Steroidogenic acute regulatory protein: its presence and function in brain neurosteroidogenesis*

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Summary. Neurosteroids are synthesized *de novo* and involved in a variety of physiological functions in the central and peripheral nervous systems. Although the steroidogenic acute regulatory protein (StAR) plays an essential role in the steroidogenesis of peripheral endocrine glands, its presence and role in the brain had been previously questioned because of difficulties in detecting it. However, a number of recent studies have confirmed the presence of StAR in rodent and human brains. Moreover, there is evidence suggesting that StAR plays a role in steroidogenesis in the brain, as it does in peripheral endocrine organs. The present review presents data regarding the presence and role of StAR in brain steroidogenesis, demonstrating the essential characteristics of the protein.

The term 'neurosteroid' was coined by Baulieu in 1981 to designate a steroid hormone intermediate, dehydroepiandrosterone sulfate (DHEA-S), which was found in the brain at concentrations independent of its plasma levels (Baulieu, 1981). Now it was known that many neurosteroids are synthesized *de novo* in the central nervous system (CNS) (Paul and Purdy, 1992). Although natural or

synthetic steroids that can alter neuronal membrane excitability are considered as 'neuroactive steroids', irrespective of their origin, neurosteroids are now defined as steroids, the accumulation of which in the CNS and peripheral nervous systems (PNS) occurs independently, at least in part, of any supply by steroidogenic endocrine glands, and which can be synthesized *de novo* in the nervous system from sterol precursors (Baulieu, 1997).

Neurosteroids are associated with a range of physiological functions including neurotransmission *via* specific receptors (Majewska, 1992; Monnet *et al.*, 1995), neural growth and differentiation (Bologa *et al.*, 1987), neural plasticity (McEwen *et al.*, 1995; Garcia-Segura *et al.*, 1995), behaviors including memory performance and aggressiveness (Young *et al.*, 1991; Robel *et al.*, 1995), homeostatic effects during stress responses (Broekhoven and Verkes, 2003), and regulatory functions during normal development (Olsen, 1983; Hutchison, 1991). Importantly, the effects of neurosteroids can be mediated without involvement of classic steroid hormone receptors (Majewska *et al.*, 1986).

Steroid hormones are synthesized in the adrenals, gonads, placenta and central nervous system. Organ-specific steroid hormones with different physiological effects are synthesized in each tissue, depending upon the set of enzymes present in it. Regardless of tissue origin, a common feature of all steroid hormones is that they are synthesized from a common precursor, namely cholesterol. Indeed, it is the delivery of cholesterol to the site of its first enzymatic conversion that constitutes the rate-limiting and hormonally acutely-regulated step in steroidogenesis (Karaboyas and Koritz, 1965; Brownie *et al.*, 1972; Simpson, 1979; Crivello and Jefcoate, 1980; Privalle *et al.*, 1983; Jefcoate *et al.*, 1987)—Although the rate-limiting step in steroidogenesis was previously thought to be pregnenolone production (Stone and Hechter, 1954; Karaboyas and Koritz, 1965).

In greater detail, the first step in all steroidogenesis is

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the delivery of cellular cholesterol in the outer mitochondrial membrane to the inner mitochondrial membrane, the site of the cytochrome P450 side chain cleavage enzyme (P450scc) which converts cholesterol to pregnenolone (Lambeth *et al.*, 1987; Privalle *et al.*, 1987). The P450scc-linked monooxygenase system is located on the matrix side of the inner mitochondrial membrane. Delivery of cholesterol to P450scc is an assisted process that requires *de novo* protein synthesis (Ferguson, 1962, 1963; Garren *et al.*, 1965, 1966).

The identity of the protein that assists the translocation of cytoplasmic cholesterol to the mitochondria inner membrane remained a mystery for almost four decades. Of several candidate proteins, the steroidogenic acute regulatory protein (StAR) has been regarded as the most likely one. First identified as a 30 kDa phosphoprotein associated with the mitochondria (Krueger and Orme-Johnson, 1983; Pon *et al.*, 1986; Pon and Orme-Johnson, 1988; Stocco and Kilgore, 1988; Epstein and Orme-Johnson, 1991; Stocco and Sodeman, 1991), StAR was purified, and the gene was cloned and sequenced in 1994 (Clark *et al.*, 1994). Its expression in MA-10 or COS-1 cells resulted in significant increases in steroid hormone synthesis in the absence of hormone stimulation such as the leutenizing hormone and follicle stimulating hormone, suggesting that StAR stimulated cholesterol transport to P450scc (Clark *et al.*, 1994; Sugawara *et al.*, 1995; Lin *et al.*, 1995; Stocco and Clark, 1996). Perhaps the most compelling evidence for a role by StAR in mediating cholesterol transfer and hence regulating steroid hormone biosynthesis was the finding that mutations in the StAR gene result in the potentially lethal condition known as congenital lipoid adrenal hyperplasia (Lin *et al.*, 1995). This disease is characterized by an almost complete lack of steroid hormone synthesis, and this blockage was found to be at the locus of cholesterol delivery to P450scc. Moreover, StAR 'knockout' mice have been generated and their phenotype is essentially identical to that seen in the human condition (Caron *et al.*, 1997).

