Psychopharmacology (2014) 231:3273–3280
DOI 10.1007/s00213-014-3558-6

ORIGINAL INVESTIGATION

Effects of different ethanol-administration regimes on mRNA and protein levels of steroid 5α-reductase isozymes in prefrontal cortex of adolescent male rats

Pilar Sánchez · Beatriz Castro · Jesús M. Torres · Esperanza Ortega

Received: 13 December 2013 / Accepted: 17 March 2014 / Published online: 9 April 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract
Rationale Underage drinking is a leading public health problem in developed countries. An increasing proportion of adolescents consume alcoholic beverages every weekend. Increased anxiety, irritability, and depression among adolescents may induce them to seek for the anxiolytic and rewarding properties of alcohol. Allopregnanolone (AlloP) shares rewarding effects of ethanol and modulates ethanol intake. The rate-limiting enzyme in the biosynthesis of AlloP is steroid 5α-reductase (5α-R), which is expressed as three isozymes, 5α-R1, 5α-R2, and 5α-R3.
Objective The objective of this study was to quantify the expression levels of 5α-R isozymes in prefrontal cortex (PFC) of adolescent male rats after three different regimes of ethanol administration.
Methods Adolescent male Wistar rats were administered with ethanol (4 g/kg) or saline intraperitoneally for 1 day (acute), for 7 days (chronic), or every 72 h for 14 days (chronic intermittent). Messenger (m)RNA and protein levels of 5α-R isozymes were measured by quantitative RT-PCR and Western blot, respectively.
Results Ethanol significantly increased mRNA and protein levels of 5α-R1, 5α-R2, and 5α-R3 in the three different regimes of ethanol administration, being higher in the chronic intermittent regime in comparison with the others.
Conclusions The expression of the AlloP-biosynthetic enzyme 5α-Rs increases in the prefrontal cortex of adolescent male rats under acute, chronic, and chronic intermittent regime of ethanol administration. The latter is very interesting because it mimics the teenage drinking behavior.

Keywords 5α-reductase isozymes · Allopregnanolone · Adolescence · Regimes of ethanol administration · Prefrontal cortex

P. Sánchez · B. Castro · J. M. Torres (✉) · E. Ortega (✉)
Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Granada, Avda. de Madrid s/n, 18012 Granada, Spain
e-mail: torrespi@ugr.es
e-mail: esortega@ugr.es

J. M. Torres · E. Ortega
Institute of Neurosciences, Faculty of Medicine, University of Granada, Granada, Spain

Introduction

Abuse and alcohol dependence by adolescent is a widespread and growing phenomenon in developed countries (Bailey and Rachal 1993), where almost 35 % of habitual drinkers are under 16 years old (Shope et al. 1994). Adolescence is a critical developmental period characterized by specific behavioral and neurobiological changes that might predispose adolescents to experiment with drugs and to be especially vulnerable to the long-term consequences of drugs of abuse (Quoilin et al. 2013). In many cases, all these changes lead to a difficult life stage for them, being more susceptible to changes in mood and behavior (i.e., shyness) or psychological disorders (i.e., anxiety, depression) and therefore to alcohol consumption (Fröjd et al. 2011; Pereira et al. 2013). Alcohol also facilitates social relationships in adolescents (Kuntsche et al. 2005) and hence is consumed mainly at weekends (Torres and Ortega 2003a, 2004).

Ethanol has multiple effects on neuronal cell physiology and signaling that contribute to the risk of alcohol dependence. One of ethanol's most potent effects on neuronal signaling is its positive modulation of γ-aminobutyric acid type A receptor ($GABA_A$-R), producing sedative and anxiolytic effects (VanDoren et al. 2000). There is evidence for interaction between ethanol and neuroactive steroids at $GABA_A$-R (Grobin et al. 1998; Morrow et al. 1999). Allopregnanolone (AlloP), the 3α,5α-reduced neurosteroid of progesterone, is

among the most potent known ligands of the $GABA_A$-R complex in the central nervous system (CNS) and has anesthetic, anxiolytic, sedative, and anti-convulsant effects, similar to the action of benzodiazepines and barbiturates (Paul and Purdy 1992).

