

## STEROIDOGENIC ACUTE REGULATORY PROTEIN IN THE BRAIN

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**Abstract**—The nervous system synthesizes steroids that regulate the development and function of neurons and glia, and have neuroprotective properties. The first step in steroidogenesis involves the delivery of free cholesterol to the inner mitochondrial membrane where it can be converted into pregnenolone by the enzyme cytochrome P450side chain cleavage. The peripheral-type benzodiazepine receptor and the steroidogenic acute regulatory protein are involved in this process and appear to function in a coordinated manner. Steroidogenic acute regulatory protein mRNA and protein are widely expressed throughout the adult brain. Steroidogenic acute regulatory protein expression has been detected in many neuronal populations, in ependymocytes, in some astroglial cells, in Schwann cells from peripheral nerves and in proliferating cells of the developing and adult brain. Steroidogenic acute regulatory protein is colocalized in the same neural cells with P450side chain cleavage and with other steroidogenic enzymes. Steroidogenic acute regulatory protein expression in the brain shows marked changes with development, aging and injury. The steroidogenic acute regulatory protein gene may be under the control of diverse mechanisms in different neural cell types, since its expression is upregulated by cyclic AMP (cAMP) in gliomas and astrocytes in culture and downregulated by cyclic AMP (cAMP) in Schwann cells. In addition, activation of *N*-methyl-D-aspartate receptors, and the consequent rise in intracellular calcium levels, activates steroidogenic acute regulatory protein and steroidogenesis in hippocampal neurons. In conclusion, steroidogenic acute regulatory protein is regulated in the nervous system by different physiological and pathological conditions and may play an important role during brain development, aging and after injury. © 2005 Published by Elsevier Ltd on behalf of IBRO.

**Key words:** neurodegeneration, neurogenesis, oxysterols, peripheral-type benzodiazepine receptor, pregnenolone, steroidogenesis.

It is now well accepted that the nervous system is a steroidogenic tissue (Baulieu, 1997, 1998; Mellon et al., 2001; Stoffel-Wagner, 2003). Both the central and the peripheral

nervous systems express enzymes involved in the synthesis and metabolism of steroids. These include the cytochrome P450side chain cleavage (P450scc) enzyme, which catalyzes the conversion of cholesterol into pregnenolone, the precursor for glucocorticoids, mineralocorticoids and sex steroids. The synthesis of pregnenolone is the first enzymatic step of steroidogenesis. However, there is a previous step that is rate limiting and hormonally regulated: the transfer of cholesterol from the outer to the inner mitochondrial membrane, where P450scc is located. Proteins located in the mitochondrial membranes, such as the peripheral benzodiazepine receptor (PBR) and the steroidogenic acute regulatory protein (StAR), allow cholesterol to cross the hydrophilic intermembrane space (Haut et al., 2005).

PBR was initially described on peripheral tissues as a second binding site for diazepam, which binds with higher affinity to GABA<sub>A</sub> receptors on the nervous system (Braestrup and Squires, 1977). Since then, many studies have demonstrated that PBR is pharmacologically and structurally distinct from the central benzodiazepine/GABA<sub>A</sub> receptors. PBR is an 18-kDa peptide located predominantly in the mitochondrial membranes (Anholt et al., 1986; Papadopoulos et al., 1994) and represents a critical component of the permeability transition pore, a multiprotein complex implicated in the regulation of apoptosis (Gallegue et al., 2003; Chelli et al., 2004; Kunduzova et al., 2004; Marselli et al., 2004; Veenman et al., 2004). In addition, PBR has been related with the regulation of several physiological events, including the control of steroidogenesis (Papadopoulos et al., 1997; Brown and Papadopoulos, 2001; Casellas et al., 2002; Lacapere and Papadopoulos, 2003).

