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Allopregnanolone: State of the art

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Abstract

Allopregnanolone, a neuroactive steroid derived from progesterone, is synthesized within the nervous tissue, by means of specific enzymes. Contrary to progesterone and its first metabolite dihydroprogesterone, allopregnanolone is able to interact with GABA-A receptor and not with the classical progesterone receptor. This suggests that the effect of progesterone administration may be due to activation of progesterone receptor, or of GABA-A receptor, or both. However, this is rarely considered in the experimental studies. Here we summarize and discuss the hot topics involving the actions of allopregnanolone within the nervous tissue. One major role of this neuroactive steroid is neuroprotection in case of lesion, ischemia or peripheral neuropathies (i.e., diabetes). In addition, allopregnanolone may reduce the symptoms of neurodegenerative diseases (e.g., Alzheimer, Parkinson, Niemann-Pick type C, multiple sclerosis) in animal models and now translational studies are developed for its therapeutic use. Allopregnanolone may exert a beneficial effect also in case of neuropathic pain and it is also a potential candidate for the treatment of mood and anxiety disorders. Finally, this neuroactive steroid seems to have important physiological roles in the early differentiation of some neural circuits (in particular at hippocampal level), and to reduce stress during pregnancy. In conclusion, it appears that allopregnanolone is a key regulator of physiological functions and may have interesting therapeutic perspectives for neurodegenerative and psychiatric disorders.

Abbreviations: DHP, dihydroprogesterone; PROG, progesterone; TSPO, translocator protein of 18 kDa

Keywords: Neuroactive steroid; Neurodegeneration; Neuroprotection; Psychiatric disorders; Pain; Hippocampal development; Pregnancy

Allopregnanolone, also known as tetrahydroprogesterone, was for a long time considered one of the many intermediate metabolites of the steroidogenic pathways, arising from progesterone (PROG), the gonadal hormone responsible for sexual receptivity. The principal sources of both PROG and allopregnanolone were considered the steroidogenic tissues as the ovary, testis and adrenal glands (Ficher and Steinberger, 1971). In the early 80s, it was shown that the brain is capable of de novo biosynthesis of steroids. The first clue of neurosteroidogenesis came from the observations that pregnenolone, dehydroepiandrosterone, and their sulphate derivatives accumulate in the brain of castrated and adrenalectomized rats (Corpechot et al., 1981). The confirmation of the existence of steroidogenic pathways within the brain was obtained during the year by the demonstration of the presence of the specific steroidogenic enzymes in both neurons and glial cells (Pelletier, 2010).

