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Biosynthesis and Biological Actions of Neurosteroids in the Brain

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Peripheral steroid hormones act on brain tissues through intracellular receptor-mediated mechanisms to regulate several important brain neuronal functions. Therefore, the brain is considered to be a target site of steroid hormones. In contrast to this classical concept, new findings over the past decade have established that the brain itself also synthesizes steroids *de novo* from cholesterol through mechanisms at least partly independent of peripheral steroidogenic glands. The pioneering discovery of Baulieu and his colleagues and our studies have opened the door of a new research field in the brain. Such steroids synthesized *de novo* in the brain, as well as other areas of the nervous system, are called neurosteroids. Subsequently, biological actions of neurosteroids have become clear by a series of our studies using an excellent cellular model system. Here I summarizes the advances made in our understanding of biosynthesis and biological actions of neurosteroids in the brain.

New concept: Neurosteroidogenesis in vertebrate brains

Baulieu and colleagues first found that several steroids, such as pregnenolone, dehydroepiandrosterone, and their sulfate and lipoidal esters, highly accumulated within the brain of mammals. The brain content of these steroids remained constant even after the removal of peripheral steroids. We therefore looked for steroids formed from cholesterol in the brain of several vertebrates. The concept of *de novo* steroidogenesis from cholesterol in vertebrate brains derived from our observations made in the 1990s. Independently, other groups, such as Vaudry's laboratory and Schlinger's laboratory, also contributed to this area. The formation of several neurosteroids from cholesterol is now known to occur in a variety of vertebrates.

An excellent model for the study of neurosteroids

To analyze neurosteroid actions in the brain, we need data on the specific synthesis in particular sites of the brain at particular times. Such informations are crucial to develop hypotheses predicting the potential roles of particular neurosteroids in the developing and adult brains. Thus, studies for this exciting area of brain research should be focused on the mode of action of neurosteroids produced locally in the identified neurosteroidogenic cells underlying important brain functions.

Glial cells were first accepted to be the primary site for neurosteroid formation in the brain, but the concept of neurosteroidogenesis in brain neurons has remained unclear. We demonstrated that the Purkinje cell, a typical cerebellar neuron, is a major site for neurosteroid formation in both mammalian and nonmammalian vertebrates. This neuron expresses several kinds of neurosteroidogenic enzymes, such as cytochrome P450 side-chain cleavage enzyme (P450scc), 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase (3β-HSD), etc., and produces pregnenolone, pregnenolone sulfate, progesterone and progesterone metabolite(s). Thus, our studies provided the first evidence for neuronal neurosteroidogenesis in the brain. Furthermore, the Purkinje neuron is known to play an important role in the process of memory and learning. Therefore, this neuron is a useful cellular model for understanding biological actions of neurosteroids in the brain.

Genomic and nongenomic actions of neurosteroids

Cytochrome P450scc appeared in the mammalian Purkinje neuron immediately after differentiation and the expression of this enzyme persisted during neonatal development into adulthood, indicating the constant production of pregnenolone and its sulfate. To clarify the mode of action of neurosteroids, we examined the effects of pregnenolone and its sulfate on synaptic currents in Purkinje neurons using the rat. Pregnenolone sulfate, produced in Purkinje neurons, modulated GABAergic neurotransmission by nongenomic actions on GABAergic neurons rather than by genomic mechanisms. Until recently, we believed that all steroid hormones regulate biological functions by genomic mechanisms. However, it is now accepted that pregnenolone sulfate and other neurosteroids mediate their actions through ion-gated channel receptors.

Interestingly, this neuron actively synthesized progesterone, as a product of an increase of 3β-HSD activity, only during a limited neonatal period, when cerebellar cortical formation occurs dramatically. Our *in vitro and in vivo* studies demonstrated that progesterone, produced as a neurosteroid in the Purkinje neuron during neonatal life, promotes both dendritic outgrowth and synaptogenesis in this neuron through intranuclear receptor-mediated mechanisms during cerebellar development. Such organizing actions contribute to the formation of the cerebellar neuronal circuit. This is the first demonstration of cerebellar cortical organization by a neurosteroid.

Conclusions: *De novo* steroidogenesis from cholesterol is a conserved property of vertebrate brains, and such steroids synthesized *de novo* in the brain are called neurosteroids. This new concept derived from observations made by Baulieu's laboratory and this laboratory. Our studies further gave the opportunity to understand biosynthesis and biological actions of neurosteroids produced in the Purkinje neuron, a major site of neurosteroidogenesis in the brain. These served an excellent cellular model for understanding the plasticity of brain cells in relation to actions of neurosteroids, because Purkinje neurons play an important role in the process of memory and learning. Recent behavioral studies using neurosteroidogenic enzyme and/or neurosteroid receptor knock-out animals and electrophysiological studies on the occurrence of long-term potentiation (LTP) and/or long-term depression (LTD) are providing new information for understanding physiological roles of neurosteroids in the brain.