LOCALIZATION AND ACTIVITIES OF NEUROSTEROIDOGENIC SYSTEMS IN THE HIPPOCAMPAL NEURONS

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ABSTRACT

Neurosteroids in the hippocampus may be promising neuromodulators which influence learning and memory. Neurosteroidogenesis has, however, not been well elucidated due to the extremely low levels of steroidogenic proteins and *their activities in the brain. Here we show the first* demonstration of the presence and activity of neurosteroidogenic systems in the adult rat hippocampus. Significant localization of cytochrome P450scc was observed in pyramidal neurons in the CA1 - CA3 regions with immunohistochemical staining of slices. We also showed co-localization of P450scc with redox partners in pyramidal neurons. The distributions of astroglial cells and oligodendroglial cells showed very different patterns from that of the P450scc-containing cells. The expression of P450scc with redox partners in mitochondria was confirmed by Western blot analysis. Upon stimulation with N-methyl-D-aspartate (NMDA) significant production of pregnenolone (PREG) and pregnenolone sulfate (PREGS) was observed in the hippocampus. Taken together, these results strongly imply that neurosteroids are synthesized in hippocampal neurons in which neurosteroids may act as paracline modulators of neurotransmitter receptors which play essential roles in learning and memory.

INTRODUCTION

There is increasing evidence that neurosteroids modulate neurotransmission

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in the hippocampus, and that they most likely influence the learning and memory processes via their effects, which are either excitatory or inhibitory. The acute actions of neurosteroids are thought to be mediated through ion-gated channel receptors rather than through nuclear steroid receptors which promote the classic genomic actions of adrenal steroid hormones. PREGS potentiates the Ca^{2+} conductivity of the NMDA receptors and suppresses the Cl^- conductivity of the γ-aminobutyric acid (GABA) receptors in cultured rat hippocampal neurons. In combination, these actions could facilitate excitation of neurons at the postsynaptic level. Dehydroepiandrosterone (DHEA) potentiates the GABA-induced Cl^- current but DHEA sulfate suppresses it. Neurosteroids are indicated to be effective in learning and memory. The administration of PREGS and DHEA enhanced the retention of footshock avoidance in mice when injected directly into the hippocampus. An injection of PREGS into the hippocampus temporally improves the spatial memory performance of deficient aged rats.

The location and activity of the neurosteroidogenic machinery not only in the hippocampus but also other regions of the brain has been, however, insufficiently elucidated . Many studies showed that the mRNAs of steroidogenic enzymes were expressed at low levels in the entire cerebrum and cerebellum. For example, the amount of P450scc mRNA expressed in the brain was extremely small, around $1/10^4$ -$1/10^5$ of that in the adrenal gland. Recent studies, however, revealed the presence of significant amounts of neurosteroids such as PREG, DHEA and their sulfate esters as in the mammalian brain. Neurosteroid synthesis probably starts as the conversion of cholesterol to PREG by P450scc in mitochondria. Cytochrome P450scc (CYP11A1) catalyzes the side-chain cleavage reaction of cholesterol, which is promoted by electron transfer from NADPH to P450scc through NADPH-adrenodoxin reductase (ADR) and adrenodoxin (ADX).

In the hippocampus, there has been no direct demonstration of the presence and localization of the cytochrome P450scc protein and redox proteins. The expression of mRNA of P450scc was found to be very little on a layer of pyramidal neurons and granule cells, although the mRNAs of steroidogenic acute regulating protein (StAR) and 3 β-hydroxysteroid dehydrogenase (3 β -HSD) were strongly expressed on the same layer. Since micromolar concentrations of neurosteroids may be necessary for acute regulation of

NMDA and GABA receptors, significant localization of steroidogenic proteins should be necessary for the possible paracline actions of neurosteroids.

RESULTS

Firstly, to determine the localization of cytochrome P450scc, we performed immunohistochemical staining of P450scc proteins in the hippocampi of adult male and female rats aged 3 months . We used anti-rat P450scc antibodies raised against peptides. An intense immunoreaction with anti-rat P450scc IgG was restricted to pyramidal neurons in the CA1 - CA3 regions as well as granule cells in the dentate gyrus. Neurofilament staining with anti-neuronal nuclear antigen IgG was used as a reference to determine the distribution of neurons. The co-localization of immunoreactivity against P450scc and neurofilaments showed P450scc was present in neurons. Pyramidal neurons were more densely stained than granule cells. Preadsorption of the antibody with an excess amount of purified bovine P450scc antigen resulted in a complete absence of P450scc immunoreactivity in all of the positively stained cells in the hippocampus, because our anti-rat P450scc antibodies crossreact with bovine P450scc. Non-immunized serum did not cause any positive staining of the hippocampus. We observed almost the same P450scc staining pattern in male and female rats. It should be noted that we also used polyclonal antibodies against bovine P450scc, with which we obtained essentially the same staining pattern of the hippocampus as that with anti-rat P450scc IgG.

