Alcohol 52 (2016) 63–70

Contents lists available at ScienceDirect

Alcohol

journal homepage: http://www.alcoholjournal.org/

Increased steroid hormone dehydroepiandrosterone and pregnenolone levels in post-mortem brain samples of alcoholics

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ARTICLE INFO

Article history:
Received 8 September 2015
Received in revised form
3 March 2016
Accepted 4 March 2016

Keywords:
DHEA
Pregnenolone
Testosterone
Anterior cingulate cortex
Anterior insula
μ-opioid receptor

ABSTRACT

Intra-tissue levels of steroid hormones (e.g., dehydroepiandrosterone [DHEA], pregnenolone [PREGN], and testosterone [T]) may influence the pathological changes seen in neurotransmitter systems of alcoholic brains. Our aim was to compare levels of these steroid hormones between the post-mortem brain samples of alcoholics and non-alcoholic controls. We studied steroid levels with quantitative liquid chromatography–tandem mass spectrometry (LC-MS/MS) in post-mortem brain samples of alcoholics ($N = 14$) and non-alcoholic controls ($N = 10$). Significant differences were observed between study groups in DHEA and PREGN levels (p values 0.0056 and 0.019, respectively), but not in T levels. Differences between the study groups were most prominent in the nucleus accumbens (NAC), anterior cingulate cortex (ACC), and anterior insula (AINS). DHEA levels were increased in most alcoholic subjects compared to controls. However, only a subgroup of alcoholics showed increased PREGN levels. Negative Spearman correlations between tissue levels of PREGN and previous reports of [^{3}H]naloxone binding to μ-opioid receptors were observed in the AINS, ACC, NAC, and frontal cortex (R values between −0.6 and −0.8; p values ≤ 0.002), suggesting an association between the opioid system and brain PREGN levels. Although preliminary, and from relatively small diagnostic groups, these results show significantly increased levels of DHEA and PREGN in the brains of alcoholics, and could be associated with the pathology of alcoholism.

Introduction

Allostatic alterations in the reward and stress systems are considered to be a nexus for developing alcohol dependence (Koob, 2013). Steroid hormones affect both reward and stress processes. There are bidirectional interactions between alcohol consumption and steroid hormones. Neuroactive steroids are considered to be critical for modifying behavioral responses to alcohol (Helms, Rossi, & Grant, 2012), and consumption of alcohol can influence the activity of the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes (Adinoff et al., 1990; Frias, Rodriguez, Torres, Ruiz, & Ortega, 2000; Mendelson, Mello, & Ellingboe, 1977; Välimäki, Härkönen, Eriksson, & Ylikahri, 1984). Furthermore, increased *de novo* steroidogenesis has also been reported in the rat brain after alcohol exposure (Sánchez, Castro, Torres, & Ortega, 2014; Sanna et al., 2004). However, considerable species differences exist in the way alcohol influences endogenous steroid levels (Porcu et al., 2010), and *de novo* steroidogenesis might not occur in the adult human brain (Steckelbroeck et al., 2010).

Testosterone (T) and glucocorticoid steroids have been studied widely and have been associated with the pathology of alcohol-use disorder (Edwards, Little, Richardson, & Vendruscolo, 2015; Lenz et al., 2012). However, other neuroactive steroids (e.g., dehydroepiandrosterone [DHEA] and pregnenolone [PREGN]) have been associated with alcohol consumption (Helms et al., 2012). In healthy non-alcoholic volunteers, alcohol consumption that leads to blood alcohol levels of ~0.06 mg/dL increases plasma DHEA and PREGN levels, which mediates some of the subjective effects of

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http://dx.doi.org/10.1016/j.alcohol.2016.03.002
0741-8329/

alcohol (Pierucci-Lagha et al., 2006). Furthermore, high saliva levels of DHEA have been associated with drinking to cope with stress in women (Wemm et al., 2013). Unconjugated DHEA has been associated with increases in catecholamine synthesis, decreases in monoamine oxidase (MAO) activity, enhanced activation of NMDA, and inhibition of GABA-A receptor function (Imamura & Prasad, 1998; Maninger, Wolkowitz, Reus, Epel, & Mellon, 2009; Pérez-Neri, Montes, & Ríos, 2009). Unconjugated PREGN has been associated with feedback control of endocannabinoid system functions (Vallée et al., 2014) and a reduction of acute alcohol self-administration in rodents (Besheer, Lindsay, O'Buckley, Hodge, & Morrow, 2010; Rezvani & Levin, 2014). Furthermore, neuroactive steroid levels are influenced by neurotransmitter systems that are important for alcohol-use disorder. For example, the μ-opioid receptor (MOR) antagonist naloxone increases plasma levels of PREGN in cynomolgus monkeys (Porcu, Rogers, Morrow, & Grant, 2006). However, plasma levels of steroids do not necessarily represent steroid levels in the brain (Alomary et al., 2003; Little et al., 2008), probably because the brain has *de novo* steroidogenesis and active steroid metabolism. Therefore, there is a need to also measure the levels of steroid hormones in the target tissue.

The aim of the present study was to measure differences in steroid levels in the nucleus accumbens (NAC), anterior insula (AINS), hippocampus (HIPP), frontal cortex (FC), amygdala (AMY), and anterior cingulate cortex (ACC) in post-mortem brain samples of alcoholics and non-alcoholic controls. Furthermore, T seems to play a role in psychological traits such as antisocial behavior (Yildirim & Derksen, 2012). In the present study, alcoholics were divided into two subgroups according to Cloninger's typology of alcoholism, where antisocial behavior is associated with early-onset Cloninger type 2 alcoholism, but not with the late-onset type 1 alcoholism (Cloninger, 1995). In these same study subjects, we have previously reported on differences in neurotransmitter systems associated with steroid function, including GABA-A (Laukkanen et al., 2013), NMDA receptor subunit 2B (Kupila et al., 2015), MOR binding (Laukkanen, Kärkkäinen, Kautiainen, Tiihonen, & Storvik, 2015), and brain tissue levels of endocannabinoids (Kärkkäinen et al., 2013; Lehtonen et al., 2010). The secondary aim of the present study was to calculate whether these previously published measurements are correlated with brain steroid levels. To our knowledge, steroid levels have not been previously studied in post-mortem brain samples of alcoholics.