PBR is expressed in the nervous system, predominantly in glial cells (Vowinckel et al., 1997; Kuhlmann and Guilarte, 2000; Casellas et al., 2002; Wilms et al., 2003). In addition, different forms of neural injury and different neuropathological conditions result in the induction of the expression of PBR in the areas of the nervous system involved in the neurodegenerative events. The induction of PBR expression after injury in the CNS is mainly restricted to microglia and astrocytes (Vowinckel et al., 1997; Kuhlmann and Guilarte, 2000; Casellas et al., 2002; Wilms et al., 2003), although a recent study has shown induction of PBR in dorsal root ganglion neurons following injury to the sciatic nerve (Karchewski et al., 2004). The induction of PBR expression after neural injury suggests that this molecule may be involved in the response of the neural tissue to cope with the neurodegenerative process. Indeed, it has been reported that SSR180575, a pyridazinole derivative that possesses a high affinity for the

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**Abbreviations:** ABCA1, ATP-binding cassette transporter A1; cAMP, cyclic AMP; GFAP, glial fibrillary acidic protein; LXRs, nuclear hormone liver X receptors; NMDA, *N*-methyl-D-aspartate; PBR, peripheral benzodiazepine receptor; P450scc, P450side chain cleavage; StAR, steroidogenic acute regulatory protein.

PBR, increases the survival of facial nerve motoneurons after axotomy and promotes the regeneration of peripheral nerves (Ferzaz et al., 2002). Another PBR ligand, the benzodiazepine 7-chloro-5-(4-chlorophenyl)-1,3-dihydro-1-methyl-2H-1,4-benzodiazepin-2-one (Ro5-4864), prevents the loss of neurons and the activation of microglia and astroglia induced by kainic acid in the rat hippocampal formation (Veiga et al., 2005). These neuroprotective effects of PBR ligands may be mediated by an increased steroid synthesis, although other alternative or complementary mechanisms cannot be excluded (Veiga et al., 2005).

PBR is closely associated to StAR in mitochondrial membranes (West et al., 2001) and both proteins interact in the transfer of cholesterol across the outer mitochondrial membrane to the inner mitochondrial membrane (Hauet et al., 2005). StAR was first characterized in murine MA-10 Leydig tumor cells as a mitochondrial protein responsible for the acute induction of steroidogenesis (Clark et al., 1994). StAR is formed as a 37-kDa protein, which is rapidly transported into mitochondria where it is cleaved, generating a mature 30-kDa intramitochondrial StAR protein that is inactive (Bose et al., 2002). StAR has been extensively studied in classical steroidogenic tissues such as the adrenal gland and the ovary (Stocco, 2001). In addition, recent studies have shown the expression of StAR in the central and the peripheral nervous systems. In the remaining sections of this review we will present an overview of the current knowledge on the distribution, regulation and function of StAR in the nervous system. We limit our attention to StAR, since PBR is the focus of another paper in this same issue (Papadopoulos et al., 2005).

### Expression of StAR in the nervous system

Expression of StAR has been detected in the rat sciatic nerve and in cultured Schwann cells (Benmessahel et al., 2002, 2004). These findings are in agreement with the well-characterized capacity of peripheral nerves and Schwann cells to synthesize steroids and with the effects of these molecules on nerve regeneration and myelination (Schumacher et al., 2001; Ibanez et al., 2003; Melcangi et al., 2003).

Several studies have reported the expression of StAR in the CNS as well (Furukawa et al., 1998; Kimoto et al., 2001; Wehrenberg et al., 2001; Inoue et al., 2002; Kim et al., 2002, 2003a, 2004; King et al., 2002; MacKenzie et al., 2002; Sierra et al., 2003). StAR appears to be widely distributed throughout the brain, although different levels of expression have been detected between different brain areas. Furthermore, StAR expression seems to be restricted to very specific neuronal and astroglial populations in each brain area (Sierra, 2004). High levels of StAR mRNA expression have been detected in the cerebral cortex, hippocampus, dentate gyrus, olfactory bulb, cerebellar granular layer, and cerebellar Purkinje cells of rodents (Furukawa et al., 1998; Wehrenberg et al., 2001; Kim et al., 2002, 2003a; King et al., 2002). StAR mRNA has also been detected in human brain (Inoue et al., 2002; King et al., 2002; Kim et al., 2003b). Western blot and

immunohistochemical analyses have confirmed that StAR protein is widely expressed throughout the adult CNS (Kimoto et al., 2001; Sierra et al., 2003). This may implicate that steroidogenesis is a generalized process in the CNS. In addition, StAR is colocalized in the same neural cells with cytochrome P450<sub>scc</sub> and with other steroidogenic enzymes (Furukawa et al., 1998; Wehrenberg et al., 2001; Kimoto et al., 2001; King et al., 2002, 2004a), suggesting that individual neural cells may synthesize several steroids directly from cholesterol.

