

Local Neurosteroid Production in the Hippocampus: Influence on Synaptic Plasticity of Memory

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Key Words

Hippocampus · Estradiol · Neurosteroid · Synaptic plasticity · Estrogen receptor

Abstract

In neuroendocrinology, it is believed that steroid hormones are synthesized in the gonads and/or adrenal glands, and reach the brain via the blood circulation. In contrast to this view, we are in progress of demonstrating that estrogens and androgens are also synthesized locally by cytochrome P450s in the hippocampus, and that these steroids act rapidly to modulate neuronal synaptic plasticity. We demonstrated that estrogens were locally synthesized in the adult hippocampal neurons. In the pathway of steroidogenesis, cholesterol is converted to pregnenolone (by P450scc), dehydroepiandrosterone [by P450(17 α)], androstenediol (by 17 β -hydroxysteroid dehydrogenase, 17 β -HSD), testosterone (by 3 β -HSD) and finally to estradiol (by P450arom) and dihydrotestosterone (by 5 α -reductase). The basal concen-

tration of estradiol in the hippocampus was approximately 1 nM, which was greater than that in blood plasma. Significant expression of mRNA for P450scc, P450(17 α), P450arom, 17 β -HSD, 3 β -HSD and 5 α -reductase was demonstrated by RT-PCR. Their mRNA levels in the hippocampus were 1/200–1/5,000 of those in the endocrine organs. Localization of P450(17 α) and P450arom was observed in synapses in addition to endoplasmic reticulum of principal neurons using immunoelectron microscopy. Different from slow action of gonadal estradiol which reaches the brain via the blood circulation, hippocampal neuron-derived estradiol may act locally and rapidly within the neurons. For example, 1 nM 17 β -estradiol rapidly enhanced the long-term depression (LTD) not only in CA1 but also in CA3 and dentate gyrus. The density of thin spines was selectively increased within 2 h upon application of 1 nM estradiol in CA1 pyramidal neurons. Only ER α agonist propyl-pyrazole-trinyl-phenol induced the same enhancing effect as estradiol on both LTD and spinogenesis in the CA1. ER β agonist hydroxyphenyl-propionitrile suppressed LTD and did not affect spinogenesis. Localization of estrogen receptor ER α in spines in addition to nuclei of principal neurons implies that synaptic ER α can drive rapid modulation of synaptic plasticity by endogenous estradiol.

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Presented at the Sixth International Congress of Neuroendocrinology, June 19–22, 2006, Pittsburgh, Pa., USA.

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Introduction

In recent years, increasing evidence has accumulated to support the local endogenous synthesis of estrogens and androgens in the mammalian brain, such as the hippocampus [1–5]. In 1980s, Baulieu and co-workers have proposed a neurosteroid hypothesis, suggesting that pregnenolone (PREG), progesterone and dehydroepiandrosterone (DHEA) may be endogenously synthesized in the brain due to the finding that PREG and DHEA has been found in the mammalian brain at concentrations greater than that in plasma [6, 7]. Because the concentration of PREG and DHEA does not decrease after adrenalectomy and castration, many experiments have been performed with the aim of demonstrating the *de novo* synthesis of DHEA within the brain [6, 8].

Direct demonstration of steroidogenesis in the mammalian brain had, however, long been unsuccessful, due to the extremely low levels of steroidogenic proteins in the brain [9]. In particular, sex steroids had not been considered to be brain-derived steroids, and rather thought to reach the brain exclusively via blood circulation [10]. This belief is supported by many reports suggesting the absence of cytochrome P450(17 α) (DHEA synthase) in adult mammalian brain [11, 12] and also by the observation of the complete disappearance of testosterone in the brain within 1 day after castration [10]. The hippocampus is a center of learning and memory processes, and is known to be a target for the neuromodulatory actions of sex hormones produced in the gonads. Estrogens and androgens have specific contributions to rapid action on the synaptic plasticity as neuromodulators. As both estradiol and testosterone may reach the brain via blood circulation after crossing the blood brain barrier, extensive studies have been performed to investigate their role in modulating hippocampal plasticity and function [13–18].

Because of the extremely low level of expression of P450s and hydroxysteroid dehydrogenases (HSDs) in the hippocampus, much lower than 1/1,000 of their levels in endocrine organs, many scientists had not seriously considered that hippocampal steroidogenesis plays an essential role in the hippocampal function. In order to describe biological significance of brain steroids, it is essential to improve the sensitivity of measurements by nearly 1,000-fold for immunostaining, Western blot, RT-PCR as well as purified steroid detection. Here, we describe recent progress in studies on local synthesis of estrogens and androgens in the hippocampus. We also describe the rapid action of estradiol on synaptic plasticity, which is a candidate essential role of endogenously synthesized estradiol.