Studies carried out in animal models have shown that alcohol increases AlloP levels in plasma and brain of rodents (Barbaccia et al. 1999; VanDoren et al. 2000; Finn et al. 2004; Sanna et al. 2004; Eva et al. 2008). Moreover, we previously reported that acute alcohol intoxication increases plasma AlloP levels in human adolescent of both sexes (Torres and Ortega 2003a, 2004). In agreement with these studies, increased AlloP levels produced in response to ethanol administration may contribute to the rewarding effects of ethanol, which may lead to higher alcohol consumption levels during the adolescence.

AlloP is synthesized from progesterone by steroidogenic enzymes 5α-reductase (5α-R) and 3α-hydroxysteroid dehydrogenase (3α-HSD), with the former being the rate-limiting enzyme in this reaction sequence (Mellon et al. 2001). There are three well-characterized 5α-R isozymes, type 1 (SRD5A1), type 2 (SRD5A2), and type 3 (SRD5A3), which are encoded by three different genes and located in different chromosomes. 5α-R isozymes are present in different brain regions, including cerebral cortex (Torres and Ortega 2003b, 2006; Sánchez et al. 2005; Castro et al. 2013).

Previous studies have shown that ethanol administration produces changes in brain 5α-R1, which correlates with changes in AlloP levels (Cagetti et al. 2004; Tanchuck et al. 2009). It is well established that 5α-R1 is the main isozyme implicated in the biosynthesis of 3α,5α-reduced neurosteroids (Paul and Purdy 1992). However, 5α-R2 and 5α-R3 might be also involved in the biosynthesis of 3α,5α-reduced neurosteroids (Torres and Ortega 2003b; Titus et al. 2013). Therefore, we cannot rule out that their expression levels might be also modified after alcohol consumption.

AlloP levels in the brain are key mediators of alcohol dependence (AD) (Morrow et al. 2001) and could vary depending on alcohol consumption regimen, i.e., acute, chronic, or chronic intermittent. The latter arises very interestingly to us, because it mimics the consumption regime of human adolescents, considering that their consumption mainly occur at weekends. Given the key role played by 5α-Rs in the biosynthesis of AlloP (Mellon et al. 2001), it is reasonable to speculate that different regimes of ethanol administration could exert different regulation profile in the expression levels of 5α-R isozymes, which may translate in differential AD responses.

The objective of this study was to quantify the messenger (m)RNA and protein levels of 5α-R isozymes in prefrontal cortex of adolescent male rats after acute, chronic, and chronic intermittent ethanol administration.

Materials and methods

Animals and treatments

Animals were treated humanely and with regard for alleviation of suffering. All procedures were performed strictly in accordance with recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Animal welfare and experimental procedures were approved by the animal experimentation ethics committee of the University of Granada, Spain (Ref. 412-2012-CEEA). Thirty-day-old male Wistar rats weighing 90–110 g were housed in stainless steel cages in an air-conditioned room with fluorescent lights on from 7 a.m. to 7 p.m. Animals were provided with a standard A04 laboratory pellet chow (Panlab, Barcelona, Spain) and water ad libitum.

Alcohol was administered by a single daily intraperitoneal (ip) injection of a 20 % (vol/vol) ethanol/saline solution at a dose of 4 g/kg, in accordance with Broadwater et al. (2011). The experimental groups were established according to the regime of ethanol administration: acute, receiving ethanol (EtOH) for 1 day; chronic, receiving EtOH for 7 consecutive days; and chronic intermittent, receiving EtOH every 72 h for 14 days. Control animals were given an ip injection of an equal volume of sterile isotonic saline following the same protocol. Each group comprised 12 animals. Rats were always injected at 9 a.m. Thirty minutes after the final injection, animals were euthanized by decapitation to avoid possible adverse effects of anesthesia. The brain was removed, frozen in liquid nitrogen, and stored at −80ºC until analysis. The dissection of prefrontal cortex (PFC) areas was assessed with reference to the Atlas of Paxinos and Watson (1986).

RNA isolation

Total RNA was extracted from 25 mg of rat PFC tissue with Trizol reagent (Invitrogen), according to the manufacturer's instructions. RNA samples were then treated with Turbo DNase (Ambion) to remove any contamination with genomic DNA. RNA yield was determined spectrophotometrically by A260/A280 ratio using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher). Isolated total RNA integrity was electrophoretically verified by ethidium bromide staining.

Reverse transcription and quantitative real-time PCR

First-strand cDNA was synthesized from 1 μg of total RNA following Castro et al. (2013). Absolute quantification of 5α-R1, 5α-R2, and 5α-R3 was performed by real-time PCR using the Techne Quantica™ with SYBR Green PCR Master Mix (Promega). We amplified the target transcripts in parallel with standard curves generated following the method described by Fronhoffs et al. (2002). The amount of mRNA

was expressed as number of copies per microgram of total RNA.