The last decades of investigation have clearly supported the concept that in the nervous system, PROG is not only a physiological regulator of reproduction (Banks and Freeman, 1980, Barraclough et al., 1986, Brann and Mahesh, 1991, Micevych and Sinchak, 2008, Micevych and Sinchak, 2011 and Skinner et al., 1998) but also regulates development of neurons (Giachino et al., 2003, Tsutsui, 2012, Tsutsui et al., 2011 and Wang et al., 2008) and glial cells (Garcia-Segura et al., 1994, Ghoumari et al., 2003, Ghoumari et al., 2005 and Luquin et al., 1993), as well as myelination process (Chan et al., 1998, Chan et al., 2000, Melcangi et al., 2003, Melcangi et al., 2011a and Schumacher et al., 2012). Moreover, this neuroactive steroid also exerts important protective effects during neurodegenerative events, such as in Parkinson's disease, Alzheimer's disease, multiple sclerosis, traumatic brain injury, stroke, peripheral neuropathy etc. (Bourque et al., 2009, Brinton, 2013, Callier et al., 2001, Garay et al., 2007, Garay et al., 2009, Giatti et al., 2012, Ibanez et al., 2003, Melcangi and Garcia-Segura, 2010, Melcangi et al., 2011a and Stein, 2011). Indeed, results obtained in several experimental models of neurodegenerative diseases provided an interesting background for therapeutic strategies based on PROG. However, it is important to consider that in the nervous system PROG is actively converted by the enzyme 5α-reductase into dihydroprogesterone (DHP) and subsequently by the action of the enzymes 3α-hydroxysteroid oxidoreductase or 3β-hydroxysteroid oxidoreductase into allopregnanolone and isopregnanolone (i.e., the 3β-isomer of allopregnanolone) (Melcangi et al., 2008 and Pelletier, 2010). These enzymatic conversions have a deep impact in the mechanism of action of PROG. In fact, while DHP is still able to interact with intracellular PROG receptor, allopregnanolone and isopregnanolone interact in a different way with GABA-A receptor. Indeed, allopregnanolone is a potent ligand of this non-classical steroid receptor (Belelli and Lambert, 2005 and Lambert et al., 2003) while isopregnanolone, does not bind directly to the GABA-A receptor (Bitran et al., 1991) but it antagonizes the effect of allopregnanolone on the GABA-A receptor (Backstrom et al., 2005 and Wang et al., 2002). A major criticism in this research field is that the discrimination whether the effects are due only to activation of PROG receptor, or of GABA-A receptor, or both is not always considered in the studies. A critical discussion on the effects of PROG in the nervous system, via its classical steroid receptor, versus those exerted by allopregnanolone, via modulation of GABA-A receptors, will be also provided in this special issue (Schumacher et al., 2013). Indeed, even if the attention for the role exerted by allopregnanolone in the nervous system is continuously increasing during the last decade (i.e., about 60 manuscripts have been published each year), the number of the observations so far present in literature on this neuroactive steroid are very limited in comparison to those of PROG. For instance, allopregnanolone and PROG, have been considered beneficial in the treatment of traumatic brain injury, reducing edema, inflammation, reactive gliosis, apoptosis, and increasing antioxidant activity (Djebaili et al., 2004, He et al., 2004, VanLandingham et al., 2006 and VanLandingham et al., 2007). However, as demonstrated on parameters like for instance, apoptosis, reactive gliosis, neuron loss and Morris water maze allopregnanolone is more effective than PROG itself. Moreover, allopregnanolone has also specific effects like for instance those mechanisms involved in apoptosis, such as mitochondrial cytochrome c release (Sayeed et al., 2009).

Allopregnanolone also reduces reactive gliosis in the hippocampus of female rats injected with kainic acid (Ciriza et al., 2004). In this animal model of excitotoxicity, the inhibition of PROG metabolizing enzymes, such as 5α-reductase and 3α-hydroxysteroid oxidoreductase, blocked the antigliotic effect of PROG (Ciriza et al., 2006).

As recently demonstrated, allopregnanolone, as well as PROG, exert protective effect also in spinal cord trauma. Indeed, in organotypic spinal cord cultures put under injury (i.e., a weight drop model) this neuroactive steroid was efficient in decreasing membrane damage and preventing neuronal death, with a mechanism involving GABA-A receptors, as they were inhibited by selective GABA-A receptor antagonist. Whereas, PROG is acting, in the same model, through the classical PROG receptor, thus, these findings identify both receptors as important targets for neuroprotection by progestagens after spinal cord injury (Labombarda et al., 2013).

Allopregnanolone has been also considered in ischemia. Like PROG, this neuroactive steroid reduces infarction volume, improve blood brain barrier integrity as well as memory and learning (Ishrat et al., 2010, Morali et al., 2012 and Sayeed et al., 2006) and as in the case of traumatic brain injury, allopregnanolone is generally more effective than PROG (Sayeed et al., 2006). Moreover, also in this case allopregnanolone specifically decreases mitochondrial cytochrome c release (Sayeed et al., 2009).

Several observations have been also obtained on seizures. Indeed, allopregnanolone has been reported to reduce seizures in several experimental models (Beckley et al., 2008, Belelli et al., 1989, Czlonkowska et al., 2000, Frye and Scalise, 2000 and Singh et al., 2010) with potency higher than PROG itself (Lonsdale and Burnham, 2007 and Lonsdale et al., 2006).

Interesting data have been also obtained in Alzheimer's disease. In particular, observations obtained in an experimental model of this neurodegenerative disorders (i.e., 3xTg-AD mice), have shown that an acute treatment with allopregnanolone prior development of the pathology was able to increase memory and learning in association with an increase in neuronal progenitor cell proliferation at the level of the hippocampal subgranular zone (Wang et al., 2010). Translational studies for the therapeutic use of allopregnanolone are reviewed in this special issue (Irwin and Brinton, 2013). In particular, it seems crucial the dosing and treatment regimen, that has to be consistent with the temporal requirements of systems biology of regeneration in brain. Indeed, with an optimized dosing and treatment regimen, chronic administration of allopregnanolone induced neurogenesis, oligodendrogenesis, reduced neuroinflammation and beta-amyloid burden while increasing markers of white matter generation and cholesterol homeostasis (Chen et al., 2011).

