. Six of nine animals had estradiol levels below detectable levels. None of the progesterone values were below detectable levels (0.5 ng/ml).

The concentration of LH in serum was determined using established methods (35). The oLH (GDN 15) antibody was provided by G. D. Niswender (Fort Collins, CO); the LH-RP2 standard was provided by NIDDK; and the ^{125}I-oLH was obtained from Hazelton Biotech. Corp. (Vienna, VA). The lower limit of detection was 0.5 ng/ml for LH.

NMDA receptor agonist site

For determination of the NMDA neurotransmitter recognition site, slide-mounted sections were preincubated in 50 mM Tris-acetate buffer (pH 7.4) for 45 min at room temperature and dried briefly before incubation in buffer containing 125 nM ^{3}H-glutamate [25 Ci/mmol, New England Nuclear (NEN), Boston, MA] in the presence or absence of NMDA (2 μM–1 mM) for 30 min at 4 C. Tris-acetate buffer enhances the binding of ^{3}H-glutamate to the NMDA-selective subtype of glutamate receptor (36). After incubation with radiolabeled ligand, the sections were washed 4 × 5 sec in buffer on ice, rinsed in 2.5% glutaraldehyde and acetone, and dried briefly on a slide warmer. The density of NMDA receptors was defined as that amount of ^{3}H-glutamate binding that could be displaced by NMDA.

NMDA competitive antagonist site

To determine whether or not the observed estradiol-induced increase in NMDA agonist binding sites was a reflection of an increase in the total number of NMDA ion channels/receptor complexes, the densities of the competitive and noncompetitive antagonist sites were also measured. Competitive NMDA antagonist sites were measured using ^{3}H-CGP 39653 (51.3 Ci/mmol, NEN). Since CGP 39653 (a gift from Ciba Geigy, Summit, NJ) has not previously been used for autoradiography (31), preliminary studies were done to established optimal conditions for maximal binding. Brain sections (8 μm) were preincubated in 50 mM Tris-HCl, 50 mM Tris-acetate, or 5 mM Tris-HCl at varying pH to remove endogenous ligand, incubated for 0–3 h on ice with 4 nM ^{3}H-CGP 39653 in the same buffer as used for preincubation in the presence or absence of 1 mM glutamate. The sections were rinsed 3 × 5 sec, removed from the slide with a Whatman glass fiber filter, and counted in a scintillation counter. Based on the results of the preliminary binding studies with CGP 39653, the following conditions were established for autoradiography. Sections were preincubated in 50 mM Tris-HCl, pH 7.8, for 1 h at room temperature, before incubation with 8 nM ^{3}H-CGP 39653 (51.3 Ci/mmol, NEN) for 3 h at 4 C. Nonspecific binding was determined by the addition of 100 μM L-glutamate or 10 μM CGP 037849, a competitive inhibitor of NMDA (37) (gift from Ciba Geigy), to the incubation medium. Sections were rinsed 3 × 5 sec in buffer on ice and dried rapidly under a stream of cool air. Sections were exposed to LKB Ultrofilm for 2 weeks.

Noncompetitive NMDA antagonist site

The noncompetitive NMDA antagonist site was determined using MK801 which binds to the ion channel. Scatchard analysis of MK801 binding was performed in Tris-HCl buffer (pH 7.4) containing 0.2–10 nM ^{3}H-MK801 (22.5 Ci/mmol, NEN) and 50 μM each of glutamic acid, glycine, and spermidine to enhance binding to the noncompetitive antagonist binding site in the NMDA ion channel (34). Sections were preincubated in buffer for 10 min at 4 C, incubated in buffer with radiolabeled MK801 for 1 h at room temperature, washed 3 × 10 sec at 4 C, dipped in distilled water, and dried briefly on a slide warmer. One point assays were done using 4 nM MK801. Nonspecific binding (10–15%) was defined as binding in the presence of 100 μM unlabeled MK801 (gift of Merck Sharp and Dohme, Harlow, Essex, England).

