The role of neurosteroids in the pathogenesis of hepatic encephalopathy

Abstract

Hepatic Encephalopathy (HE) represents a neuropsychiatric syndrome caused by acute or chronic liver failure. Hyperammonemia plays a pivotal role in the development of HE through modulation of neurotransmission, oxidative stress, neuroinflammation, mitochondrial dysfunction, and energy deficit. Neurosteroids contribute significantly to increased GABAergic tone in HE. Ammonia, in combination with manganese and proinflammatory cytokines, stimulate neurosteroid synthesis by up-regulation of translocator protein, a component of multiprotein complex that stimulate cholesterol transport into astrocytic mitochondria. Cholesterol serves as a substrate for the synthesis of neurosteroids allopregnanolone and tetrahydro-deoxycorticosterone. After release from astrocytes, allopregnanolone and tetrahydro-deoxycorticosterone potentiate GABAergic transmission by positive allosteric modulation of GABAA receptor, thus contributing to cognitive deficit and alterations in sleep-wake cycle. Additional potential mechanisms of neurosteroid action in HE include modulation of serotonergic, cholinergic, glutamatergic, glycinergic, and opioid receptor activities, as well as modulation of gene expression. This review aimed to summarize current knowledge of the role of neurosteroids in the pathogenesis of HE.

Key words: neurosteroids, hepatic encephalopathy, ammonia, neurotransmission

Introduction

Hepatic Encephalopathy (HE) represents a complex neuropsychiatric syndrome caused by acute or chronic liver failure (1). It may be presented in three major forms: type A (caused by acute liver failure), type B (caused by porto-systemic shunt without liver disease), and type C HE (associated with liver cirrhosis) (2). Type A HE has a rapid course with deterioration of mental functions, convulsions and the loss of consciousness. On the other hand, signs and symptoms of chronic HE (usually type C) may vary from subtle cognitive dysfunction and attention deficits, which cannot be detected by the standard neurological exam (minimal HE), to the development of hepatic coma in the stage 4 of HE (2,3).

The pathogenesis of HE is complex and not fully established. In type A HE hyperammonemia, along with other toxins, induces cytotoxic brain edema due to the swelling of astrocytes, with subsequent rise in intracranial pressure (4). This may potentially lead to the fatal outcome due to the brainstem herniation in the foramen magnum.
Opposite to the acute form, type B and type C HE are not characterized by prominent brain edema, but the dominant pathological change is Alzheimer type II astrocytosis (large pale nuclei with prominent nucleoli frequently occurring in pairs, cytoplasmic vacuolization), found in both grey and white matter (5,6). Pathogenetic mechanisms involved in the development of chronic forms of HE are closely interrelated and include: alterations in neurotransmission (7), oxidative stress (8,9), energy deficit (10), neuroinflammation (11) and mitochondrial dysfunction (12,13). Alterations in neurotransmission have an important role in the pathogenesis of types B and C HE and include changes in glutamatergic (14), GABAergic (14), cholinergic (15,16), serotonergic (17), dopaminergic (18), adrenergic (19) and purinergic transmission (20). Neurosteroids contribute significantly to changes in neurotransmission found in HE. This review aimed to summarize current knowledge of the role of neurosteroids in the pathogenesis of HE.

Synthesis of neurosteroids

Neurosteroids have been initially defined as steroid compounds synthesized within brain, that remain elevated in the central nervous system after ablation of peripheral sources (21). They may be classified, according to the structural features, into three major groups: pregnane neurosteroids (allopregnanolone, tetrahydro-deoxy-corticosterone), androstane neurosteroids (androstanediol, etiocholanolone) and sulphated neurosteroids (pregnenolone sulphate, dehydroepiandrosterone sulphate) (22,23). The initial step in neurosteroid synthesis includes the transport of cholesterol into the mitochondria, by the action of Translocator protein (TSPO) formerly known as Peripheral-type benzodiazepine receptor. TSPO associates with Voltage-dependent anion channel (VDAC) and Adenine nucleotide carrier (ANC) to form a heteromeric complex on outer mitochondrial membrane, which allows the transfer of cholesterol through the hydrophilic intermembrane space (24). After transfer cytochrome P450 cholesterol side chain cleavage enzyme (P450scc) catalyzes the conversion of cholesterol to pregnenolone, a rate-limiting step in neurosteroid synthesis. Pregnenolone is further converted to progesterone, Allopregnanolone (ALLO) and Tetrahydro-deoxy-corticosterone (THDOC) in series of enzymatic reactions, which occur in the smooth endoplasmic reticulum and mitochondria (24,25) (figure 1).

