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HIPPOCAMPAL SYNTHESIS OF ESTROGENS AND ANDROGENS WHICH ARE PARACRINE MODULATORS OF SYNAPTIC PLASTICITY: SYNAPTOCRINOLOGY

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Abstract—Hippocampal pyramidal neurons and granule neurons of adult male rats are equipped with a complete machinery for the synthesis of pregnenolone, dehydroepiandrosterone, testosterone, dihydrotestosterone and 17β-estradiol. Both estrogens and androgens are synthesized in male hippocampus. These brain steroids are synthesized by cytochrome P450s (P450scc, P45017α and P450arom), hydroxysteroid dehydrogenases and reductases from endogenous cholesterol. The expression levels of enzymes are as low as 1/300–1/1000 of those in endocrine organs. Synthesis is dependent on the acute Ca^{2+} influx upon neuron–neuron communication via NMDA receptors. Estradiol is particularly important because estradiol rapidly modulates neuronal synaptic transmission such as long-term potentiation via synaptic estrogen receptors. Xenoestrogens may also act via estrogen-driven signaling pathways.

Key words: neurosteroid, P450, hippocampus, estradiol, endocrine disrupter.

This article describes local endogenous synthesis of estrogens and androgens in the mammalian brain, particularly hippocampus, in relation to their rapid action as modulators of the synaptic plasticity. The hippocampus is essentially involved in learning and memory processes, and is known to be a target for the neuromodulatory actions of hormones produced in the gonads. 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 (Woolley and McEwen, 1994; Woolley, 1998; Foy et al., 1999; Pozzo-Miller et al., 1999; Bi et al., 2000; Shibuya et al., 2003). In addition to endocrine-derived hormones, recent experiments have demonstrated that hippocampal neurons may also be exposed to locally synthesized brain steroids, such as pregnenolone (PREG) (Baulieu, 1997; Kimoto et al., 2001; Kawato et al., 2003). Dehydroepiandrosterone (DHEA) has also been found in the mammalian brain at concentrations greater than that in plasma (Corpechot et al., 1981; Baulieu, 1997). 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 (Corpechot et al., 1981; Robel et al., 1987). Direct demonstration of steroidogenesis in the mammalian brain had, however, been difficult, due to the extremely low levels of steroidogenic proteins in the brain (Warner and Gustafsson, 1995). Sex steroids had not been considered to be brain-derived steroids, because of many reports suggesting the absence of cytochrome P45017α in adult mammalian brain (Le Goascogne et al., 1991; Mellon and Deschepper, 1993). In particular, since sex steroids cannot be synthesized without P45017α, which converts PREG to DHEA, they are thought to reach the brain via blood circulation (Baulieu and Robel, 1998). On the other hand, frog brain has been reported to synthesize testosterone from PREG (Mensah-Nyagan et al., 1996). There has been an exceptional report suggesting that human brain tissue has an ability of conversion of androstenedione to testosterone (Steckelbroeck et al., 1999).

To date, the term ‘neurosteroids’ has been used to refer to steroids produced both in the brain and in the peripheral nerves and Schwann cells (Morfin et al., 1992; Koenig et al., 1995). Here, ‘brain neurosteroid’ refer to a steroid that is synthesized de novo in the brain by P450 systems.

Localization of steroidogenic systems in the adult rat hippocampus

All experiments were conducted along with the institutional ethical guidelines. All efforts were made to minimize the number of animals per group and any potential suffering of those subjects.

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 (Warner and Gustafsson, 1995).

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Abbreviations: BPA, bisphenol A; DES, diethylstilbestrol; DHEA, dehydroepiandrosterone; LTP, long-term potentiation; PBR, peripheral benzodiazepine receptor; PREG, pregnenolone; RNase, ribonuclease; StAR, steroidogenic acute regulatory protein; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase.

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

The concentration of P450scc mRNA expressed in the brain is reported to be only 10^{-4}-10^{-5} of that in the adrenal gland (Mellon and Deschepper, 1993; Sanne and Krueger, 1995). Our analysis showed approximately $1/10^3$ for P450scc (Ishii, Furukawa and Kawato, unpublished observations). As a result, the presence of P450scc mRNA could be demonstrated only by RT-PCR method. The ribonuclease (RNase) protection assay for P450scc in the hippocampus, which was performed using a ^{32}P-labeled rat P450scc antisense riboprobe, however, yielded no detectable specific hybridization signals (Furukawa et al., 1998). 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 levels in the adrenal gland (Furukawa et al., 1998; King et al., 2003).