The PCR profile was as follows: denaturation at 94ºC for 30 s; annealing at 55ºC for the Srd5a1 gene, 55ºC for the Srd5a2 gene, and 50ºC for the Srd5a3 gene for 30 s; and extension at 72ºC for 30 s. The number of cycles was 40 in all cases. At the end of the amplification phase, a melting curve analysis was carried out on the products formed in order to confirm that a single PCR product was detected by the SYBR Green dye. Primers for 5α-R1 (Srd5a1 GenbankNM_017070.3), 5α-R2 (Srd5a2 Genbank NM_022711.4), and 5α-R3 (Srd5a3 GenbankNM_001013990.1) were designed using Primer 3 Plus free software. The primer sequences (5′-3′) are given in Table 1.

Electrophoresis and Western blot analysis

Protein extraction was performed as previously described (Castro et al. 2013). Protein concentration was determined by the dye-binding method of Bradford (1976) with BSA as standard using Bio-Rad protein assay reagent (Bio-Rad Laboratories, Inc., Richmond, CA, USA). Aliquots of samples containing 50 mg of proteins were subjected to 12 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot following Castro et al. (2013). The blots were incubated overnight at 4ºC with primary antibodies at a dilution of 1:500 for 5α-R1, 1:500 for 5α-R2, 1:1,000 for 5α-R3, and 1:1,000 for β-actin, in T-PBS containing 0.5 % non-fat dry milk. The blots were incubated for 1 h with the appropriate anti-IgG horseradish peroxidase (HRP)-conjugated secondary antibody at a dilution of 1:5,000. The blots were visualized using enhanced chemiluminescence detection system according to the supplier's instructions (ECL-Plus, GE Healthcare, USA). The ImageJ program (http://rsb.info.nih.gov/ij/) was used for quantitative analysis of the bands. To account for any differences in loading, target band densitometries were divided by actin densitometries obtained from the same lane. These corrected densitometries were normalized to controls in each experiment.

Antibodies: goat anti-5α-R1 (Abcam ab110123), rabbit anti-5α-R2 (Santa Cruz sc-20659), and anti-5α-R3 (Novus Biological NBP-69612). A mouse anti-β-actin antibody (Thermo Scientific BA3R) was used as loading control. Goat anti-mouse, goat anti-rabbit, and donkey anti-goat IgG HRP conjugated (Santa Cruz) were used as secondary antibodies.

Statistical analysis

One-way ANOVA was used to compare means among multiple groups, applying post hoc pairwise comparisons with Bonferroni's penalization, where results were significant. A value of $p<0.05$ was considered to be of statistical significance. Data are expressed as mean±SE. All statistical analyses were performed using SigmaPlot for Windows v.11.0 (SPSS Inc., Chicago, IL, USA).

Results

Effects of different alcohol administration regimes on 5α-R1

Figure 1 depicts the effects of the different alcohol administration regimes on mRNA (panel A) and protein levels (panel B) of 5α-R1 isozyme in PFC of adolescent male rats. 5α-R1 mRNA levels were significantly increased after acute, chronic, and chronic intermittent ethanol administration in comparison with their respective controls, with the higher increase observed for the chronic intermittent treatment. 5α-R1 mRNA levels were also significantly increased in the chronic intermittent-treated group in comparison with the acute- and chronic-treated groups. No significant differences in 5α-R1 mRNA levels were found in acute versus the chronic treatments. No significant differences in 5α-R1 mRNA levels were found between the different control groups. For this reason, we only run for protein measurement the control sample for the chronic intermittent treatment.

5α-R1 protein levels were significantly increased after acute, chronic, and chronic intermittent ethanol administration in comparison with the control group. 5α-R1 protein levels were also significantly increased in the chronic intermittent-treated group in comparison with the acute and chronic-treated groups. No significant differences in 5α-R1 protein levels were found in acute versus chronic treatments.