Figure 1. Synthesis of neurosteroids in astrocytes. Neurosteroidogenesis starts with uptake of cholesterol into astrocytic mitochondria mediated by TSPO. Cholesterol is then converted to neurosteroids through a series of enzymatic reactions (TSPO, Translocator protein; VDAC, Voltage-dependent anion channel; ANC, Adenine nucleotide carrier; DOC, Deoxycorticosterone; 5α-DHDOC, 5α-dihydrodeoxycorticosterone; THDOC, Tetrahydro-deoxycortico-sterone; 5α-DHPROG, 5α-dihydro-progesterone) (modified according to Ahboucha et al.)(24).
Neurosteroids were found to exhibit some fundamental effects on the myelination, synaptic organization (26-28). They participate in the regulation of food intake, sexual behavior, anxiety, body temperature, blood pressure (29) and may also exert neuroprotective effects in Parkinson's disease, Alzheimer's disease, epilepsy and stroke (23).

Neurosteroids and HE

The involvement of neurosteroids in the pathogenesis of HE was first suggested by Zaman (30), as an alternative to the hypothesis that "endogenous benzodiazepines", mediate increased GABAergic tone in HE. In accordance with the supposed role of neurosteroids in HE, later studies have confirmed the increased brain level of pregnenolone, ALLO and THDOC in experimental models of HE induced by thioacetamide, (TAA) (31) together with liver ischemia caused by portacaval anastomosis (PCA) and hepatic artery ligation (24). An increase in pregnenolone and ALLO was also found in autopsied brain tissue from cirrhotic patients who died in hepatic coma (32). Ahboucha et al. (24) have demonstrated that brain levels of neurosteroids correlate with the severity of HE. Furthermore, the blockage of neurosteroid synthesis by finasteride, 5α-reductase inhibitor, was found to improve the course of TAA-induced HE and to reduce mortality in rats (33). Finasteride pretreatment ameliorates motor activity, preserves vital reflexes and completely prevents the development of hepatic coma. Motor changes are followed by both an increase in mean voltage of EEG bands and a decrease in delta band proportion in total power density. All findings correspond to mild stages of HE (33,34). Together, these findings point out the significant role of neurosteroids in the pathogenesis of HE.

Various studies (24,35-37) have shown that neurosteroid levels in HE are elevated due to increased neurosteroid synthesis consistently, partly caused by the up-regulation of TSPO on astrocytic mitochondrial membrane. This was, firstly demonstrated by Itzhak et al. (35), who have found increased binding site densities of the isoquinoline ligand [3H]PK11195 and the peripheral benzodiazepine ligand [3H]Ro5-4864 in brains of TAA-treated mice. TSPO expression was found to be increased in a region-selective manner in the PCA model of HE, with the highest increase in the cerebellum and pons, with the lowest increase in the striatum (38,39). Region-selective up-regulation of TSPO was also found in humans with different etiologies of cirrhosis, but with highest increase in right dorsolateral prefrontal cortex, pallidum and putamen (40). Increased TSPO expression in the brain is mediated by hyperammonemia in combination with proinflammatory cytokines (IL-1β, TNF-α) in acute, and manganese in chronic forms of HE (24). This is evident by increased densities of [3H]PK11195 or [3H]Ro5-4864 binding sites in all experimental models (35,38), as well as in human HE (40), whenever hyperammonemia develops. Similar changes occur after treatment of cultured astrocytes with pathophysiologically relevant concentrations of manganese (41) and proinflammatory cytokines (24,42).

Apart from the up-regulation of TSPO, increased neurosteroid synthesis in HE may be also mediated by increased blood-brain transfer of lipophylic neuroactive steroids (43). Activation of TSPO, by its "endogenous ligands" such as diazepam binding inhibitor and its processing product octadecaneuropeptide, have also been postulated to stimulate neurosteroid synthesis (44). Both peptide levels were found to be elevated in the cerebrospinal fluid of HE patients (45) and in brain extracts from animals models of HE (46). Peripheral sources of neurosteroids may also contribute to the pathogenesis of HE (44). Although these additional mechanisms cannot be excluded, the majority of data suggest that the up-regulation of TSPO by hyperammonemia and other toxins plays a pivotal role in the increased synthesis of neurosteroids in HE.

Mechanisms of neurosteroid action in HE include modulation of various membrane receptor activities, including GABAA, N-methyl-D-aspartate (NMDA), and serotoninergic receptors (nongenomic effects), as well as the modulation of intracellular receptor activities, which can regulate gene expression (genomic effects) (14,24). Additionally, neurosteroids may modulate brain oxidative stress and neuroinflammation in HE (16,24,33).

Neurosteroids and GABAergic transmission

The effects of neurosteroids on GABAergic transmission have been most extensively studied. Earlier studies postulated that "endogenous benzodiazepines" mediate GABAergic transmission in HE (47), thus suggesting that administration of flumazenil, benzodiazepine site antagonist, may have beneficial effects in HE (48). However, the role of "endogenous benzodiazepines" has been seriously questioned, since neuroprotective effect of flumazenil in HE was not consistent (24). Further opposing the role of benzodiazepine-like ligands in alterations of GABAergic transmission was the fact that "endogenous benzodiazepines", were not elevated in the plasma, brain and cerebrospinal fluid of HE patients (49). These findings shed light on neurosteroids as potential mediators of changes in GABAergic transmission in HE. It is known that ALLO and THDOC bind to a specific neurosteroid site on GABAA receptor and potentiate GABAergic transmission in HE, as positive allosteric modulators of GABAA receptors (39,50). Radiometric assays in autopsied brain tissue, from cirrhotic patients who died
in hepatic coma, have shown that neurosteroids do not change neither GABA nor benzodiazepine recognition sites, they just modulate both sites on GABAA receptor complex (39,51). The interaction between neurosteroids and benzodiazepine binding site has been further confirmed by consistently beneficial effects of Ro15-4513, benzodiazepine partial inverse agonist, on the course of HE. Ro15-4513 reduces the modulatory activity of neurosteroids and attenuates the effects of ALLO on GA- 