Glutamate sites

For determination of glutamate binding sites, sections were preincubated in Tris-acetate buffer (pH 7.4) for 45 min to remove endogenous ligand, incubated in buffer and 125 nM ^{3}H-glutamate (25 Ci/mmol, NEN) with or without varying concentrations of unlabeled glutamate (0.0125 − 1 μM) for 30 min at 4 C, rinsed 4 × 5 sec in buffer at 4 C, rinsed in 2.5% glutaraldehyde in acetone, and dried.

Kainate receptors

For determination of kainate receptors, sections were preincubated for 1 h in buffer before incubation with 20 nM ^{3}H-kainate (58 Ci/mmol, NEN) in 0.31 M Tris-citrate buffer, pH 7.2, for 2 h on ice (38). Nonspecific binding was determined in the presence of 0.1 mM L-glutamate. Sections were rinsed 2 × 1 min in ice-cold buffer, dried under a stream of cool air, and exposed to film for 4–5 weeks.

CNQX sites

Competitive antagonist binding for AMPA receptors was determined using 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) with methods as described by Kerwin *et al.* (38) with minor modifications. Sections were preincubated in buffer to remove endogenous ligands, incubated in 50 mM Tris-HCl, pH 7.3, containing 20 nM ^{3}H-CNQX (17.3 Ci/mmol, NEN) for 1 h on ice, rinsed 3 × 5 sec, dried under a stream of cool air, and exposed to film for 10–14 days. Nonspecific binding was determined by the addition of 1 mM L-glutamate to the incubation buffer.

Autoradiography

After exposure to labeled sections and ^{3}H-standards for 1–6 weeks, the films (LKB Ultrofilm, LKB, Inc., Gaithersburg, MD) were developed, and fixed using conventional photographic techniques. Quantitative analysis of the films was done using an image analysis system (Image Technology Corp., Deer Park, NY). A fixed window was placed over the brain region of interest, and the relative optical density was measured and converted to concentrations in fmoles/mg protein using the tritiated standards. Bilateral measurements were made on 4 sections for total binding and 2 sections for nonspecific binding for each animal (10 animals/group).

Data analysis

Analysis of the receptor binding data was performed using LIGAND PC (Peter J. Munson, NICHHD). Statistical analysis of the data was performed using a one-way analysis of variance for effect of treatment followed by the Ryan, Einot, Gabriel, and Welsch multiple F test (39).

Results

Serum concentrations of estradiol were 6.3 ± 0.4, 18.6 ± 1.8, and 16.8 ± 1.1 pg/ml in ovariectomized, estradiol, and estradiol plus progesterone-treated animals, respectively. In the ovariectomized rats, six of nine animals had levels of

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estradiol below the sensitivity of our assay (5 pg/ml); thus the 6.3 pg/ml mean level is an overestimate of serum estradiol levels. Progesterone levels were 4.6 ± 0.9, 12.3 ± 3.2, and 440 ± 67 ng/ml in ovariectomized, estradiol, and estradiol plus progesterone-treated rats respectively. Treatment with estradiol alone significantly ($P < 0.05$) increased the levels of progesterone in the serum compared to ovariectomized rats. The source of this progesterone may be the adrenal gland. LH levels were 12.8 ± 1.3, 19.5 ± 2.4, and 81.1 ± 6.2 in ovariectomized, estradiol-treated, and estradiol plus progesterone-treated animals.

Two days of estradiol treatment increased the density of NMDA displaceable glutamate binding sites by 30% in the CA1 region of the hippocampus (Fig. 1). In the estradiol plus progesterone-treated animals the density of glutamate binding to NMDA sites was increased 45%, but this was not a significant increase over the density of NMDA sites observed in animals receiving estradiol alone. Ovarian steroid treatment had no effect on the density of receptors in the dentate gyrus. Analysis of NMDA-displaceable ^{3}H-glutamate binding in the CA1 region of the hippocampus demonstrated an estradiol-induced increase in the maximum number of binding sites and no change in the inhibition constant (K_i) (Table 1).