StAR immunoreactivity has been detected in several neuronal populations, in ependymocytes and in some astroglial cells in the brain (King et al., 2002; Sierra et al., 2003). The immunoreactive signal is located in the cytoplasm and has a punctuate aspect compatible with mitochondrial localization. Strong StAR immunoreactivity is observed in the soma of large neurons, such as motoneurons of the motor cranial nerves, large rombencephalic motoneurons, neurons of the deep cerebellar nuclei, Purkinje cells of the cerebellar cortex and pyramidal neurons of the cerebral cortex. These large neurons are characterized by high mitochondrial content. In general, there is a good agreement between data from *in situ* mRNA localization and data from immunohistochemical studies. Table 1 shows the areas of the brain where neuronal somas with high StAR expression have been detected. These include (i), areas involved in the transmission, processing and integration of sensory information, such as the olfactory bulb, olfactory nuclei, somatosensory cerebral cortex, lateral geniculate nucleus, superior olive, superior colliculus, vestibular and cochlear nuclei or the spinal trigeminal nucleus; (ii), areas involved in motor coordination and control, such as the motor cerebral cortex, the globus pallidus, some thalamic nuclei, the cerebellum or several motor nuclei in the brainstem; (iii), areas involved in the control of brain activity and cognition such as the locus coeruleus, the reticular formation and the hippocampal formation. Therefore, StAR is expressed in brain areas with different functions. This broad distribution of StAR corresponds to what should be expected, considering the variety of brain functions affected by locally produced steroids (Baulieu, 1998; Compagnone and Mellon, 2000; Mellon et al., 2001; Stoffel-Wagner, 2003).

Interestingly, the hypothalamus, a key center for the regulation of neuroendocrine control, which is under the regulation of hormonal steroids, shows a relatively low expression of StAR. Although neurons in some hypothalamic nuclei, such as the arcuate nucleus, express StAR, in general the expression levels detected are low in most hypothalamic nuclei, compared with other brain areas. The low level of expression of StAR in areas with a high sensitivity to steroids, such as the hypothalamus, or the amygdala, is intriguing. It is possible that areas whose function is mainly under the control of hormonal steroids may express low StAR levels and therefore, a low production of local steroids. In contrast, StAR expression may be higher in areas where the impact of locally produced steroids predominates over the impact of peripheral steroids. However, it should be noted that other proteins that contain the

**Table 1.** Areas of the rat brain that show the strongest StAR expression in neurons, based on *in situ* localization of mRNA and/or on immunoreactive signal. (According to Furukawa et al., 1998; Kimoto et al 2001; Wchronberg et al., 2001; Kim et al., 2002, 2003a; King et al., 2002; Sierra et al., 2003; King et al., 2004a)

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Telencephalon
Olfactory bulb (mitral cells)
Olfactory nuclei
Motor cortex
Somatosensory cortex
Hippocampal formation
Globus pallidus
Ventral pallidum
Septum medial
Diagonal band
Nucleus of the lateral olfactory tract
Diencephalon
Lateral habenular nucleus
Dorsal anterior thalamic nucleus
Central lateral nucleus
Lateral geniculate nucleus
Intralaminar nucleus
Laterodorsal nucleus
Subthalamic nucleus
Sustantia innominata
Lateral posterior nucleus
Posterior thalamic area
Preoptic area
Arcuate nucleus
Mesencephalon
Superior colliculus
Central gray
Locus coeruleus
Darkschewitsch's nucleus
III Nucleus
Anterior tegmental nucleus
Interfascicular nucleus
Paranigral nucleus
Reticular formation
Pons
VI Nucleus
VII Nucleus
Trapezoid body
Superior olive
Neurons from the reticular formation
Medulla oblongata
V spinal nucleus
Vestibular nuclei
Choclear nucleus
Vestibulo-cochlear ganglion
X nucleus
XII nucleus
Cerebellum
Nuclei
Cortex (Purkinje cells)