Materials and methods

Study subjects and diagnostics

The selection and collection of these post-mortem human brains, psychological diagnostics, and sample preservation methods have been described in detail (Lehtonen et al., 2010; Mantere et al., 2002; Storvik, Häkkinen, Tupala, & Tiihonen, 2009). Briefly, left hemispheres were obtained during clinical necropsy at the Department of Forensic Medicine, University of Oulu, Finland, and the Department of Forensic Medicine, University of Eastern Finland, Finland. This portion of the study was approved by the Ethics Committees of the University of Oulu (27.12.1997; latest amendment Dnro 125/2009) and the National Board of Medicolegal Affairs, Helsinki, Finland (Dnro 3020/322/96 and 3141/32/200/98). The brains were removed, cleaned of the dura, and divided at the midsagittal plane. The left hemisphere was placed on a glass plate before freezing at −75 °C. None of the hemispheres exhibited damage or neuroanatomical abnormalities. Brain samples were cryo-sectioned into 100-μm cantomeatal slices that were allowed to air dry before storage at −25 °C with dehydrating agents until use.

The study groups consisted of Cloninger type 1 alcoholics ($N = 6$, four males and two females; age at the time of death [AGE] 57.5 ± 12.1 years [mean ± SD]; post-mortem interval [PMI] 13.9 ± 3.0 h; blood alcohol concentration [BAC] 2.7 ± 1.4 mass/mass ‰ [1 mass/mass ‰ is equivalent to approximately 106 mg of alcohol in 1 dL of blood]), type 2 alcoholics ($N = 8$, all male; AGE 34.6 ± 11.4 years; PMI 14.1 ± 3.2 h; BAC 1.8 ± 1.3‰), and a non-alcoholic control group ($N = 10$; eight males and two females; AGE 53.5 ± 10.1 years; PMI 14.8 ± 8.8 h; BAC 0.04 ± 0.12‰) (Table 1). Two physicians reviewed medical records and anamnestic data, which included extant criminal records. Alcoholism, determined by frequent medical appointments due to alcohol-related problems, was coded according to DSM-IV criteria (American Psychiatric Association, 1994) and further sub-classified as type 1 or type 2 alcoholism according to Cloninger's typology, which resembles Babor and Early/Late onset typologies of alcoholism (Cloninger, 1995; Leggio, Kenna, Fenton, Bonenfant, & Swift, 2009). The two main separating criteria for the present study were early onset of alcohol abuse (<25 years old) and a record of severe antisocial behavior for type 2 alcoholics. Subjects with psychotic disorders or any other neurological disease, those taking medication that could affect the CNS (such as neuroleptics or antidepressants), and subjects with severe inflammation as a cause of death (e.g., acute pancreatitis or pneumonia) were excluded. All type 1 and six type 2 alcoholics had ethanol in their blood at their time of death. One type 2 alcoholic had an abstinence period of 5 days and another had abstained for 3–7 days. One of the controls had a small amount of ethanol in his blood at the time of death (0.36‰ blood alcohol content). Two of the type 1 and three of the type 2 alcoholics had traces of benzodiazepines in their blood samples. Evaluations for the duration of heavy alcohol use, family histories of alcohol misuse, and tobacco smoking, based on medical records, were considered to be unreliable and thus not considered in the final analysis.

Table 1
The study subjects: age at time of death, post-mortem interval (PMI), blood alcohol concentration (BAC), and cause of death.

Group and subject	Sex	Age (years)	PMI (h)	BAC (‰)	Cause of death
Non-alcoholic controls					
1	Male	55	5.5	0.0	Acute myocardial infarction
2	Male	45	9.5	0.0	Acute myocardial infarction
3	Male	77	7.5	0.0	Acute myocardial infarction
4	Female	57	11.0	0.0	Acute myocardial infarction
5	Male	50	18.5	0.0	Acute myocardial infarction
6	Female	60	12.0	0.0	Acute myocardial infarction
7	Male	49	33.0	0.4	Acute myocardial infarction
8	Male	53	29.0	0.0	Acute myocardial infarction
9	Male	53	11.0	0.0	Acute myocardial infarction
10	Male	36	11.0	0.0	Dissection of aorta
Alcoholics					
Type 1					
1	Male	45	12.0	1.5	Suicide by hanging
2	Male	42	14.8	0.8	Acute myocardial infarction
3	Male	76	10.5	3.2	Acute myocardial infarction
4	Female	56	19.0	4.1	Ethanol intoxication
5	Male	69	16.0	4.7	Ethanol intoxication
6	Female	57	11.0	2.0	Right subdural hemorrhage
Type 2					
7	Male	49	12.0	1.7	Fibrotic degeneration of myocardium
8	Male	37	9.5	3.0	Gunshot wound
9	Male	47	15.5	3.0	Knife wound
10	Male	20	14.5	1.3	Knife wound
11	Male	46	18.0	0.0	Suicide by hanging
12	Male	18	9.5	1.5	Heart rupture (car accident)
13	Male	32	16.5	3.6	Suicide by hanging
14	Male	28	17.5	0.0	Suicide by hanging