In spite of its potential importance, neurosteroid biosynthesis has been poorly understood. Actually, past difficul-

ties in identifying StAR in the brain led to doubts as to whether this protein was really involved in brain steroidogenesis. However, recent studies have shown that StAR is present in the mammalian brain, and in this review we summarize the findings of those studies and present evidence indicating a role for StAR in brain steroidogenesis.

1. StAR expression in rodent brains

1.1. Adult brains

Furukawa *et al.* (1998) cloned two types of StAR cDNA (1.3 and 3.4 kb) by library screening from the rat. Using a Northern blot analysis of the rat brain, Kim *et al.* (2003a) detected StAR mRNA transcripts of 1.6 and 3.6 kb, which were the same sizes as transcripts found in adrenal glands and gonads. The difference in transcript sizes observed using the two methods may be due to polyadenylation (Mizutani *et al.*, 1997).

StAR mRNA transcripts have been identified in the following regions of the adult rat brain: cerebral cortical cells, mitral cells of the olfactory bulb, cells of the caudate putamen, several nuclei in the hypothalamus (the median preoptic, lateral habenular, arcuate hypothalamic and paraventricular nuclei in particular), neuronal cells of CA1, 2, 3, and dentate gyrus of the hippocampus, cerebellar granule cells, and Purkinje cells (Furukawa *et al.*, 1998; Kim *et al.*, 2003a; Fig. 1). These findings are generally consistent with those of immunohistochemical studies on the localization of the StAR protein in rat and mouse brains (Wehrenberg *et al.*, 2001; Kimoto *et al.*, 2001; King *et al.*, 2002; Sierra *et al.*, 2003). The data from these studies indicate that the StAR expression pattern is similar to that of the P450scc expression pattern (Le Goascogne *et al.*, 1987; Iwahashi *et al.*, 1993; Compagnone *et al.*, 1995). Indeed, StAR and P450scc were shown to co-localize in some hippocampus and cerebellum neurons (Furukawa *et al.*, 1998; Kimoto *et al.*, 2001), strongly demonstrating that StAR may acutely regulate steroidogenesis in the CNS, as has been established in peripheral tissues.

Fig. 1. *In situ* localization of StAR mRNA in the rat brain (Kim *et al.*, 2003a). **A:** Schematic localization of StAR mRNA in the rat brain. The symbols, “*”, represent StAR mRNA signals. **B:** Hippocampus. **B-a:** Bregma -4.80 mm. Scale bar: 300 μ m. **B-b:** Field CA1 of Ammon's horn. Magnified representation of box b in B-a. Scale bars for B-b-e: 60 μ m. **B-c:** Field CA2 of Ammon's horn. Magnified representation of box c in B-a. **(B-d):** Field CA3 of Ammon's horn. Magnified representation of box d in B-a. **B-e:** Dentate gyrus and polymorph layer. Magnified representation of box e in B-a. **C:** Cerebellum and pons. **C-a:** Bregma -10.52 mm. Scale bar: 300 μ m. **C-b:** Cerebellar Purkinje cells. Magnified representation of box b in C-a. Scale bar for C-b-e: 60 μ m. **C-c:** Cerebellar Purkinje cell and cerebellar granular cell layer. Magnified representation of box c in C-a. **C-d:** Vestibular nucleus. Magnified representation of box c in C-a. **C-e:** Gigantocellular reticular nucleus. Magnified representation of box e in C-a.

Fig. 1. Legend on the opposite page.

1.2. Developing brain

While the amount of StAR mRNA in the rat brain was found to be much less than that in peripheral endocrine organs, it is expressed from birth and, in general, appears to gradually increase during postnatal development (Kim *et al.*, 2002). StAR mRNA expression has been shown to increase fairly linearly in the hypothalamus from birth to adulthood, while the expression increases in a sigmoidal fashion in the olfactory bulb during the same period of development (Kim *et al.*, 2002). Immunohistochemical studies have shown that the StAR protein first appears in the rat brain on postnatal day 10, and that the expression then either increases or remains constant until adulthood (Sierra *et al.*, 2003). More recent immunohistochemical studies have shown StAR protein expression in the neonatal mouse brain (King *et al.*, 2004).