Data from preliminary binding assays with CGP 39653 demonstrated that the binding of CGP 39653 is pH dependent with maximal binding occurring between pH 7.8 and 8.2 (Fig. 2). Binding increases over time and saturates between 1 and 2 h, remaining elevated through 3 h (data not shown), and binding was greater in 50 mM Tris-HCl compare to 5 mM Tris-HCl (Fig. 3) or 50 mM Tris-acetate (Fig. 2). Autoradiograms generated using ^{3}H-CGP 39653 to label the NMDA competitive antagonist sites (Fig. 4) were specific, reproducible, and the distribution of binding sites paralleled those observed previously for another NMDA competitive antagonist CPP (40).

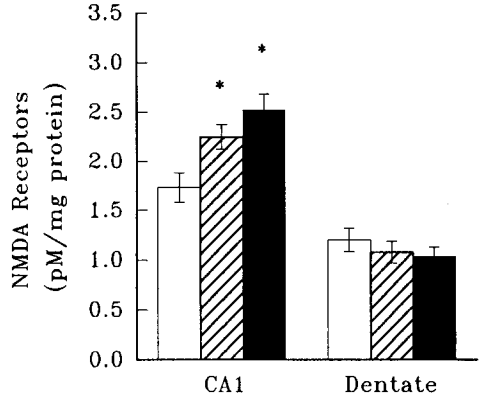

FIG. 1. NMDA binding sites in the CA1 and dentate gyrus of the hippocampus. Treatment with estradiol (*hatched bars*) increased NMDA sites by 30% compared to ovariectomized rats (*open bars*) in the CA1 region. The level of NMDA binding after estradiol plus progesterone treatment (*filled bars*) was not significantly different from levels after treatment with estradiol alone. No steroid-induced changes in NMDA binding occurred in the dentate gyrus compared to ovariectomized animals (*, $P < 0.05$).

TABLE 1. NMDA binding in Ca1 stratum radiatum of the hippocampus

Treatment	K_I (μM)	B_{max} (pmoles/mg protein)
Ovariectomy	15 ± 3	2.3 ± 0.2
Estradiol	19 ± 4	3.7 ± 0.9[a]
Progesterone	18 ± 4	3.3 ± 0.7[a]

[a] $P < 0.05$ compared to ovariectomy.

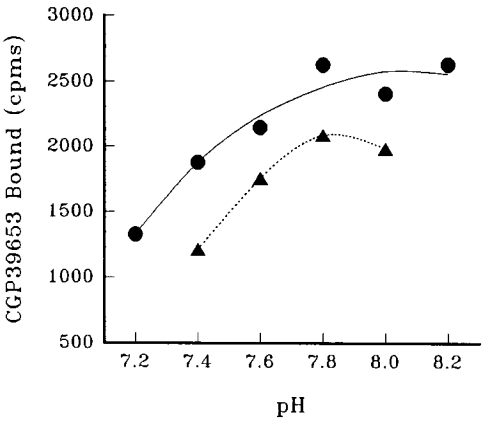

FIG. 2. Specific binding of ^{3}H-CGP 39653 (51.3 Ci/mmol) in whole coronal sections of brain from regions containing rostral hippocampus. The binding of CGP 39653 is greater in 50 mM Tris-HCl (*circles*) than in 50 mM Tris-acetate (*triangles*) buffer. The binding is pH dependent with maximal binding between pH 7.8 and 8.2.

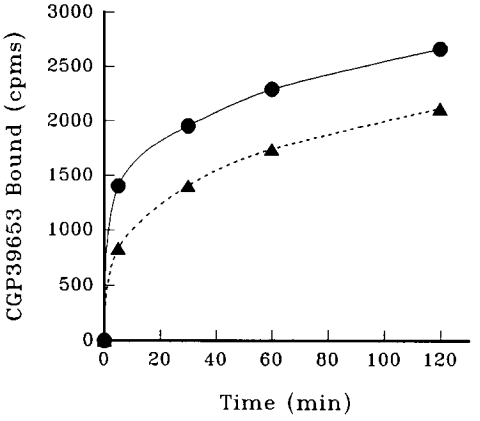

FIG. 3. Total binding of ^{3}H-CGP 39653 (51.3 Ci/mmol) in 50 mM Tris-HCl (*circles*) and 5 mM Tris-HCl (*triangles*) in coronal slices of brain through the region of rostral hippocampus. Binding was greater in 50 mM Tris-HCl, and saturation occurred by 2 h and remained elevated for at least 3 h (data for 3-h time point not shown).