Effects of different alcohol administration regimes on 5α-R2

Figure 2 depicts the effects of the different alcohol administration regimes on mRNA (panel A) and protein levels (panel

Table 1 Primer sequences (5′-3′) for PCR amplification

Primer	Forward primer	Reverse primer
5α-R1	GAGATATTCAGCTGAGACCC	TTAGTATGTGGGCAGCTTGG
5α-R2	ATTTGTGTGGCAGAGAGAGG	TTGATTGACTGCCTGGATGG
5α-R3	TGCCCATCAGTATAAGTGCC	TCACCATAAAGCTCGAACCAG

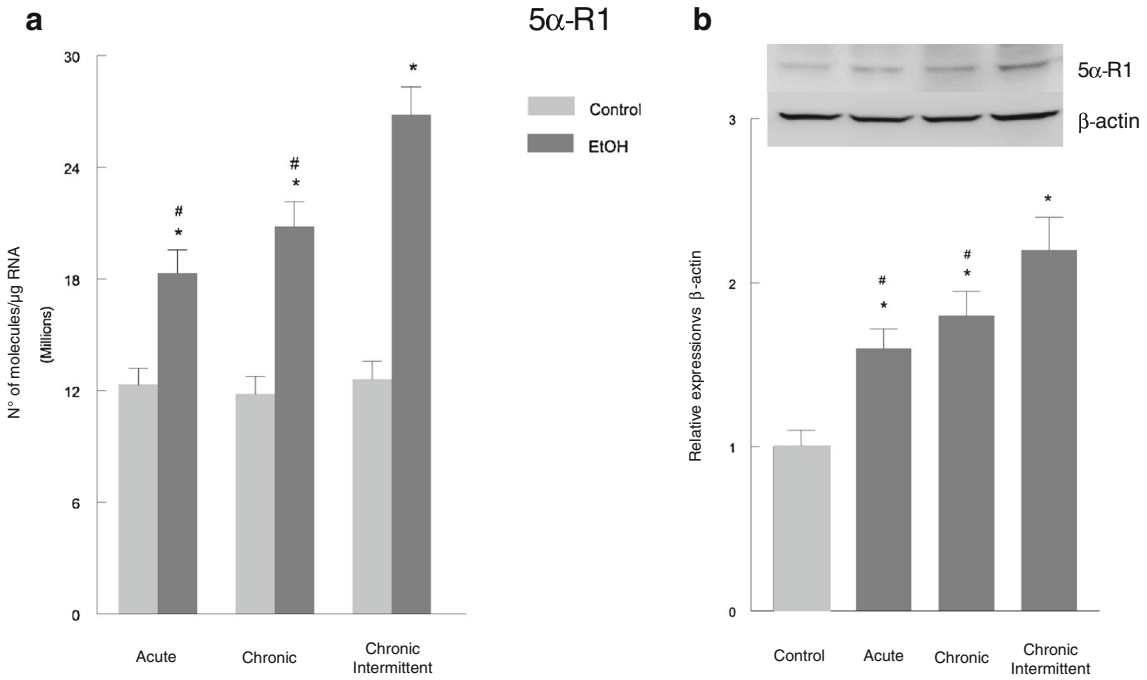

Fig. 1 Effects of acute, chronic, and chronic intermittent alcohol administration on mRNA (**a**) and protein levels (**b**) of steroid 5α-reductase type 1 (5α-R1) in prefrontal cortex of adolescent male rats. *at least $p<0.05$ vs. their controls. #at least $p<0.05$ vs. chronic intermittent-treated group

B) of 5α-R2 isozyme in PFC of adolescent male rats. 5α-R2 mRNA levels were significantly increased after acute, chronic, and chronic intermittent ethanol administration in comparison with their respective controls, with the higher increase observed for the chronic intermittent treatment. 5α-R2 mRNA levels were also significantly increased in the chronic intermittent-treated group in comparison with the acute- and chronic-treated groups. No significant differences in 5α-R2 mRNA levels were found in acute versus chronic treatments. No significant differences in 5α-R2 mRNA levels were found between the different control groups.