Table 1. Comparison of relative mRNA expression level between steroidogenic enzymes in the adult (3-month-old) rat

	Hippocampus	Hypothalamus	Ad/Te/Ov/Li
P450scc	1	3	50,000 (Ad)
P450(17 α)	1	3	300 (Te)
P450arom	1	3	300 (Ov)
17 β -HSD (type 1)	1	3	200 (Ov)
17 β -HSD (type 3)	1	5	300 (Te)
3 β -HSD (type 1)	1	3	5,000 (Ov)
5 α -reductase (type 1)	1	2	5 (Li)
ER α	1	5	15 (Ov)
ER β	1	4	80 (Ov)

The level in the hippocampus is normalized to be 1. Ad = Adrenal gland; Te = testis; Ov = ovary; Li = liver.

Values of mRNA expression level are approximate values obtained from semiquantitative RT-PCR analyses [3; Ishii and Kawato, unpubl. results].

Steroid Synthesis Systems in the Adult Rat Hippocampus

Expression of Transcripts for Steroidogenic Enzymes

Highly sensitive molecular biology investigations are necessary for determination of the presence of steroidogenic enzymes, because of the very low level of expression of the mRNAs in the cerebrum and cerebellum [9].

Collectively from many studies, the relative level of mRNA expressed in the hippocampus has been suggested to be lowest for cytochrome P450scc and 3 β -HSD, and highest for steroidogenic acute regulatory protein (StAR) and 5 α -reductase, with that of P450arom expressed at an intermediate level (table 1).

The concentration of P450scc mRNA expressed in the brain is reported to be only 10^{-4} – 10^{-5} of that in the adrenal gland [12, 19]. Our analysis showed approximately 1/50,000 for the P450scc level in the adult hippocampus as compared with that in the adrenal gland (table 1). As a result, the presence of P450scc mRNA could be demonstrated only by the RT-PCR method. The ribonuclease (RNase) protection assay for P450scc in the hippocampus, which was performed using a 32 P-labelled rat P450scc antisense riboprobe, however, yielded no detectable specific hybridization signals [20]. On the other hand, because StAR is most abundant, not only the PCR amplification but also the RNase protection assay demonstrated the presence of StAR transcripts with an expression level of approximately 1/200 of the level in the adrenal gland [20, 21].

Concerning P450(17 α), many attempts to demonstrate the immunohistochemical reactivity in the rat brain had been unsuccessful for almost two decades [11]. The mRNAs for P450(17 α) had not been detected in adult rat brain by either RNase protection assays or RT-PCR [12]. The expression of the mRNA for P450(17 α) had been reported by many laboratories as only transient, occurring during rat embryonic and neonatal development [22–24]. We overcame this difficulty by carefully choosing the sequence of primer pairs which have high specificity by minimizing Gibbs free energy upon recombination of a 3'-primer with cDNA, using computer calculation [3]. In the hippocampal tissues from adult male rats aged 3 months, we observed the P450(17 α) transcripts expressed approximately 1/300 [3], when compared with those expressed in the testis.

The role of P450arom (estrogen synthase) in the hippocampus had also not been well elucidated, primarily because many studies had indicated the absence of P450arom in the adult rat and mouse hippocampus. The significant expression of mRNA for P450arom in the pyramidal and granule neurons of the adult rat hippocampus, however, has recently been demonstrated using *in situ* hybridization [25]. The level of the mRNA expression in the adult mouse hippocampus was approximately half of that in neonatal stages [26]. We observed that the P450arom transcripts expressed approximately 1/300 [3], as compared with those expressed in the ovary by using carefully designed primer pairs for RT-PCR.

The presence of mRNAs for 17 β -HSD type 1 and 3 has been demonstrated in the human and rat hippocampus [27]. We investigated the expression level of mRNA transcripts for 17 β -HSD (types 1–4) using carefully designed primer pairs in the hippocampus from adult male rats. The mRNA level of 17 β -HSD transcripts observed was approximately 1/200, relative to the level in the ovary for 17 β -HSD (type 1), 1/300, relative to the level in the testis for 17 β -HSD (type 3), respectively [3].