In contrast to NMDA agonist sites, no estrogen-dependent alterations in the density of binding sites for CGP 39653 were observed in any region of the hippocampus (Fig. 5). In addition, estradiol and progesterone had no effect on the density of MK801 binding in the CA1 region of the hippocampus, and decreased MK801 binding in the dentate gyrus (Fig. 6). Scatchard analysis of MK801 binding in the CA1 and dentate regions of the hippocampus demonstrated a significant decrease in the maximal number of binding sites in the dentate gyrus in the steroid-treated animals (Table 2). There is a small but significant decrease in the dissociation constant (K_d) of binding in the same region. Estradiol and

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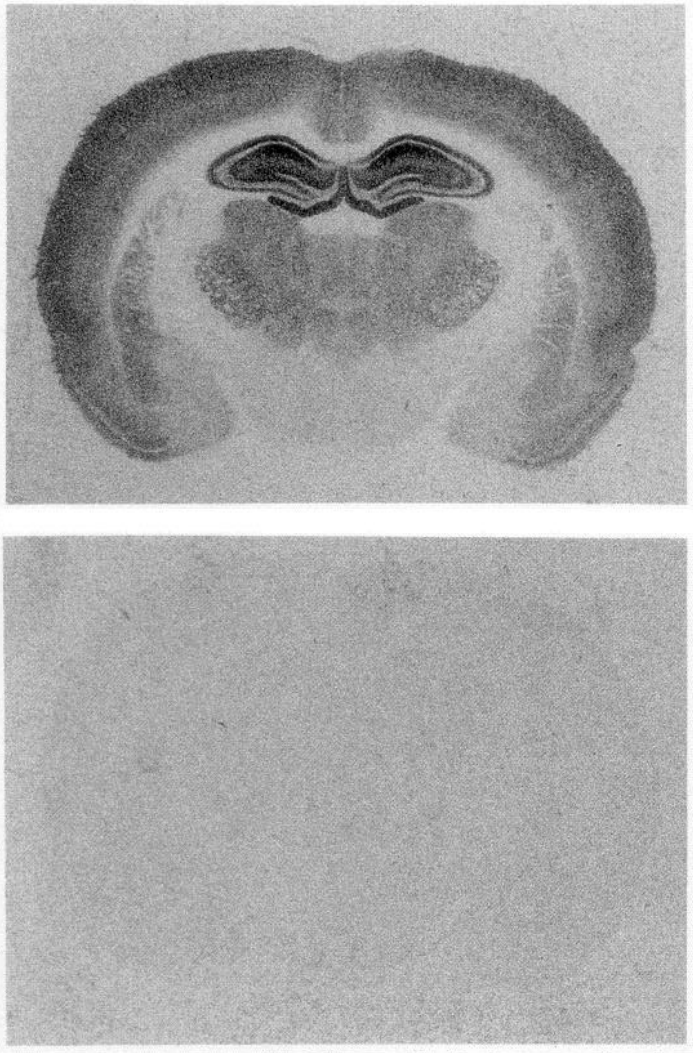

FIG. 4. Autoradiogram of ^{3}H-CGP 39653 binding in a coronal section of rat brain through the rostral hippocampal region. The lower figure is the nonspecific binding in the presence of 10 μM CGP 037849 (an NMDA competitive antagonist).

estradiol plus progesterone had no effect on the K_d nor the maximal number of MK801 binding sites in the CA1 region of the hippocampus. These data indicate that not only are the antagonist binding sites regulated independently of the agonist site, but that this regulation is limited to specific brain regions.

No steroid-induced differences were detected in the non-NMDA glutamate receptors labelled by kainate (Fig. 7) or CNQX (Fig. 8) in any region of the hippocampus. A remaining possibility is that estradiol influences only the agonist binding site recognized by AMPA or the metabotropic glutamate receptor site activated by quisqualate or ibotenate (29, 41).