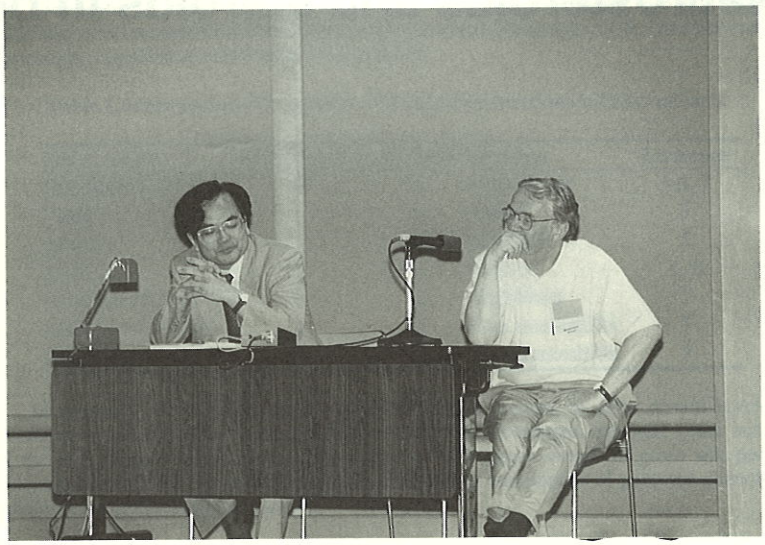
Secondly, we investigated the presence of redox partners of P450scc in pyramidal neurons. Immunolabeling was performed for ADR and ADX, which transfer electrons to P450scc. Antibodies against ADR and ADX stained neurons whose distribution coincided with that of the P450scc-immunoreactive cells. These results support that pyramidal neurons have a complete P450scc system, which catalyzes the conversion of cholesterol to PREG driven by electron transport from NADPH to P450scc through ADR and ADX.

In contrast, antibodies against glial fibrillary acidic protein (GFAP), a marker protein of astroglial cells, stained astro-shaped cells in the stratum radiatum and the stratum oriens in the hippocampus. The antibodies against myelin basic protein (MBP), a marker protein of oligodendroglial cells, stained many long fibril cells in the hippocampus. The distributions and shapes of GFAP-reactive cells and MBP-reactive cells are very different from those of

P450scc-reactive cells, indicating that P450scc-containing cells are neither astroglial cells nor oligodendroglial cells.

Thirdly, we examined biochemically the presence of steroidogenic proteins by Western immunoblot analysis in isolated mitochondria from the hippocampus and cerebellum. As illustrated in Fig. 4, protein bands were observed for P450scc, ADR and ADX. The electrophoretic mobility of the P450scc band for the hippocampus was almost the same as that of the purified boyine adrenocortical P450scc, whose molecular mass was about 54 kDa. The molecular weights of ADR and ADX were around 54 kDa and 12 kDa, respectively.

Fourthly, we examined the activity of the neurosteroidogenic system in the hippocampus. The basal concentrations of PREG and PREGS were measured in homogenates of the whole hippocampus, cerebellum and plasma by means of a specific radioimmunoassay (RIA) involving antibodies against PREG. Significant concentrations of PREG were observed in the hippocampus (4.4 ± 0.3 pmol/mg protein; mean $\pm$ SEM) and cerebellum (3.7 ± 0.6 pmol/mg protein). Comparable levels of PREGS to that of PREG were observed. The concentration of PREGS was 3.6 ± 0.2 pmol/mg protein in the hippocampus. The concentrations of PREG and PREGS in plasma were 0.027 ± 0.001 pmol/μl and 0.016 ± 0.001 pmol/μl, respectively, which were considerably lower than those in the hippocampus. The NMDA-stimulated production of PREG and PREGS was investigated in hippocampal tissues. Upon stimulation with 100 μM NMDA for 30 min, the hippocampus produced 8.4 ± 0.8 pmol of PREG and 3.7 ± 0.5 pmol of PREGS per mg of protein, respectively, resulting in increases in the levels of PREG and PREGS to about 2 to 3-fold the basal levels. Significance of the NMDA-induced production of PREG and PREGS was confirmed by Student's t-test ($p < 0.001$). NMDA receptor-mediated Ca^{2+} influx probably enhanced PREG synthesis, as judged when Ca^{2+} signal-induced enhancement was extensively examined in the retina and adrenocortical cells. The basal PREG concentration was also measured in mitochondria isolated from the hippocampus, cerebellum and testis. They contained 160.1 ± 24.3, 203.7 ± 8.4, and 635.0 ± 28.4 pmol PREG/mg protein, respectively, implying that the local concentration of PREG in mitochondria was about 40-fold greater than the bulk concentration in hippocampal tissue homogenates.



Prof. S. Kawato and Prof. M. Waterman
Chairman,
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