Concerning P45017α, many attempts to demonstrate the immunohistochemical reactivity in the rat brain had been unsuccessful (Le Goascogne et al., 1991). The mRNAs for P45017α had not been detected in adult rat brain by either RNase protection assays or RT-PCR (Mellon and Deschepper, 1993). The expression of the mRNA for P45017α had been reported by many laboratories as only transient, occurring during rat embryonic and neonatal development (Compagnone et al., 1995; Zwain and Yen, 1999a,b). We overcame this difficulty by carefully designing primer pairs which were free from three-dimensional loop formation, using computer calculation (Hojo et al., 2004). In the hippocampal tissues from adult male rats aged 3 months, we observed the P450 transcripts expressed approximately 1/300 for P45017α (Hojo et al., 2004), when compared with those expressed in the testis. It should be noted that approx. 1.5-fold of P45017α transcripts was expressed in the hypothalamus as compared with those in the hippocampus.

The role of P450arom 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. Recently, however, the significant expression of mRNA for P450arom in the pyramidal and granule neurons of the adult rat hippocampus has been demonstrated using *in situ* hybridization (Wehrenberg et al., 2001). The level of the mRNA expression in the adult mouse hippocampus was approximately half of that in neonatal stages (Ivanova and Beyer, 2000). We observed the P450 transcripts expressed approximately 1/300 for P450arom (Hojo et al., 2004), when compared with those expressed in the ovary by using carefully designed primer pairs for RT-PCR. Note that approx. 1.5-fold of P450arom transcripts was expressed in the hypothalamus as compared with those in the hippocampus.

The presence of mRNAs for 17β-hydroxysteroid dehydrogenase (17β-HSD) types 1 and 3 has also been demonstrated in the human and rat hippocampus (Beyenburg et al., 2000). We investigated the expression level of mRNA transcripts for 17β-HSD (types 1–4) by using carefully designed primer pairs in the hippocampus from adult male rats. The mRNA level of 17β-HSD transcripts observed was approximately 1/10, relative to the level in the ovary for 17β-HSD (type 1), 1/200–1/300, relative to the level in the testis for 17β-HSD (type 3), respectively (Hojo et al., 2004).

The localization in neurons of several steroidogenic proteins has been demonstrated by means of *in situ* hybridization. For example, mRNAs for both StAR and 3β-HSD mRNA (10^{-2}-10^{-3} of the levels in the adrenal gland) were observed to be localized along the pyramidal cell layer in the CA1–CA3 regions and the granule cell layer in the dentate gyrus of rats (Furukawa et al., 1998) and mice (King et al., 2003).

Glial cells have been considered to play an important role in steroidogenesis, as many reports have indicated the presence of mRNA for P450scc, P45017α, 3β-HSD, and 17β-HSD in cultures of astrocytes and oligodendrocytes from embryonic and neonatal brains (Jung-Testas et al., 1989; Baulieu, 1997; Zwain and Yen, 1999a,b). Although a similar level of P45017α mRNA had been reported to be expressed in both astrocytes and neurons in primary cell cultures from the brain of neonatal rats, neurons had exhibited a much lower metabolic activity than astrocytes for the conversion of PREG to DHEA (Zwain and Yen, 1999a,b).

These extensive investigations are possible because primary glial cell cultures can easily be obtained for embryonic and neonatal brains. As a result, however, direct information is not available from these studies regarding the biosynthesis system of neurosteroids in 'adult' rat brain.

Neuronal localization of enzymes investigated with immunohistochemical and Western immunoblot analysis. The role of neurons in steroid synthesis had not yet been clearly determined in mammalian brain, although there had been some reports indicating the expression of several steroidogenic enzymes in non-mammalian brains (Mensah-Nyagan et al., 1994) and rat brain neurons (Guennoun et al., 1995; Tsutsui et al., 2000). We overcame many difficulties of nonspecific immunostaining by using purified antibodies (instead of using non-purified 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), P45017α (CYP17A) and P450arom (CYP19) was observed in pyramidal neurons in the CA1-CA3 regions, as well as in granule cells in the dentate gyrus, by means of the immunohistochemical staining of hippocampal slices (Fig. 1) (Kimoto et al., 2001; Kawato et al., 2002, 2003; Hojo et al., 2004). The co-localization of immunoreactivity against P450s and NeuN confirmed the presence of P450s in these neurons (Kimoto et al., 2001; Kawato et al., 2002; Hojo et al., 2004). StAR