The localization in neurons of several steroidogenic proteins has been demonstrated by means of *in situ* hybridization. For example, mRNAs for both StAR and 3 β -HSD mRNA (10^{-2} for StAR and 10^{-3} for 3 β -HSD of the levels in the adrenal gland) have been observed to be localized along the pyramidal cell layer in the CA1–CA3 regions and the granule cell layer in the dentate gyrus (DG) of rats [20] and mice [21].

Glial cells have been considered to play an important role in steroidogenesis, as many reports have indicated the presence of mRNA for P450scc, P450(17 α) 3 β -HSD, and 17 β -HSD in cultures of astrocytes and oligodendro-

cytes from embryonic and neonatal brains [7, 23, 24, 28]. Although similar levels of P450(17 α) mRNA had been reported to be expressed in both astrocytes and neurons in primary cell cultures from the brains of neonatal rats, a much lower metabolic activity had been observed in neurons than astrocytes for the conversion of PREG to DHEA [23, 24].

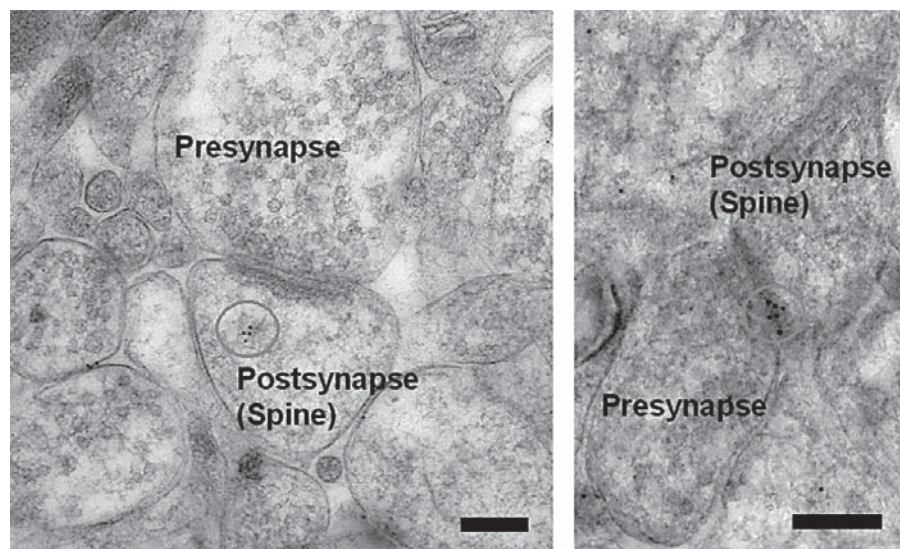
These investigations are available on primary glial cell cultures which are easily prepared from embryonic and neonatal brains. However, information regarding the biosynthesis system of neurosteroids in 'adult' rat brain is not directly available from these cell culture studies.

Neuronal Localization of Enzymes Investigated with Immunostaining

The role of neurons in steroid synthesis had not yet been clearly determined in the mammalian brain, although some reports suggested the expression of several steroidogenic enzymes in nonmammalian brains [29] and rat brain neurons [30, 31]. We overcame many difficulties of nonspecific immunostaining by using affinity column-purified antibodies (instead of using nonpurified antisera) with a slightly higher Triton X-100 concentration (0.5%) in order to obtain a good penetration of IgG, as well as using fresh frozen slices of hippocampus (instead of using paraffin sections) from adult male rats. A significant localization of cytochromes P450scc (CYP11A1), P450(17 α) (CYP17A) and P450arom (CYP19) was observed in pyramidal neurons in the CA1–CA3 regions, as well as in granule cells in the DG, by means of the immunohistochemical staining of hippocampal slices (fig. 1) [1–3, 32]. The co-localization of immunoreactivity against P450s and NeuN (marker protein of neuronal nuclei) confirmed the presence of P450s in these neurons [1–3]. StAR was co-localized with P450s [1, 21]. These results imply that pyramidal neurons and granule cells are equipped with complete steroidogenic systems which catalyze the conversion of cholesterol to PREG, DHEA, testosterone and estradiol.

An immunoelectron microscopic analysis using postembedding immunogold method was performed in order to determine the intraneuronal localization of P450(17 α) in the hippocampal neurons of adult male rats. Surprisingly, we observed that both P450(17 α) and P450arom were localized not only in the endoplasmic reticulum but also in the presynaptic region as well as the postsynaptic region of pyramidal neurons in the CA1–CA3 regions and of granule neurons in the DG (fig. 1). These results suggest a possibility of 'synaptocrine' mechanisms of synthesis of estrogens and androgens, in addi-

Fig. 1. Immunoelectron microscopic analysis of the distribution of ER α (left, CA1 region) and P450arom (right, CA1 region) within axospinous synapses, in the stratum radiatum of the hippocampus. Gold particles (indicated with circles) were localized in the spines (postsynaptic region) of pyramidal neurons, and in some cases, gold particles were affiliated within the postsynaptic density (data not shown). Gold particles were also found in the axon terminal (data not shown). Scale bar: 200 nm.



tion to classical endocrine mechanisms in which sex steroids reach the brain via blood circulation.