The mammalian nervous system is particularly sensitive to steroids during the late fetal and early postnatal periods. At this stage of development, many neuronal and glial cells are undifferentiated, and gonadal steroids influence the survival and maturation of these cells (Arnold and Gorski, 1984; McEwen *et al.*, 1979; McEwen, 1991). Steroid hormones supplied by peripheral steroidogenic organs can cross the blood-brain barrier due to their lipid solubility and can regulate several important brain-neuron functions during development (Fuxe *et al.*, 1981; McEwen, 1991). However, peripheral endocrine organs may not be the sole governor of steroid effects since the developing brain also contains high levels of P450 aromatase, and a significant amount of gonadally derived testosterone is converted to estrogen in the brain (Harada and Yamada, 1992). The aromatization of testosterone is a necessary event in the masculinization of the rodent brain (Naftolin *et al.*, 1975). Besides aromatase, many other steroidogenic enzymes are expressed in the brain from the early embryonic stage through to adulthood (Compagnone and Mellon, 2000).

More interestingly, StAR mRNA and protein have been shown to be expressed in discrete rat brain cell populations during the postnatal period (Kim *et al.* 2002, Sierra *et al.* 2003), and it appears that steroids are synthesized *de novo* in the brain from birth to adulthood. These findings indicate that early postnatal development may also be affected by steroids synthesized *de novo* in the brain. Furthermore, the increased expression of StAR mRNA in the postnatal period suggests that it might have a role in regulating neurosteroidogenesis associated with neuronal growth and differentiation during postnatal rat brain development (Kim *et al.*, 2002).

2. StAR expression in human brain and brain tumor

The human brain is as much a steroidogenic organ as the endocrine glands. Recently, steroidogenic enzymes known to be expressed in adrenal glands were also found to be expressed in the brain (Brown *et al.*, 2000; Yu *et al.*, 2002). Steroid hormones are known to participate in several physiological and pathological processes in the brain, particularly including tumor cell growth (Langeveld *et al.*, 1992; Nygren *et al.*, 1997; Pinski *et al.*, 1993). In addition, steroid receptors are present in the normal human brain and in tumors, and neurosteroidogenesis also occurs *de novo* (Baulieu, 1981; Weidenfeld and Schiller, 1984; Khalid *et al.*, 1997; Assimakopoulou *et al.*, 1998). Immunohistochemical studies have shown that the StAR protein is expressed in the normal human brain tissue (Inoue *et al.*, 2002, King *et al.*, 2002). More recently, Kim *et al.* (2003b) reported StAR mRNA and protein expression in the normal human brain and intracranial tumor tissue. These studies showed that StAR mRNA was expressed at very low levels in the white matter of the normal human brain, and was more highly expressed in oligodendrogliomas and malignant glial tumors, suggesting that StAR may play a role in human glioma tumorigenesis. These findings are consistent with those showing that cholesterol is required for glioma cell proliferation (Nygren *et al.*, 1997). Furthermore, the glucocorticoid agonist, dexamethasone, stimulated human glioma cell proliferation, while the glucocorticoid antagonist, RU38486, abolished the effect of dexamethasone (Langeveld *et al.*, 1992, Pinski *et al.*, 1993). Therefore, the steroid hormones that promote tumor cell proliferation can be derived from surrounding micro-vessels in brain tissue, or from the tumor itself through *de novo* synthesis. In addition, StAR expression was found to be greater in glioblastoma-multiforms, the most malignant form of astrocytoma, compared with anaplastic astrocytomas (Kim *et al.*, 2003b, Fig. 2). Taken together, all this evidence suggests that increased StAR expression might correlate with tumor progression and intratumoral steroidogenesis in malignant astrocytomas (Kim *et al.*, 2003b).

3. Regulation of StAR expression and its potential roles in the brain

3.1. Acute regulation of brain StAR expression

Clear evidence for the active transcription of StAR under circumstances demanding neurosteroidogenetic activation would strongly support the idea that StAR has a functional

Fig. 2. Immunohistochemistry of StAR in the human brain (Kim *et al.*, 2003b). **A** and **B**: Normal human brain gray matter. **C**: Normal human brain white matter. **D**: Anaplastic astrocytoma. **E** and **F**: Glioblastoma. **G–I**: Double immunofluorescence of anaplastic astrocytoma using anti-StAR (red) and anti-GFAP (green) antibody. Double positive cells are yellow in **I**. Scale bars in **A–I**=50 μ m

significance in the brain. The concentration of brain neurosteroids such as 3 α -hydroxy-5 α -pregnanolone (allopregnanolone) increases in various stress paradigms, including that of acute alcohol administration (Purdy *et al.*, 1991; Barbaccia *et al.*, 1996; VanDoren *et al.*, 2000). VanDoren *et al.* (2000) reported that elevated brain allopregnanolone levels did not correlate with either plasma corticosterone or progesterone levels or with plasma allopregnanolone levels, and suggested that brain steroids are regulated differently from circulating steroids. Acute alcohol administration can alter levels of brain steroidogenic enzymes either directly

or indirectly, and such changes could explain their regulatory roles in coping with environmental changes. In fact, Kim *et al.* (2003a) showed confirmed rapid changes in the mRNA expression of StAR as well as other steroidogenic enzymes including P450_{scc}, 5 α -reductase type I, aromatase, 3 β -hydroxysteroid dehydrogenase (3 β HSD) and 3 α -hydroxysteroid dehydrogenase after acute alcohol injection (Fig. 3). *De novo* neurosteroidogenesis may be anticipated under stressful circumstances in order to maintain brain homeostasis. Indeed, activation of a set of steroidogenic enzymes responsible for *de novo* synthesis has been reported

