Neurochemistry International 61 (2012) 48–53

Contents lists available at SciVerse ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

Reduced brain levels of DHEAS in hepatic coma patients: Significance for increased GABAergic tone in hepatic encephalopathy

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ARTICLE INFO

Article history:
Received 22 December 2011
Received in revised form 25 March 2012
Accepted 26 March 2012
Available online 3 April 2012

Keywords:
Hepatic encephalopathy
Neurosteroids
Allopregnanolone
DHEAS
Increased GABAergic tone

ABSTRACT

Increased neurosteroids with allosteric modulatory activity on $GABA_A$ receptors such as 3α–5α tertrahydroprogesterone; allopregnanolone (ALLO), are candidates to explain the phenomenon of "increased GABAergic tone" in hepatic encephalopathy (HE). However, it is not known how changes of other $GABA_A$ receptor modulators such as dehydroepiandrosterone sulfate (DHEAS) contribute to altered GABAergic tone in HE. Concentrations of DHEAS were measured by radioimmunoassay in frontal cortex samples obtained at autopsy from 11 cirrhotic patients who died in hepatic coma and from an equal number of controls matched for age, gender, and autopsy delay intervals free from hepatic or neurological diseases. To assess whether reduced brain DHEAS contributes to increased GABAergic tone, *in vitro* patch clamp recordings in rat prefrontal cortex neurons were performed. A significant reduction of DHEAS (5.81 ± 0.88 ng/g tissue) compared to control values (9.70 ± 0.79 ng/g, $p < 0.01$) was found. Brain levels of DHEAS in patients with liver disease who died without HE (11.43 ± 1.74 ng/g tissue), and in a patient who died in uremic coma (12.56 ng/g tissue) were within the control range. Increasing ALLO enhances GABAergic tonic currents concentration-dependently, but increasing DHEAS reduces these currents. High concentrations of DHEAS (50 μM) reduce GABAergic tonic currents in the presence of ALLO, whereas reduced concentrations of DHEAS (1 μM) further stimulate these currents. These findings demonstrate that decreased concentrations of DHEAS together with increased brain concentrations of ALLO increase GABAergic tonic currents synergistically; suggesting that reduced brain DHEAS could further increase GABAergic tone in human HE.

1. Introduction

Hepatic encephalopathy (HE) is a neuropsychiatric complication of chronic liver failure of different etiologies which occurs as a consequence of altered brain signaling mechanisms involving glutamatergic, serotoninergic, catecholaminergic and γ-aminobutyric acid (GABA)ergic systems (Hazell and Butterworth, 1999). Evidence for the existence of "increased GABAergic tone" in HE was provided by the report of similarities between visual evoked potentials in rats with thioacetamide-induced acute failure and animals treated with $GABA_A$ receptor agonists (Schafer et al., 1984), together with reports of a beneficial effect of benzodiazepine ligands acting as antagonists such as flumazenil and the partial inverse agonist Ro15-4513 in several animal models of HE (Ahboucha and Butterworth, 2008).

It was recently proposed that increased GABAergic tone could also result from neurosteroids acting at the $GABA_A$ receptor complex such as the progesterone metabolites ALLO and tertrahydrodeoxycorticosterone, substances that have been shown to accumulate in brain in humans who died in hepatic coma (Ahboucha et al., 2005), and in chronic experimental HE (Ahboucha et al., 2008a). Neurosteroids are produced in the brain endogenously and may influence CNS function in several ways having the capacity to

Abbreviations: ALLO, allopregnanolone (3α–5α tertrahydroprogesterone); HE, hepatic encephalopathy; DHEAS, dehydroepiandrosterone sulfate; GABA, γ-aminobutyric acid; $GABA_A$, GABA type A receptors; LD, liver disease; mACSF, modified artificial cerebrospinal fluid; mPFC, medial prefrontal cortex; sIPSCs, spontaneous $GABA_A$ receptor-mediated currents.

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http://dx.doi.org/10.1016/j.neuint.2012.03.020

regulate transcription via nuclear receptors, and to influence neuronal excitability by actions on ligand-gated ion channels including $GABA_A$ receptors (Rupprecht and Holsboer, 1999). It has been demonstrated recently that $GABA_A$ receptors mediate both phasic inhibitory synaptic transmission and a more persistent tonic extrasynaptic inhibition, and neurosteroids may play a significant role for tonic inhibition particularly in dynamic regulation of neurotransmission (Belelli et al., 2009).

Unlike ALLO and tertrahydrodeoxycorticosterone, which are positive allosteric modulators of $GABA_A$ receptors, the neurosteroid dehydroepiandrosterone sulphate (DHEAS) is a negative allosteric modulator of these receptors (Demirgören et al., 1991; Majewska et al., 1990; Park-Chung et al., 1999). DHEAS represents an abundant circulating steroid hormone synthesized in the periphery and which is locally produced in the brain in humans and other vertebrates (Fig. 1) (Do Rego et al., 2009). Furthermore, DHEAS has been consistently reported to be decreased in circulation in patients with chronic liver diseases (Ahboucha et al., 2008b; Bannister et al., 1985; Becker et al., 1991; Charlton et al., 2008; Franz et al., 1979; Zietz et al., 2003) and to be associated with symptoms such as fatigue in these patients (Ahboucha et al., 2008b; Butterworth et al., 2009). We hypothesize that brain alterations of the levels of DHEAS may further compromise GABAergic function in HE patients. In order to evaluate this hypothesis, frontal cortical levels of DHEAS were measured in patients who died in hepatic coma and controls. In addition, the effect of altered levels of DHEAS was assessed *in vitro* on GABAergic tonic currents in rat prefrontal cortical neurons.