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StAR-related lipid transfer (START) domain might be involved in steroidogenesis (Strauss et al., 2003) and that their regional pattern of expression in the brain remain to be determined.

Although StAR is predominantly expressed by neurons in the CNS, astrocytes also express StAR, both *in vivo* and *in vitro* (King et al., 2002; Sierra et al., 2003). Immunore-

activity for both StAR and the astroglial marker glial fibrillary acidic protein (GFAP), is observed in the gray matter of specific brain areas such as the molecular layer of the hippocampus and the superficial layers of the cerebral cortex. In the white matter, StAR expression is exclusively restricted to astrocytes. In some white matter areas in proximity to the pia, long StAR immunoreactive cell processes are observed perpendicular to the brain surface. These cell processes are immunoreactive for both GFAP and vimentin and probably correspond to marginal glia. In other areas of white matter, including the hippocampal fimbria, the corpus callosum or the corticospinal tract, immunoreactivity for both GFAP and StAR is observed in the soma and processes of astrocytes (Sierra et al., 2003). This suggests that a subpopulation of astroglial cells is able to synthesize steroids from cholesterol *in vivo*, in agreement with the ability of astrocytes to synthesize pregnenolone, progesterone, dehydroepiandrosterone, androstenedione and testosterone *in vitro* (Zwain and Yen, 1999) and with the localization of P450scc in brain white matter (Le Goascogne et al., 1987). The local production of steroids, such as progesterone, by astrocytes in the white matter may influence oligodendrocyte differentiation and myelination (Jung-Testas et al., 1996; Gago et al., 2001). However, despite the fact that oligodendrocytes express several steroidogenic enzymes and may produce pregnenolone *in vitro* (Zwain and Yen, 1999), StAR expression has not been observed *in vivo* in cells stained with the oligodendrocytic marker RIP (Sierra et al., 2003). Finally, it should be mentioned that StAR (Kim et al., 2003b) and other steroidogenic enzymes (Papadopoulos et al., 1992; Zhang et al., 1995; Yague et al., 2004) are expressed in gliomas and may be potentially involved in tumor progression.

#### Developmental changes and effects of aging and neurodegeneration

Steroids are known to affect neuronal and glial differentiation and the formation of neuronal connectivity (Garcia-Segura et al., 1994; Baulieu, 1997, 1998; McEwen and Alves, 1999; Compagnone and Mellon, 2000; Mellon et al., 2001). In this regard, it is of interest that StAR is expressed in the developing brain (Kim et al., 2002; Sierra et al., 2003; King et al., 2004a). One of the possible roles of StAR in developing nervous system is the regulation of neurogenesis. This is suggested by its expression in germinal layers of the developing brain. In addition, StAR is expressed in the subventricular zone of the adult brain, in proliferating cells that incorporate BrdU (Sierra et al., 2003). Furthermore, steroids are known to affect glial and neuronal proliferation in the adult brain (Garcia-Estrada et al., 1999; Tanapat et al., 1999; Banasr et al., 2001; Brannvall et al., 2002; Karishma and Herbert, 2002; Giachino et al., 2003, 2004; Mayo et al., 2005). Therefore, it is possible that activation of StAR may contribute to the regulation of cell proliferation in the nervous system.