To determine whether treatment with estradiol increased the number of NMDA sites in another more traditionally estrogen-responsive region in a manner parallel to that in the hippocampus, the densities of NMDA displaceable glutamate binding sites were measured in the medial preoptic

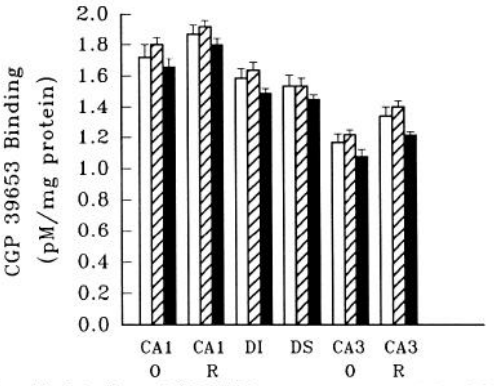

FIG. 5. Specific binding of ^{3}H-CGP 39653, a competitive NMDA antagonist, in the hippocampus. The *open bars* represent binding in ovariectomized rats, the *hatched bars* estradiol-treated ovariectomized animals, and the *filled bars* estradiol- plus progesterone-treated ovariectomized animals. Abbreviations: O, stratum oriens; R, stratum radiatum; DI, inferior limb of the dentate gyrus stratum moleculare; DS, superior limb of the dentate. No steroid-induced differences in competitive antagonist binding sites were observed in any region of the hippocampus.

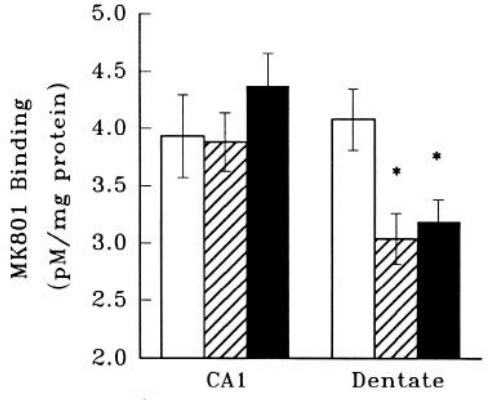

FIG. 6. Specific binding of ^{3}H-MK801 to the noncompetitive NMDA antagonist site located within the ion channel in the hippocampus of ovariectomized (*open bars*), estradiol-treated (*hatched bars*), and estradiol- plus progesterone-treated (*solid bars*) ovariectomized rats. Estradiol treatment decreased the level of MK801 binding in the dentate gyrus but had no effect on MK801 binding in the CA1 region of the hippocampus. (*, $P < 0.05$ compared to ovariectomized group).

TABLE 2. Scatchard analysis of MK801 binding in the dentate gyrus of the hippocampus

Treatment	K_d (nM)	B_{max} (pmoles/mg protein)
Ovariectomy	2.8 ± 0.4	5.8 ± 0.6
Estradiol	1.9 ± 0.5	4.2 ± 0.4[a]
Progesterone	1.3 ± 0.2[a]	3.4 ± 0.2[a]

[a] $P < 0.05$ compared to ovariectomy.

nucleus of the hypothalamus. In the preoptic nucleus, where NMDA binding represents less than 15% of total ^{3}H-glutamate binding even in buffer systems which enhance NMDA binding, there is a steroid-induced increase in ^{3}H-glutamate binding sites (Fig. 9), but this binding is not displaceable by NMDA. From this we may conclude that estradiol does not change the density of NMDA binding sites in the medial

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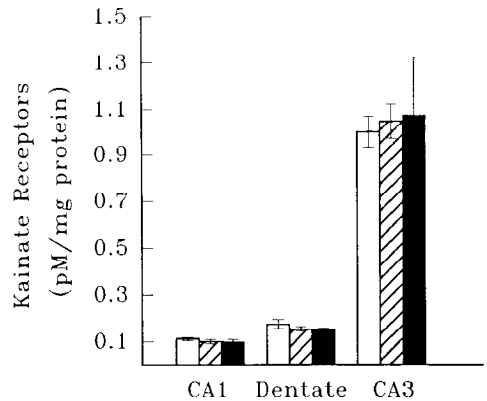

FIG. 7. Specific binding of ^{3}H-kainate (20 nM) in the hippocampus in ovariectomized (*open bars*), estradiol-treated (*hatched bars*), and estradiol- plus progesterone-treated (*filled bars*) ovariectomized rats. The greatest level of kainate binding was observed in the mossy fiber pathway to the CA3 region of the hippocampus. Treatment with ovarian hormones had no effect on kainate binding in any region.