The existence of these steroidogenic proteins was confirmed by Western immunoblot analyses. A single protein band was observed for each of these P450s [1–3]. The resulting molecular weights obtained for P450scc, P450(17 α) and P450arom were nearly identical to those obtained from peripheral steroidogenic organs. The relative levels of these P450s in the hippocampus were approximately 1/500 (P450scc) and 1/300 [P450(17 α) and P450arom] of that in the testis [P450scc and P450(17 α)] and the ovary (P450arom), respectively.

For decades, neurosteroidogenesis had been extensively studied in glial cells. From our observation in adult hippocampus, the distributions of astroglial cells and oligodendroglial cells displayed very different patterns from those characteristics of the cells containing P450scc, P450(17 α) and P450arom [1, 3]. This indicates that the majority of P450-containing cells are neither astroglial cells nor oligodendroglial cells.

Synthesis of Estrogens and Androgens in the Hippocampus

A direct demonstration of the neuronal synthesis of DHEA in adult mammals was for the first time reported by our group [2, 3]. It had been assumed that DHEA and the sex steroids are supplied to the hypothalamus, via the blood circulation, where they are converted to estradiol by P450arom [7, 10]. The absence of P450(17 α) activity in

the brain of adult mammals has been reported in a number of studies [10, 11, 29, 33]. Incubations of [3 H]-PREG with brain slices, homogenates and microsomes, primary cultures of mixed glial cells, or astrocytes and neurons from rat and mouse embryos had failed to produce a radioactive metabolite [3 H]-DHEA [10].

We challenged to demonstrate the synthesis of DHEA, testosterone and estradiol in the hippocampal slices by means of HPLC analysis [2, 3]. The purification of neurosteroids from very fatty brain tissues required the application of a set of sophisticated methods, which included purification with organic solvent, column chromatography, and HPLC [1, 3, 34]. The significant conversion from [3 H]-PREG to [3 H]-DHEA, from [3 H]-DHEA to [3 H]-androstenediol, [3 H]-testosterone and [3 H]-estradiol was observed after incubation with the slices for 5 h (fig. 2) [3]. The conversion from [3 H]-testosterone to [3 H]-estradiol and [3 H]-dihydrotestosterone was also demonstrated. These activities were abolished by the application of specific inhibitors of cytochrome P450s. Interestingly, [3 H]-estradiol was rather stably present and not significantly converted to other steroid metabolites. On the other hand, dihydrotestosterone was rapidly converted to 3 α ,5 α -androstenediol.

We determined the concentration of DHEA and estradiol as well as PREG in the acute hippocampal slices from adult male rats by means of RIA or mass spectroscopy (LC/MS/MS) after careful purification of steroids with HPLC. The basal concentrations of PREG, DHEA and estradiol in the male rat hippocampus were approximately 18, 0.3 and 0.6 nM, which were 6–10 times greater than

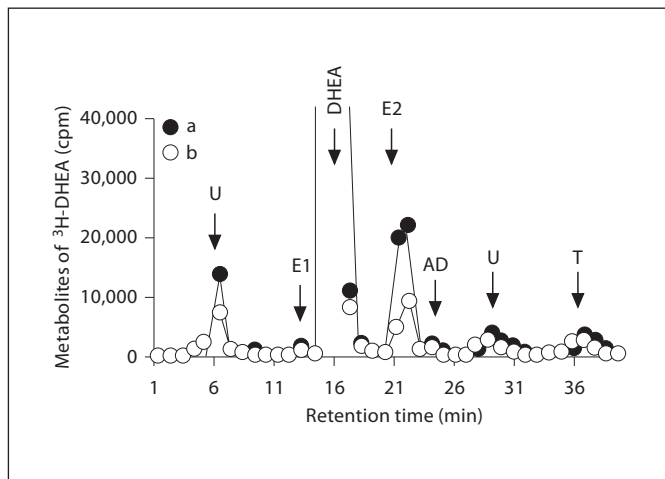


Fig. 2. Synthesis of estradiol and testosterone in adult hippocampal slices. HPLC analysis shows the profile of ^3H -DHEA metabolites in the absence (line a) or in the presence (line b) of fadrozole (inhibitor of P450arom), after incubation of slices for 5 h at 30°C . E2 = Estradiol; T = testosterone; AD = androstenedione; E1 = estrone; U = unknown metabolites. The vertical axis indicates ^3H radioactivity.