It is also noteworthy that important changes in the pattern and/or level of expression of StAR occur in different brain areas during postnatal development (Kim et al.,

2002; Sierra et al., 2003). Although the significance of these developmental changes remains to be clarified, it is probable that changes in StAR expression may affect developmental processes by the modification of local steroid levels. For instance, in the developing cerebellar cortex StAR is transiently expressed in the external granular layer (Sierra et al., 2003), where granule cells proliferate and initiate their migration toward the internal granular layer. Purkinje cells also express StAR in developing and adult rats (Furukawa et al., 1998; Sierra et al., 2003). However, the intensity of StAR immunostaining in these cells is higher at P10 and P20 than in 2-month-old rats (Sierra et al., 2003). Recent studies have shown that Purkinje cells have an active steroidogenic activity, in particular during the developmental period (Tsutsui et al., 2003, 2004). Purkinje cells synthesize progesterone and estradiol de novo from cholesterol during neonatal life. In turn, these steroids regulate the maturation of Purkinje cell dendrites, dendritic spines and synaptic contacts (Tsutsui et al., 2003, 2004). Changes in the pattern of expression of StAR in the developing cerebellar cortex may therefore be related with the proliferation and migration of granule neurons, with the differentiation of Purkinje and granule cells and with the formation of synaptic connectivity in the molecular layer.

Important changes in the expression of StAR occur as well during brain aging. In 24-month-old rats StAR immunoreactivity is increased in hippocampal and cortical neurons as well as in glial processes compared with young animals (Sierra et al., 2003). In contrast, StAR expression is decreased in the aged cerebellum (Dertien et al., 2004). It is unknown what is the physiological significance of these changes in StAR expression in the brain of aging animals and whether they are associated to differences in steroidogenesis.

Among other functions, steroidogenesis in the nervous system may play a neuroprotective role, since pregnenolone and its derivatives have neuroprotective properties (Compagnone and Mellon, 2000; Garcia-Segura et al., 2001; Schumacher et al., 2003; Stoffel-Wagner, 2003; Maggi et al., 2004). As mentioned before, it is well established that PBR is upregulated in the nervous system after injury (Vowinckel et al., 1997; Kuhlmann and Guilarte, 2000; Casellas et al., 2002; Wilms et al., 2003; Karchewski et al., 2004). Recent studies have shown that StAR expression is also responsive to neurodegenerative insults: StAR mRNA and protein levels increase acutely and transiently in the brain after injury (Sierra et al., 2003). This raises the possibility that the upregulation of the expression of PBR and StAR and the subsequent formation of neuroprotective steroids may be part of the mechanisms used by the nervous system to cope with neurodegeneration. In addition, the upregulation of the expression of StAR and PBR after injury may be coordinated with the upregulation of steroidogenic enzymes and steroid receptors (di Michele et al., 2000; Blurton-Jones and Tuszyński, 2001; Garcia-Ovejero et al., 2002; Garcia-Segura et al., 2003).

### Molecular mechanisms that regulate StAR expression by neural cells

The mechanisms that regulate StAR expression and steroidogenesis in the nervous system need to be studied with more detail. Probably, factors that regulate StAR expression may also affect the expression and/or activity of steroidogenic enzymes. For instance, one of such factors is ethanol, which induces parallel changes in the mRNA levels of a variety of steroidogenic enzymes and StAR in several brain regions (Kim et al., 2003a).

A potent inducer of StAR expression in steroidogenic tissues is cyclic AMP (cAMP) (Stocco, 2001). cAMP has been also shown to upregulate StAR expression in gliomas and astrocytes in culture (King et al., 2002). In contrast, StAR expression is downregulated by cAMP in Schwann cells (Benmessahel et al., 2002, 2004), suggesting that the mechanisms controlling StAR may differ between different neural cell types.

Not much is known on the mechanisms that regulate StAR expression in neurons. An important and interesting finding is that the activation of *N*-methyl-D-aspartate (NMDA) receptors in hippocampal neurons, results in the processing of full-length StAR (37-kDa) to the truncated 30-kDa form. NMDA application to hippocampal tissue induces a reduction in the levels of the full-length form of StAR in the mitochondria. This reduction is accompanied by a parallel increase in the levels of the truncated form (Kimoto et al., 2001). In addition, this change is associated to an increase in the synthesis of pregnenolone (Kimoto et al., 2001), suggesting that the processing of StAR may coincide with the cholesterol transfer from the outer to the inner membranes of the mitochondria and the initiation of steroidogenesis. These findings suggest that StAR expression may be regulated in neurons by synaptic activity and the consequent activation of NMDA receptors. Furthermore, since the activation of NMDA receptors induces the influx of  $Ca^{2+}$  in hippocampal neurons, it is likely that  $Ca^{2+}$  signaling drives the neuronal steroidogenic reactions. Indeed, inhibition of  $Ca^{2+}$  influx through NMDA receptors by MK-801, suppressed the processing of StAR (Kimoto et al., 2001).