Fig. 3. RT-PCR analysis of steroidogenic enzyme mRNAs after an acute alcohol administration in the frontal cortex (Kim *et al.*, 2003a). The left panels show photographs of PCR products on agarose gels and the right panels show a densitometric analysis of each mRNA levels are presented as arbitrary units (A.U.) that were normalized to the corresponding GAPDH signal. SF-1, steroidogenic factor-1; StAR, steroidogenic acute regulatory protein; P450scc, cholesterol side chain cleavage enzyme; 3βHSD, 3β-hydroxysteroid dehydrogenase; 5αR-I, 5α-reductase; P450aro, aromatase; 3α HSD, 3α-hydroxy steroid dehydrogenase. Represented data: mean+S.E.M. ($n=3$). * $P=0.05$ (vs. '0' time point); ** $P=0.01$ (vs. '0' time point).

(Kim *et al.*, 2003b, Fig. 3). A rapid increase in brain StAR mRNA expression is noteworthy, considering its rate-limiting role in peripheral steroidogenic systems.

3.2. Constitutive expression of brain StAR in the absence of peripheral endocrine glands

To our knowledge, the concept of 'neurosteroids' was first proposed on the basis data generated using endocrine organ depletion models (Corpechot *et al.*, 1983). In a recent review, Baulieu commented that the continued presence of steroid compounds several weeks after the surgical removal of adrenal glands and gonads was due to their *de novo* synthesis in the brain (Baulieu, 1997). Therefore, neurosteroidogenesis is independently maintained in the brain after endocrine gland depletion. Pertinent to the present review, Kim *et al.* (2003a) showed that StAR mRNA was expressed in various brain regions in the absence of adrenal glands and gonads after surgery. These data indicate that StAR is constitutively expressed in the brain at an extremely low level, and suggest that StAR independently acts to maintain brain steroid levels regardless of the existence of adrenal glands and gonads.

3.3. Steroidogenic factor-1 and StAR expression in the brain

Steroidogenic factor-1 (SF-1) is an orphan steroid receptor originally shown to regulate transcription of StAR and

essential steroidogenic enzymes in peripheral endocrine organs (Peter and Dubuis, 2000). Since SF-1 and StAR co-localize in the rat hippocampus (Wehrenberg *et al.*, 2001), the transcription of StAR and other steroidogenic enzymes may be regulated by SF-1 in the brain, as an SF-1 an dose in peripheral endocrine glands. Kim *et al.* (2003a) found that acute alcohol administration resulted in an increase in SF-1 transcription in the frontal cortex and hippocampus, suggesting that SF-1 may activate the transcription of steroidogenic genes including StAR, P450scc and 3β HSD. Indeed, they also found increased StAR and P450scc mRNA expression in both regions. However, Zhang *et al.* (1995) reported that the brain contains an additional factor not found in the adrenal or testis that binds to the rat P450scc promoter and increases its basal and cAMP-mediated transcription. In the hypothalamus, while SF-1 expression did not increase after alcohol injection, StAR and P450scc mRNA expressions were shown to increase (Kim *et al.*, 2003a). These data suggest that StAR and P450scc expressions in the hypothalamus are not regulated by SF-1, even though SF-1 is normally expressed in the ventromedial nucleus and is essential for its development (Ikeda *et al.*, 1994).

In summary, StAR expression appears to be linked to distinct events in brain tissue, and there is strong evidence that it plays a role in the regulation of neurosteroidogenesis (Fig. 4). While it appears that StAR is involved at the start of the neurosteroidogenic pathway, there remain many

Fig. 4. Schematic diagram of putative mechanism in early stages in brain neurosteroidogenesis. The numerical assigned events on this diagram represent a possible sequence that occurs upon acute neurosteroidogenesis in neural cells.

questions regarding the exact mechanisms underlying the regulation of neurosteroidogenesis by StAR. In addition, little is known of the mechanisms that regulate StAR expression and activity in the brain, particularly in response to externally initiated environmental changes including stress, drugs, and injuries. Such knowledge will serve in the development of strategies for manipulating brain neurosteroidogenesis clinically in order to overcome various neural disorders.

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