those typical of plasma [1–3]. To demonstrate the rapid net production of neurosteroids upon synaptic stimulation, the NMDA-induced production of PREG and estradiol was investigated in hippocampal slices [1, 3]. Upon stimulation with NMDA for 30 min, the hippocampal level of PREG and estradiol increased to approximately twofold of the basal levels. This implies that the NMDA-induced Ca^{2+} influx drives net production of PREG and estradiol. Estradiol synthesis has also been demonstrated in cultured hippocampal slices in the absence and presence of letrozole, an inhibitor of P450arom. After 4 days of treatment with letrozole, the amount of estradiol released into the medium was significantly decreased [5].

Interestingly, PREG sulfate and DHEA sulfate have been reported to be absent in the rat brain as measured by direct mass spectroscopic analysis, although cholesterol sulfate is present [35–37]. In many previous publications, PREG sulfate or DHEA sulfate has been determined indirectly, i.e. by measuring PREG or DHEA after solvolysis of water-soluble fractions which may contain some PREG derivatives different from sulfated steroids [1, 6, 7, 35, 37]. Because numerous publications report that sulfated steroids are very effective for neuromodulation, careful considerations should be made [10, 38, 39].

It is necessary to consider whether the local concentration of brain neurosteroids is sufficiently high to allow

action as local mediators. The concentration of estradiol detected in the hippocampus is about 0.6 nM (basal) and 1.3 nM after the NMDA stimulation, respectively. The local concentration of estradiol immediately after the synthesis in the pyramidal neurons is likely to be approximately tenfold higher than the bulk concentration of 1.3 nM, due to the relatively small volume of the P450-immunoreactive cells in the total hippocampus. These considerations suggest that the local concentration of estradiol could be as high as 1–10 nM. These levels are sufficient to allow estradiol to act as a local mediator that modulates synaptic transmission [13, 15, 17, 40, 41]. Functional differences between blood-derived estradiol (reproductive modulator) and brain-synthesized estradiol (neuronal modulator) may be due to the time-dependence of their levels. The brain is filled with a low concentration of blood-derived estradiol whose level changes depending on the circadian rhythm, while endogenous synthesis of estradiol is a transient event occurring mainly during synaptic transmission which drives Ca^{2+} influx [3].

Modulation of Synaptic Plasticity by Estrogens

Evidence is emerging that 17β -estradiol exerts a rapid influence (0.5–1 h) on the synaptic transmission of adult rat hippocampal neurons, as demonstrated by means of electrophysiology [15, 17, 40–42]. In case of the enhancement of long-term potentiation (LTP) by 1–10 nM estradiol in CA1 pyramidal neurons, an immediate increase by approximately 20% has been observed upon the onset of estradiol perfusion in the initial slope of the excitatory postsynaptic potential (EPSP), which has been attendant upon a further approximately 130% increase upon high-frequency tetanic stimulation of Schaffer collaterals [13, 17]. However, it should be noted that if we subtract the 20% immediate increase in EPSP slope upon the onset of estradiol perfusion before the tetanic stimulation, the enhancement of LTP by estradiol is not significant. In other words, the magnitude of pure tetanic stimulation-induced LTP is nearly the same in the presence and in the absence of estradiol.

On the other hand, we demonstrated, for the first time, a significant rapid enhancement of the long-term depression (LTD) by 1–10 nM 17β -estradiol perfusion in adult male rat hippocampal CA1, CA3 and DG (fig. 1) [43]. Recordings were performed using novel 64 multielectrodes (MED64, Panasonic, Japan) particularly arranged to stimulate the Schaffer collaterals in the stratum radiatum