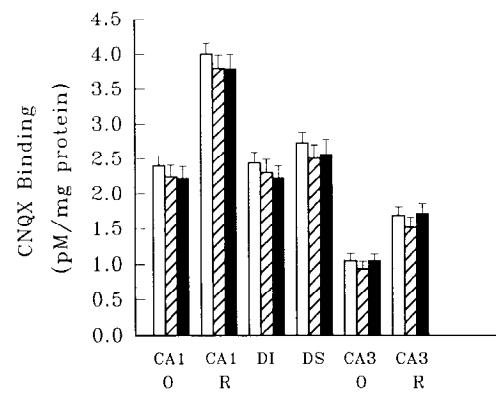

FIG. 8. Specific binding of ^{3}H-CNQX, a competitive antagonist to non-NMDA glutamate receptors, in ovariectomized (*open bars*), estradiol-treated (*hatched bars*), and estradiol- plus progesterone-treated rats (*filled bars*). Estradiol and progesterone had no effect on CNQX binding in any region of the hippocampus examined. Abbreviations: O, stratum oriens; R, stratum radiatum; DI, inferior limb of the dentate; DS, superior limb of the dentate gyrus.

preoptic nucleus of the hypothalamus but increases one or more of the non-NMDA glutamate sites.

Discussion

Estradiol (and/or estradiol plus progesterone) appears to regulate the NMDA but not the non-NMDA glutamate iontropic receptors in the hippocampus. Furthermore, the present data suggests strongly that the sites on the NMDA receptor/ion channel complex are regulated independently since the neurotransmitter recognition site (identified by NMDA binding), the competitive antagonist site (identified by CGP 39653), and the ion channel inhibitory site (identified by the noncompetitive antagonist MK801), do not change in parallel in response to treatment with estradiol. In addition, the effects of ovarian hormones on these receptors exhibit regional specificity in the hippocampus: NMDA agonist sites are increased in CA1; competitive antagonist sites do not change in the CA1; and MK801 sites are decreased in

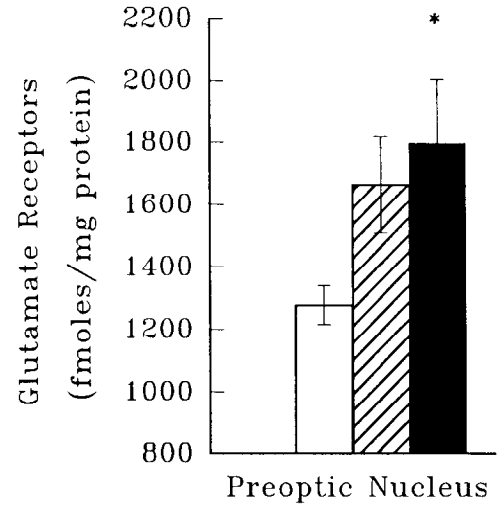

FIG. 9. Specific ^{3}H-glutamate binding in the medial preoptic nucleus of the hypothalamus in ovariectomized (*open bars*), estradiol-treated (*hatched bars*), and estradiol- plus progesterone-treated animals (*filled bars*). Glutamate binding increased in estradiol- plus progesterone-treated animals compared to ovariectomized controls, whereas estradiol-treated animals had intermediate levels of glutamate binding sites. (*, $p < 0.05$ compared to ovariectomized group).

the dentate gyrus. In contrast, non-NMDA glutamate receptors are regulated by estradiol in the medial preoptic nucleus of the hypothalamus. The fact that estradiol treatment caused a small but significant increase in circulating progesterone levels raises the possibility that the combined elevations of both hormones produced the changes in glutamate binding to NMDA receptors. Whether these neuroendocrine influences of estradiol and progesterone are mediated by a steroid receptor/genomic event or through membrane effects has not been determined.