BAA-induced chloride currents in hippocampal neurons (52). Neurosteroids also act in synergy with ammonia at the benzodiazepine site, evident as a dose-dependent AL- 

LO-induced increase in the binding of (3H)flumazenil in the presence of ammonia (39).

Recent studies revealed a reduced synthesis of dehi- 

droepiandrosterone sulphate (DHEAS), negative allo-

steric modulator of GABAA receptor, in HE (53). In summary, increased synthesis of ALLO and THDOC, positive allosteric modulators and reduced synthesis of DHEAS, negative allosteric modulator of GABAA recep-

tor, potentiate GABAergic transmission and contribute to the cognitive dysfunction and alterations in sleep-

wake cycle in HE (14).

Other potentially relevant effects of neurosteroids in HE

It is known that neurosteroids apart from GAB-

AA affect the function of various receptors in the brain, including serotonin 5-HT3 receptors, NMDA receptors, glycine receptors and opioid receptors (54). However, the relevance of these effects in the pathogenesis of HE has not been fully established. Interactions between neuro-

steroids and serotoninergic transmission have been most studied. It is suggested that HE is accompanied with increased serotoninergic transmission, based on an improvement of HE after administration of nonselective serotonin antagonist methysergide (55) and selective 5-HT3 receptor antagonist ondansetron (17). Furthermore, administration of venlafaxine, serotonin reuptake inhibitor, worsens HE and increases serotonin concentration in synaptic clefts in PCA rats (56). This increase is associated with increased level of ALLO (57), thus sug-

gest that neurosteroids may be involved in the modula-

tion of serotoninergic transmission in HE.

Neurosteroids may also modulate cholinergic transmission in HE, in a region-selective manner. Acetylcholinesterase activity was found to rise in the thala-

mus and caudate nucleus in TAA-induced HE in rats, the effect that can be impeded by finasteride, neurosteroid synthesis inhibitor (16). The contribution of this effect to motor and cognitive changes in HE should be further investigated.

HE is associated with altered expression of sever-

al key astrocytic and neuronal proteins, including Glial fibrillary acidic protein (GFAP), aquaporin IV, transport-
ers for glucose (GLUT1), glycine (GLYT-1) and glutamat-
e (EAAT-2), as well as enzymes Monoaminooxidase A (MAO-A) and nitric oxide synthase (39,58). Although the role of neurosteroids in regulating the expression of these proteins is still blurred, it is clear that neurosteroids could change the expression of several genes (genomic effects). Progesterone, its metabolites and possibly other neurosteroids bind to progesterone or pregnane X recep-
tors and change the expression of GFAP and aquaporin IV. Additionally, GLUT-1 and MAO-A gene expression may be altered by activation of glucocorticoid receptors (24). Genomic effects of neurosteroids have been postulated to contribute to astrocyte swelling in HE (59). However, this hypothesis remains controversial, since ALLO and proges-
terone were found to reduce brain edema in other patho-

logical states, such as traumatic brain injury (24,60).

Neurosteroids may have a dual region-specific role in oxidative brain injury in HE (16). While finasteride pre
treatment reduces lipid peroxidation in the cerebral cortex, it increases oxidative lipid damage in the thala-
mus in TAA-induced liver failure. Prooxidative effect of neurosteroids in the cortex could be mediated by reduced catalase activity (16). The significance of these effects in the pathogenesis of HE should be further investigated.

Conclusion

Neurosteroids play an important role in the patho-
genesis of HE. Hyperammonemia, manganese and proinflammatory cytokines up-regulate TSPO in outer mitochondrial membrane of astrocytes, thus enabling increased cholesterol uptake into mitochondria. Chole-

setrol serves as a substrate for the synthesis of neuroste-

roids, which are then released from astrocytes and exert nongenomic and genomic effects in neurons. Among these effects, the most important seems to be the modula-
tion of GABAergic transmission. ALLO and THDOC, as positive allosteric modulators of GABAA receptors, potentiate GABAergic transmission and contribute to cognitive dysfunction and alterations in sleep-wake cycle in HE (14,24,39,44). Additionally, neurosteroids modu-
late the activity of serotonin, glycine, glutamate, opioid receptors, as well as the expression of key neuronal and astrocytic genes (39,54,58), but the contribution of these effects to the pathogenesis of HE should be further inves-
tigated. Inhibitors of neurosteroid synthesis (16,33) or neurosteroid site antagonists at GABAA receptor (44) could have beneficial effects on the course of HE.
References

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