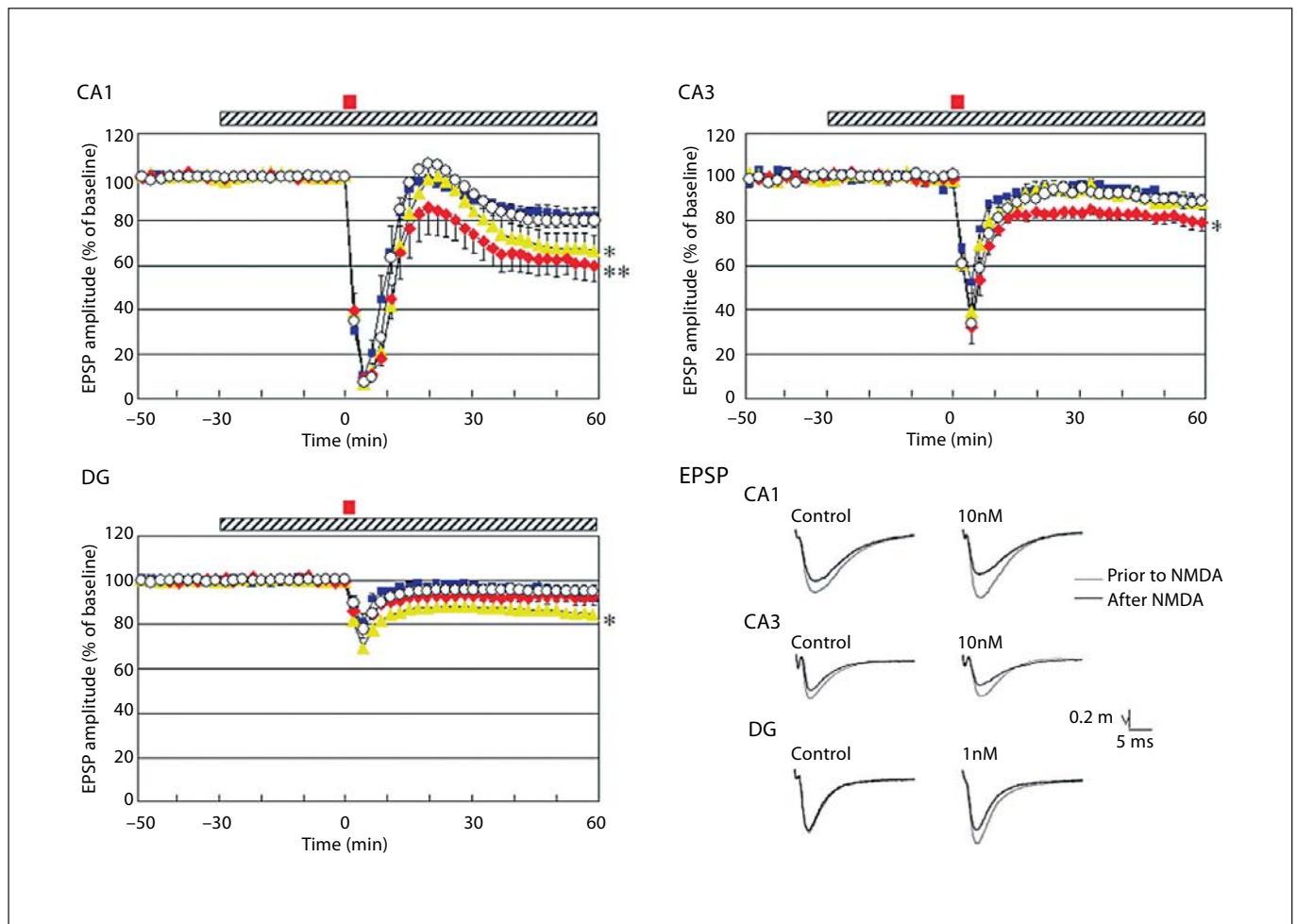


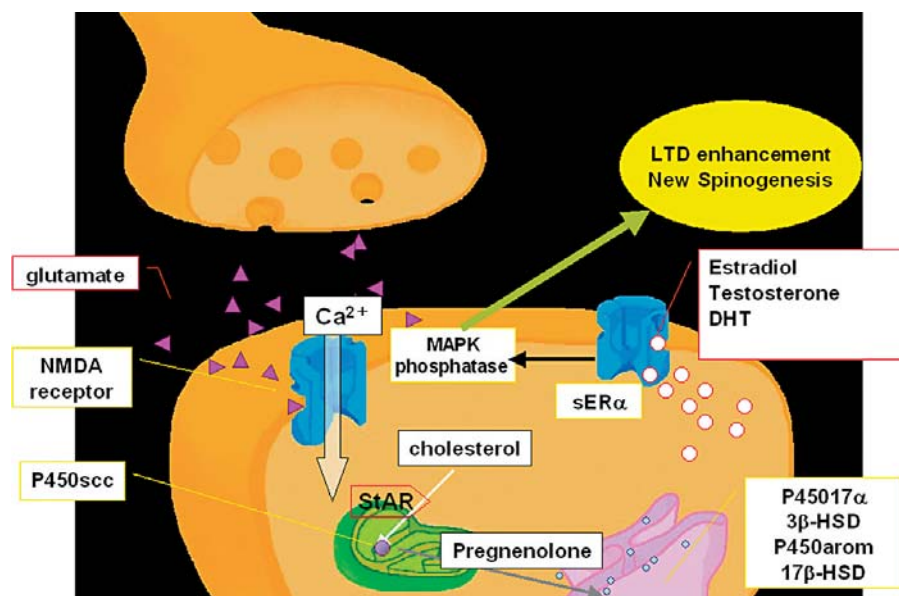
Fig. 3. Rapid enhancement of LTD by 17β -estradiol in the CA1 (CA1), CA3 (CA3) and DG (A-DG) of the same hippocampal slice. Estradiol concentration was 0 nM (open circle, $n = 17$) and 0.1 nM (blue closed square, $n = 8$), 1 nM (yellow closed triangle, $n = 11$) and 10 nM (red closed diamond, $n = 10$), respectively. EPSP traces in the presence of 10 nM estradiol, showing sample recordings taken prior to ($t = -40$ min) and after ($t = 60$ min) NMDA stimulation. The number of independent experiments is indicated as n . Vertical axis indicates maximal amplitude of EPSP. Here,

100% refers to the EPSP value at $t = -40$ min prior to NMDA stimulation, irrespective of the test condition. LTD was induced by $30 \mu\text{M}$ NMDA perfusion at time, $t = 0$ to 3 min (closed bar above the graph). Hatched bar above the graph indicates period of time during which estradiol was administered. The significance of the estradiol effect was confirmed at 60 min via statistical analysis using ANOVAs ($* p < 0.05$; $** p < 0.01$) as indicated in the figure. Illustrated data points and error bars represent the mean \pm SEM from n of independent slices.

of CA1, the recurrent collateral fibers in the stratum radiatum of CA3, and the medial perforant pathways in the molecular layer of DG. LTD was induced pharmacologically by the transient application (3 min) of NMDA. This LTD was induced by the activation of phosphatase due to a moderate Ca^{2+} influx through NMDA receptors [44]. The plateau EPSP amplitude at 60 min after the NMDA application was 80.4% (CA1), 88.8% (CA3) and 95.1% (DG). A 30-min preperfusion of 10 nM estradiol significantly enhanced LTD resulting in the residual EPSP am-

plitude at 60 min of 59.7% (CA1), 79.1% (CA3) and 92.2% (DG) (fig. 3) [43]. Investigations using specific estrogen agonists indicated that the contribution of $\text{ER}\alpha$ (but not $\text{ER}\beta$) was essential to these estradiol effects. Propyl-pyrazole-trinyl-phenol ($\text{ER}\alpha$ agonist) [45] at 100 nM exhibited a significant LTD enhancement in CA1, while hydroxyphenyl-propionitrile (DPN, $\text{ER}\beta$ agonist) did induce a suppression of LTD in CA1, implying that the contribution of $\text{ER}\beta$ was opposite to that of $\text{ER}\alpha$ in the estradiol effect on LTD.