Other evidence supports the possibility that the agonist and competitive antagonist sites are two distinct binding sites. First, the distribution of sites labeled by NMDA displaceable glutamate is anatomically distinct from that labelled with the competitive antagonists CPP (33) or CGP 39653. Second, the polyamine spermine can enhance competitive antagonist binding while inhibiting agonist binding (42), supporting the idea that these are two sites are regulated differentially. Third, radiation inactivation analysis suggests that agonist and antagonists bind two different sized proteins (43). Fourth, recent work on purification, isolation, and cloning of a putative NMDA receptor subunit, demonstrated that the glutamate and CPP binding sites were on separate subunits (30, 44). The NMDA subunit cloned by Kumar *et al.* (44) exhibits no substantial homology with the non-NMDA glutamate receptor, contains the glutamate binding site, and requires reconstitution with three other subunits (one of which contains the CPP or competitive antagonist binding site) for functional activity (30, 44). Independently, Moriyoshi *et al.* (45) have cloned an NMDA receptor subunit that has significant homology to the GluR1–7 clones for the non-NMDA iontropic glutamate receptor subunits, and the single protein has functional activity when expressed in *Xenopus* oocytes. The Moriyoshi *et al.* (45) clone is clearly an NMDA receptor subunit, but it remains to be determined whether or

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not the Kumar *et al.* clone (44) is a functional NMDA receptor, and/or whether one subunit may contain all of the agonist, antagonist, and modulatory sites.

Estradiol may regulate the NMDA receptor by altering the stoichiometry of the subunits and/or by increasing gene transcription, translation and synthesis of the agonist-containing subunit. Based on prior investigations with other ligand-gated ion channel receptors such as the AMPA/kainate and $GABA_A$ receptors, it was predicted that the NMDA ion channel receptor complex was a hetero-oligomer with subunits coded by different genes. Each subunit of both the $GABA_A$ and AMPA/kainate appear to bind the different agonists and modulators of their respective receptors, but the ion channel activity induced is specific for each ligand and subunit (46, 47), and only the ion currents produced by hetero-oligomers simulate ion currents of the native receptors (47). In addition, subunits of both $GABA_A$ and AMPA/kainate receptors exhibit regional specificity in the hippocampus (48, 49). Evidence exists for heterogeneity and regional specificity of the NMDA receptors as well (33, 50). The NMDA receptors appear to have a parallel structure, and the effects of estrogen may be limited to specific subunit genes. Alternatively, the effects of estradiol and/or progesterone may be mediated by alterations in the glycine or the polyamine modulatory sites or the release of endogenous ligands for these sites. Glycine enhances NMDA-displaceable ^{3}H-glutamate binding sites (33), but the effect of glycine has been reported to be an affinity rather than a binding density change (51).

The estradiol-induced decrease in MK801 binding sites in the dentate gyrus is interesting. Compared to the CA1 region, the dentate gyrus has not been identified as a hippocampal region particularly responsive to ovarian steroids. In contrast, regions of the entorhinal cortex and the amygdala which provide the significant afferents to the dentate gyrus of the hippocampus (52) contain high levels of estrogen receptors (53). The alteration in MK801 binding sites in the dentate may occur indirectly in response to ovarian hormone-induced changes in the afferent input such as increased release of glutamate or glycine from these regions. Glutamate increases the affinity of MK801 for its binding site, and this effect is enhanced by glycine (51). The increase in affinity of MK801 for its binding site has been interpreted as increased activation of channel opening allowing for enhanced NMDA stimulation (54).

In summary, the CA1 region of the hippocampus appears to be a prime target for ovarian steroids. Binding sites (53), immunoreactivity (55), and mRNA (56) for the estrogen receptor are present in the hippocampus allowing for a direct site of action for estradiol. Estradiol induces progestin receptors (57), increases $GABA_A$ receptors (58), increases dendritic spine density (59), induces synapse formation (60), and increases the excitability of neurons (25) exclusively in the CA1 region. Present data showing estradiol-induced increases in NMDA binding sites resulting in increased sensitivity to glutamate in CA1 provides for an additional mechanism for estradiol's influence on learning, memory, and epileptic seizure activities mediated by this region of the hippocampus.

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