2. Materials and methods

2.1. Patient brains

Frontal cortical samples were obtained at autopsy from 27 subjects namely 11 cirrhotic patients who died in hepatic coma designated HE, 4 patients with liver disease (LD) of comparable etiology and severity but without encephalopathy at the time of death, one patient who died in uremic coma, designated UC and 11 control subjects matched for age and postmortem delay intervals who were free from hepatic, renal or neurological diseases. Causes of death included mainly pneumonia, septic shock and gastrointestinal bleeding. Other causes of death were aortic aneurism, sudden death, pulmonary abscess and myocardial infarction (Table 1). Brain tissue was supplied by the Saint-Luc Hospital (Montreal, Canada) and University of Maryland (MD, USA) Human Brain Banks. All cases were matched for age, gender, post-mortem time, and disease state and all had been free from alcohol or benzodiazepine intake for at least 14 days prior to death. Diagnosis of HE was confirmed neuropathologically by the presence of Alzheimer-type II astrocytosis and all procedures were approved by the University of Montreal (CHUM) Research and Ethics Board. The experimental protocols were also approved by the Animal Ethics Committee of the University of Cagliari.

2.2. Animals

Male Sprague Dawley CD rats (Charles River, Como, Italy) were used at 21–40 days of age (body mass, 70–110 g). Animals were allowed to acclimatize to the new housing conditions for at least 1 week after arrival at the animal facility. They were housed six per cage under an artificial 12 h light/dark cycle (lights on from 8:00 AM to 8:00 PM) and at a constant temperature of 22 ± 2 °C and a relative humidity (65%) of all times with *ad libitum* food access. Animal care and handling throughout the experimental procedures were in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC).

2.3. Measurement of DHEAS by radioimmunoassay

Brain samples (200 mg) were homogenized in methanol (40% in H_2O) using a small electric pellet pestle® motor (Kontes, IL, USA) on ice and then centrifuged for 15 min at 12,000 rpm at 4 °C. Solid phase extraction was performed as previously reported (Butterworth et al., 2009). After centrifugation of brain homogenates, supernatants were passed through micro-elution cartridges previously conditioned with 100% methanol and water, respectively. Samples were passed through the columns under vacuum and the eluate was discarded. The columns were then washed with 50% methanol/H_2O. The steroid fraction was eluted with pure methanol and evaporated to dryness at 40 °C in a speed vacuum system. Brain DHEAS was measured using a radioimmunoassay kit (DHEAS-CT) (Diagnostic System Laboratories Inc., TX, USA).

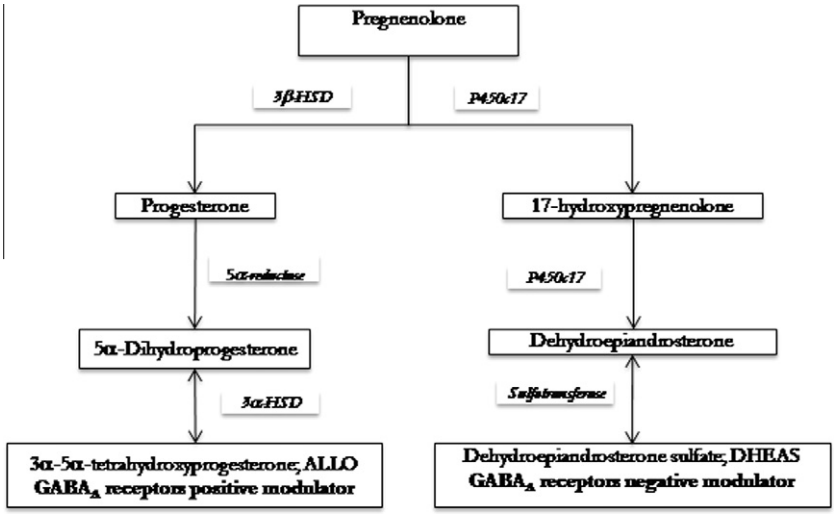

Fig. 1. Schematic representation of neurosteroid synthetic pathways of relevance for the present study. Two neurosteroids with $GABA_A$ receptor negative and positive modulatory activities namely dehydroepiandrosterone sulfate (DHEAS) and 3α–5α-tetrahydroprogesterone (ALLO), respectively, are both produced from the neurosteroid precursor pregnenolone. In vertebrates, conversion of pregnenolone to these neurosteroids may involve several enzymes including hydroxysteroid dehydrogenase (HSD), cytochrome Pc_{17} ($P450c_{17}$), 5α-reductase and/or sulfotransferase.