Fig. 4. Schematic illustration of the synaptic synthesis of neurosteroids, and the modulation of the synaptic transmission of neurons by neurosteroids. AMPA type glutamate receptors are omitted for clarity. StAR, PBR and P450scc are present in the mitochondria. P450(17 α), 3 β -HSD, 17 β -HSD and P450arom are localized in the membranes in the synaptic compartment. The site of rapid action for estradiol is synaptic ER α . The site of delayed action for estradiol is ER α present in the cytoplasm and nuclei.



We also demonstrated that dendritic spines were rapidly modulated by estradiol application, using single spine analysis of lucifer yellow-injected neurons in adult male hippocampal slices [43, 46]. Following a 2-hour treatment with estradiol in the stratum radiatum of the CA1 region, the treated dendrites have significantly more spines at 1 nM estradiol (1.31 spines/ μ m) than dendrites at 0 nM estradiol (0.85 spines/ μ m) [43]. Propyl-pyrazole-trinyl-phenol at 100 nM induced a significant enhancement of the spine density to 1.20 spines/ μ m. However, DPN at 100 nM increased the spine density only slightly (0.95 spines/ μ m). Blocking of ER α by 1 μ M ICI 182,780 and of NMDA receptors by MK-801 completely suppressed the enhancing effect of estradiol on the spine density. Blocking of phosphorylation of MAP kinase by 50 μ M PD98059 completely prevented the estradiol-induced spinogenesis. The morphological changes in CA1 spines induced by 2-hour estradiol treatments were also assessed. In control slices (0 nM estradiol), the relative population of spines was approximately 24% for mushroom spine, 62% for thin spine, 1% for filopodium, and 13% for stubby spine. Upon 1 nM estradiol treatment, the density of thin spine was selectively increased, from 0.57 spines/ μ m to 0.97 spines/ μ m, while the density of mushroom and stubby was not significantly altered. Filopodium was increased from almost null (0.01 spines/ μ m) to 0.11 spines/ μ m. Interestingly, in CA3 pyramidal neurons, the total density of thorns of thorny excrescences (spine-like postsynaptic structures in CA3), having contacts with mossy fiber terminals originated from granule

