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Organizing Actions of Neurosteroids Synthesized De Novo in the Cerebellar Purkinje Neuron

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The great deal was known about the brain as a target site of steroid hormones more than 10 years before. On the other hand, new finding from several laboratories over the past decade have established unequivocally that the nervous system itself forms steroids de novo from cholesterol. The pioneering discovery of Baulieu and his colleagues using mammals and Tsutsui and his colleagues using nonmammals have opened the door of a new research field. The new concept that steroids could be synthesized de novo in the brain derived from observations made by Baulieu and his colleagues. They found that several steroids such as pregnenolone, dehydroepiandrosterone, and their sulfate and lipoidal esters highly accumulated within the brain of several mammalian species. These results suggested that the brain can synthesize steroids de novo from cholesterol. In contrast to mammalian studies, little has been known regarding de novo steroidogenesis in the brain of nonmammalian vertebrates. Therefore, Tsutsui and his colleagues looked for steroids formed from cholesterol in the brain of birds, amphibians and fishes (this study). The formation of several steroids from cholesterol is now known to occur in both mammalian and nonmammalian vertebrates. Such steroids synthesized in vertebrate brains are called 'neurosteroids'.

Little information is available for neurosteroidogenesis in the central nervous system (CNS) of lower vertebrates. Therefore, in the present study, the enzymatic activity and localization of 3bHSD, a key steroidogenic enzyme, was examined in the CNS of adult male zebrafish to clarify central progesterone biosynthesis. Biochemical studies together with HPLC analysis revealed that the zebrafish brain converted pregnenolone to progesterone, suggesting the enzymatic activity of 3bHSD. This conversion was significantly reduced by trilostane, a specific inhibitor of 3bHSD. By using Western immunoblotting with the polyclonal antiserum directed against purified bovine adrenal 3bHSD, a 3bHSD-like substance was found in homogenates of the zebrafish brain. Immunocytochemical analysis was then undertaken to investigate the localization of the 3bHSD-like substance in the zebrafish brain and spinal cord. An intense immunoreaction was observed in somata of Purkinje neurons. Clusters of immunoreactive cell bodies were also localized in the dorsal telencephalic areas (D), central posterior thalamic nucleus (CP), preoptic nuclei (NPO), posterior tuberal nucleus (PTN), paraventricular organ (PVO), nucleus of medial longitudinal fascicle (NMLF). A widespread distribution of immunoreactive fibers was found throughout the brain and spinal cord. In addition, positively stained cells were restricted to other organs, such as the pituitary and retina. Preabsorbing the antiserum with purified bovine adrenal microsome resulted in a complete absence of 3bHSD-like immunoreactivity. These results suggest that the fish CNS possesses steroidogenic enzyme 3bHSD and produces progesterone. The present study further provides the first immunocytochemical mapping of the site of 3bHSD expression in the fish

CNS and suggests that the Purkinje cell, a typical cerebellar neuron, is an important neurosteroidogenic cell in vertebrates.

De novo steroidogenesis from cholesterol is a conserved property of vertebrate brains, and such steroids synthesized de novo in the brain are called neurosteroids. The identification of neurosteroidogenic cells is essential to the understanding of the physiological role of neurosteroids in the brain. Tsutsui et al. have demonstrated recently that neuronal neurosteroidogenesis occurs in the brain and indicated that the Purkinje neuron actively synthesizes several neurosteroids de novo from cholesterol in vertebrates. Interestingly, in the rat, this neuron actively synthesizes progesterone de novo from cholesterol only during neonatal life, when cerebellar cortical formation occurs most markedly. Therefore, in this study, the possible organizing actions of progesterone during cerebellar development have been examined. In vitro studies using cerebellar slice cultures from newborn rats showed that progesterone promotes dose-dependent dendritic outgrowth of Purkinje neurons but dose not affect their somata. This effect was blocked by the anti-progestin RU 486 (mifepristone). In vivo administration of progesterone to pups further revealed an increase in the density of Purkinje spine synapses electron microscopically. In contrast to progesterone, there was no significant effect of 3a,5a-tetrahydroprogesterone, a progesterone metabolite, on Purkinje neuron development. These results taken together suggest that progesterone promotes neuronal growth in the cerebellum during neonatal life. Such an action of progesterone may contribute to the formation of cerebellar neuronal circuit.

In Chapter 2, it has been demonstrated that progesterone synthesized de novo in the Purkinje neuron promotes dose-dependent dendritic outgrowth of Purkinje neurons. In the present study, therefore, to understand the mode of actions of the neurosteroid, progesterone, in the cerebellum, the expression of PR in the rat cerebellum was investigated during neonatal development and in the adult. RT-PCR-Southern analyses showed the expression of PR mRNA in the cerebellum. Interestingly, the PR mRNA expression in the cerebellum was higher at 7～14 days of age than at other times. Subsequently, the site of PR expression in the cerebellum was further examined in neonatal and adult rats by using immunocytochemistry. PR-like immunoreactivity was present in the nucleus of Purkinje neurons in both neonate and adult. From these findings together with the results in Chapter 2, it can be supposed that progesterone acts directly on the Purkinje neuron through intranuclear receptor-mediated mechanisms to promote the dendritic outgrowth, spinogenesis and synaptogenesis in Purkinje neurons during cerebellar cortical formation. Thus, these organizing actions of progesterone synthesized de novo in the developing Purkinje neuron may be essential for the formation of the cerebellar neuronal circuit.

The cerebellar Purkinje neuron is a major site of neurosteroidogenesis in vertebrate brains. This neuron actively synthesizes several neurosteroids de novo from cholesterol. Progesterone synthesized de novo in the developing Purkinje neuron promotes its dendritic growth, spinogenesis and synaptogenesis through intranuclear receptor-mediated mechanisms. These organizing actions of progesterone synthesized de novo in Purkinje neurons may be essential for the formation of the cerebellar neuronal circuit during neonatal development.