The efficacy of allopregnanolone treatment has been tested also in an MPTP-lesioned mouse model of Parkinson's disease. As recently reported, an acute treatment (i.e., once/week for two weeks) with this neuroactive steroid restored the number of tyrosine hydroxylase-positive and the total number of cells in the substantia nigra pars compacta of MPTP-lesioned mice. Moreover, animals treated with allopregnanolone showed an improvement in the motor performance (Adeosun et al., 2012).

Niemann–Pick type C disease is a devasting developmental disorder with progressive and fatal neurodegeneration. Treatment with allopregnanolone in animal model of this pathology has been demonstrated to delay the onset of neurological symptoms, to increase Purkinje and granule cell survival in the cerebellum, to reduce cortical ganglioside accumulation, cholesterol accumulation and inflammation and to enhance myelination (Ahmad et al., 2005, Griffin et al., 2004 and Liao et al., 2009). Moreover, as recently demonstrated, the combination of the allopregnanolone treatment with cyclodextrin and miglustat seems to ameliorates motor but not cognitive deficits (Hovakimyan et al., 2013).

Allopregnanolone treatment exerts also beneficial effect in experimental models of multiple sclerosis, such as for instance the experimental autoimmune encephalomyelitis. As demonstrated, this neuroactive steroid is able to reduce the immunoreactivity of ionized calcium-binding adapter molecule 1, the monocytoid cell marker, and CD3e (i.e., a lymphocytic marker) in lumbar spinal cord (Noorbakhsh et al., 2011). Thus, the treatment was able to prevent the exacerbation of the immune response. In agreement, allopregnanolone treatment diminishes neurological score (Noorbakhsh et al., 2011).

Protective effects of allopregnanolone have been also reported in experimental models of peripheral diabetic neuropathy (i.e., rats rendered diabetic by streptozotocin injection). This neuroactive steroid improves sciatic nerve conduction velocity, mRNA levels of a myelin protein, such as the peripheral myelin protein 22, thermal threshold, skin innervation density (Leonelli et al., 2007). Interestingly in this context, it is also important to highlight that allopregnanolone was able to counteract myelin abnormalities observed in peripheral nerve of aged male rats. Indeed, the treatment increased the number and g ratio of myelinated fibers of small diameter as well as reduced the frequency of myelinated abnormalities (Azcoitia et al., 2003).

It is important to highlight that the beneficial effects exerted by PROG and particularly by allopregnanolone in several experimental models of central and peripheral neurodegeneration are in agreement with changes in neuroactive steroid levels observed in the nervous tissue of these experimental models (Caruso et al., 2013, Caruso et al., 2008a, Caruso et al., 2008b, Caruso et al., 2010a, Giatti et al., 2010, Labombarda et al., 2006, Meffre et al., 2007, Melcangi et al., 2011a, Melcangi et al., 2012, Pesaresi et al., 2010, Roglio et al., 2008 and Schumacher et al., 2003). In addition, metabolic pathways of PROG in the nervous system are affected by modifications in the level of gonadal hormones (Caruso et al., 2010b) and by pathology with a regional specificity and in a sex-dimorphic way (Caruso et al., 2010a, Giatti et al., 2010 and Pesaresi et al., 2010). Thus, as discussed in this special issue (Melcangi et al., 2013), these findings may provide a background to design sex-specific therapies based on PROG metabolites.