cells, decreased dramatically to approximately 70% upon 2-hour treatments with 1 nM estradiol [46]. These results imply that the spine density is not always increased by the estradiol treatments and that the estradiol-induced spinogenesis is highly region specific and heterogeneous.

The rapid effect of estrogens has also been observed in vivo. MacLusky et al. [47] have demonstrated that the estradiol (60 μ g/kg) has increased the spine-synapse density due to synaptic rearrangements in ovariectomized rats after 30 min using electron micrographic analysis.

Over decades, the delayed genomic effects (1–4 days) of estradiol on synaptic plasticity have been extensively investigated. For example, supplement of estrogens in ovariectomized female rats [47–50] increases the density of spines in the stratum radiatum of CA1 pyramidal neurons, resulting in recovery of spines to the level of wild rat. In vitro investigations have also shown that spine density is increased following several days' treatment of cultured hippocampal slices with exogenous estradiol [18, 51]. The contribution of endogenous estradiol is reported by Kretz et al. [5] who have demonstrated that the suppression of endogenous estradiol synthesis by letrozole treatments for 4 days has significantly decreased the spine density in the stratum radiatum of the CA1 region in cultured slices.

What is a receptor of estradiol for its rapid action (0.5–2 h) in the hippocampus? Putative membrane estrogen receptors for rapid estradiol action has been poorly understood. After several years of careful investigations, we successfully identified the membrane estrogen receptor

ER α localized in the spines of hippocampal pyramidal and granule neurons by means of immunoelectron microscopic analysis as well as Western blot analysis using novel purified anti-ER α antibody RC-19 [46]. A postembedding immunogold electron microscopic analysis demonstrated the subcellular localization of ER α in the glutamatergic neurons in CA1, CA3 and DG. ER α was localized not only in the nuclei but also in both the dendritic spines and axon terminals of principal neurons (fig. 1). Western blot of purified postsynaptic density fractions demonstrated a single band of ER α (67 kDa) together with a MAP kinase band. Because the estradiol-induced modulation of LTD and spine morphology appeared so rapidly in the time range of 1–2 h, the synaptic ER α observed in postsynaptic density fractions and postsynaptic compartment probably plays an essential role in driving rapid processes. It should be noted that specific binding of purified RC-19 antibody to real ER α (67 kDa) in the hippocampus was qualified using ER α KO mice and MALDI-TOF mass-spectrometric analysis of RC-19-reacted proteins [43]. These analyses were essential, because we found that nonpurified MC-20 antisera, frequently used in previous investigations, often reacted with 62 kDa unknown proteins in the brain and did not

significantly react with real ER α (67 kDa). Therefore, staining patterns with nonpurified antisera may not show real ER α distribution.

Hypothetical Model of Synaptocrinology

Based on experimental observations, we illustrate in figure 4 a hypothetical model for the synaptic synthesis of brain steroid and the modulation of the synaptic transmission of neurons by brain steroid. Brain steroid synthesis proceeds in the following manner. First, glutamate release from the presynapse induces a Ca²⁺ influx through the NMDA receptors. The Ca²⁺ influx drives StAR or peripheral benzodiazepine receptor (PBR) [52] to transport cholesterol into the mitochondria, where P450scc converts cholesterol to PREG. After reaching the endoplasmic reticulum, the conversion of 'PREG \rightarrow DHEA \rightarrow androstenediol \rightarrow testosterone \rightarrow estradiol, or testosterone \rightarrow dihydrotestosterone' is performed by P450(17 α), 3 β -HSD, 17 β -HSD, P450arom and 5 α -reductase. Produced estradiol binds to synaptic ER α and drives signaling pathway including MAP kinase, etc., finally resulting in modulation of AMPA receptors or NMDA receptors.

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