### Functional implications: steroidogenesis and synthesis of oxysterols

We have seen in the previous sections of this review that StAR is regulated by different physiological and pathological conditions in the nervous system. As in other tissues, changes in StAR expression and activity affect local steroidogenesis in neural cells (Kimoto et al., 2001; Benmessahel et al., 2004). StAR allows the formation of pregnenolone by nerve cells, which in turn may be converted in other steroids, since the nervous tissue expresses the necessary enzymatic machinery to synthesize a variety of steroids (Baulieu, 1997, 1998; Mellon et al., 2001; Stoffel-Wagner, 2003). Pregnenolone and its derivatives, such as progesterone, dihydroprogesterone, tetrahydroprogesterone, dehydroepiandrosterone, testosterone and estradiol are known to affect neuronal and glial development, syn-

aptic formation, synaptic plasticity, myelination, neuronal survival, learning, memory, cognition, behavior, anxiety, depression and the response of the central and peripheral nervous systems to injury and pathology (Baulieu, 1998; Compagnone and Mellon, 2000; Garcia-Segura et al., 2001; Mellon et al., 2001; Vallee et al., 2001; Melcangi et al., 2003; Schumacher et al., 2003; Stoffel-Wagner, 2003; Maggi et al., 2004). In some cases, steroids, such as estradiol, progesterone and testosterone, act as hormonal mediators and their actions on specific brain areas, such as the hypothalamus, are involved in the control of neuroendocrine events and reproduction. However, in other cases the same steroids act as local paracrine or autocrine neuromodulators and the fine regulation of their local synthesis, exerted by StAR, may have profound influences in the development and function of the nervous system.

Although the regulation of steroidogenesis is probably the most important function of StAR in the nervous system, it is important to mention that StAR may also affect the formation of oxysterols in the mitochondria (Schroepfer, 2000). Oxysterols regulate cholesterol and lipid homeostasis through the transcription factors nuclear hormone liver X receptors (LXRs) and suppressing the production of mature sterol regulatory element binding proteins (Brown and Goldstein, 1997; Schroepfer, 2000; Edwards et al., 2002). In addition, oxysterols may be metabolized to steroids and may affect steroidogenesis by the regulation of StAR activity (Christenson et al., 1998; King et al., 2004b). Oxysterols are actively produced by the brain and may affect neural function under physiological and pathological conditions. For instance, oxysterols may reduce reactive gliosis after brain injury (Bochelen et al., 1995) and may regulate amyloid beta release by neural cells via the activation of LXRs and the consequent upregulation of the ATP-binding cassette transporter A1 (ABCA1) (Fukumoto et al., 2002; Koldamova et al., 2003; Brown et al., 2004). Interestingly, ABCA1 expression is up-regulated in neurons and glia after brain injury (Fukumoto et al., 2002), suggesting that the brain may increase its responsiveness to oxysterols under neurodegenerative conditions. On the other hand, cholesterol metabolism is altered in neurodegenerative diseases and changes in oxysterol levels in plasma and cerebrospinal fluid have been detected in patients with dementia or neurological disorders (Lütjohann et al., 2000; Leoni et al., 2002; Teunissen et al., 2003).

## CONCLUSION

StAR is widely expressed in the nervous system and may affect its physiological and pathological responses by the formation of steroids and oxysterols. Future studies should clarify the mechanisms of regulation of StAR in the nervous system and precisely define the functional outcomes of the reported changes in StAR expression with development, aging and injury.

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