Neuropathic pain is another important consequence of peripheral nerve damage. As reported, allopregnanolone exert a beneficial effect also on this component. Indeed, it suppresses neuropathic symptoms (i.e., allodynia/hyperalgesia) evoked by antineoplastic drugs, such as vincristine (Meyer et al., 2010) or oxaliplatin (Meyer et al., 2011), or by spinal nerve ligation (Kawano et al., 2011). Based on allopregnanolone ability to modulate GABA-A receptors, glycine, L- and T-type calcium channels, several studies demonstrated analgesic, neuroprotective, antidepressant and anxiolytic effects of this neuroactive steroid. In particular, as reported in this special issue (Patte-Mensah et al., 2013), allopregnanolone treatment induced beneficial actions in humans and animal models with no toxic side effects. In fact, a multi-parametric analysis revealed that allopregnanolone efficiently counteracted chemotherapy-evoked neuropathic pain in rats, thus indicating this molecule as possible candidate for therapy of neuropathic pain (Patte-Mensah et al., 2013).

Finally, allopregnanolone is also a potential candidate for the treatment of mood and anxiety disorders (Biggio et al., 2009, Eser et al., 2008, Girdler and Klatzkin, 2007, Melcangi et al., 2011b, Pinna, 2010, Turkmen et al., 2011 and Wirth, 2011). Indeed, reduced levels of allopregnanolone in the peripheral blood or cerebrospinal fluid were found to be associated with major depression, anxiety disorders, premenstrual dysphoric disorder, negative symptoms in schizophrenia, or impulsive aggression. These findings could support a therapeutic role for this neuroactive steroid. However, as discussed in this special issue (Schüle et al., 2013), pharmacokinetic obstacles, such as low bioavailability, oxidation to the ketone limit the therapeutic use. On this point of view, pharmacologic tools, such as a ligand of the translocator protein-18 kDa (TSPO) the XBD 173, that it is able to increase in situ the synthesis of neuroactive steroids may represent an interesting therapeutic perspective (Nothdurfter et al., 2012a and Nothdurfter et al., 2012b). In this context, it

is interesting to note that other ligands of TSPO, such as Ro5-4864 or etifoxine, or of the liver X receptor, such as GW 3965, which are able to increase the synthesis of neuroactive steroids, including allopregnanolone, also exert neuroprotective effects in different experimental models of neurodegeneration (Barron et al., 2013, Cermenati et al., 2010, Cermenati et al., 2013, Daugherty et al., 2013, Giatti et al., 2009, Girard et al., 2008, Girard et al., 2009, Leonelli et al., 2005, Mitro et al., 2012, Papadopoulos and Lecanu, 2009 and Veiga et al., 2005).

On the other hand, as discussed in this special issue (Backstrom et al., 2013) allopregnanolone may also induce negative mood symptoms in women with premenstrual dysphoric disorder. As suggested, a possible hypothesis for this paradoxical effect could be changes in GABA-A receptors (i.e., an upregulation of the alpha4, beta, delta subunit composition) during the luteal phase.

Finally, two further hot topics are introduced in this special issue. In particular, Darbra et al. (2013) discuss the importance of allopregnanolone levels during brain development for adolescent and adult behavior and for nervous system maturation. In fact, rat cortical levels of allopregnanolone in the forebrain of embryonic rats vary widely throughout development. During the last pregnancy period allopregnanolone levels sharply increase, and decline prior to parturition (Grobin and Morrow, 2001). In addition, it has been demonstrated that inhibiting the formation of 5α-reduced steroids during late gestation in rats reduces gestation and the number of viable pups per litter; in addition, it impairs cognitive and neuroendocrine functions in the juvenile offspring (Paris et al., 2011). Thus, changes of neonatal allopregnanolone levels in the first weeks of life may alter emotional adult behavior and sensory gating processes. Behavioral studies reviewed by Darbra et al. (2013) in this special issue show that some of these effects are related to a different functioning of the dorsal hippocampus, probably related to alterations in the expression of gamma-aminobutyric-acid receptors containing α4 and δ subunits, molecular alterations that can persist into adult age and that can, in part, explain the reported behavioral disturbances (Mòdol et al., 2012).

Brunton et al. (2013) have critically examined the role for allopregnanolone in both the maternal and fetal brain during pregnancy and development in protecting pregnancy and birth outcomes. In particular, the role of this neuroactive steroid was discussed in relation to stress exposure at this time.

In conclusion, observations here reported and discussed in the manuscripts included in this special issue further support the concept that allopregnanolone is a key regulator of physiological functions and may have interesting therapeutic perspectives in the field of neurodegenerative and psychiatric disorders.

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