5α-R2 protein levels were significantly increased after acute, chronic, and chronic intermittent ethanol administration in comparison with the control group. 5α-R2 protein levels

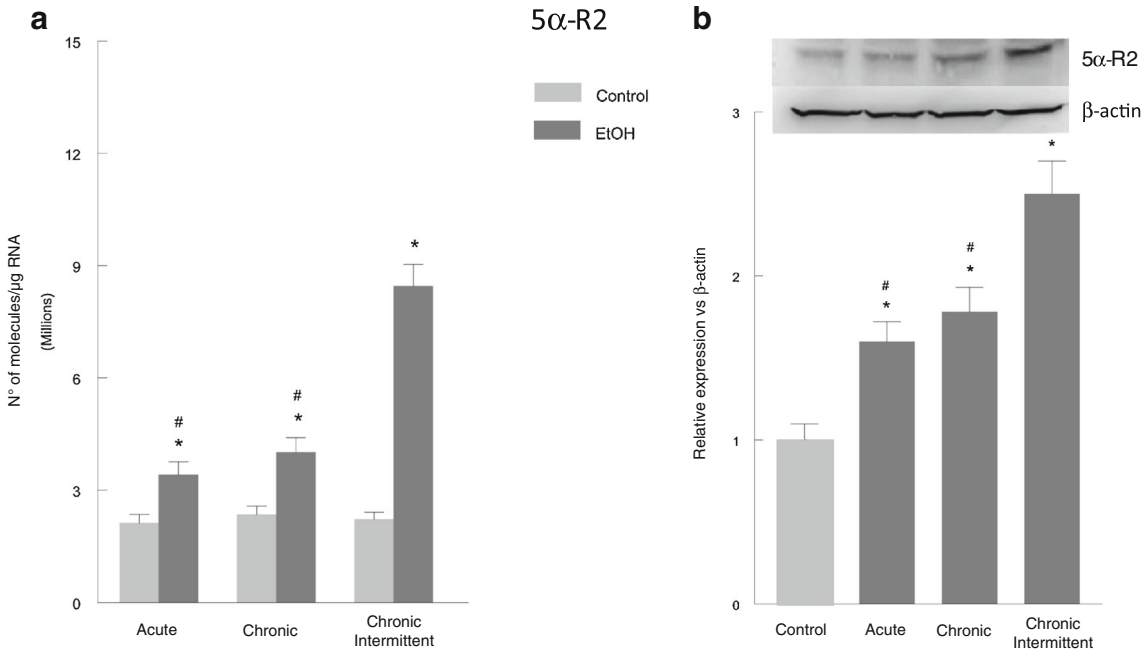

Fig. 2 Effects of acute, chronic, and chronic intermittent alcohol administration on mRNA (**a**) and protein levels (**b**) of steroid 5α-reductase type 2 (5α-R2) in prefrontal cortex of adolescent male rats. *at least $p<0.05$ vs. their controls. #at least $p<0.05$ vs. chronic intermittent-treated group

were also significantly increased in the chronic intermittent-treated group in comparison with the acute and chronic-treated groups. No significant differences in 5α-R2 protein levels were found in acute versus chronic treatments.

Effects of different alcohol administration regimes on 5α-R3

Figure 3 depicts the effects of the different alcohol administration regimes on mRNA (panel A) and protein levels (panel B) of 5α-R3 isozyme in PFC of adolescent male rats. 5α-R3 mRNA levels were significantly increased after acute, chronic, and chronic intermittent ethanol administration in comparison with their respective controls, with the higher increase observed for the chronic intermittent treatment. 5α-R3 mRNA levels were also significantly increased in the chronic intermittent-treated group in comparison with the acute- and chronic-treated groups. Significant differences were also observed in 5α-R3 mRNA levels in acute versus chronic treatments. No significant differences in 5α-R3 mRNA levels were found between the different control groups.

5α-R3 protein levels were significantly increased after acute, chronic, and chronic intermittent ethanol administration in comparison with the control group. 5α-R3 protein levels were also significantly increased in the chronic intermittent-treated group in comparison with the acute- and chronic-treated groups. Significant differences were also observed in 5α-R3 protein levels in acute versus chronic treatments.

Discussion

Regular alcohol consumption and binge drinking among adolescents are risk behaviors of particular concern. In fact, a determined regime of three or more times a week of excessive alcohol intake in adolescent predicted alcohol dependence in adulthood (Bonomo et al. 2004).

Many of the effects of alcohol are exerted through $GABA_A$-R well directly or indirectly through the increase of AlloP (Sanna et al. 2004). Growing evidences in animals suggest that changes in AlloP levels are involved in acute effects of alcohol, as well as in alcohol tolerance and dependence (Morrow et al. 2001). The question that arises now is whether the isozymes of 5α-R, enzyme which catalyzes the rate-limiting step in the biosynthesis of AlloP (Paul and Purdy 1992), could be modified by ethanol. Another interesting question to address is whether 5α-R isozymes are differentially modified depending on the regime of alcohol administration. It is of particular interest to know what happens in a regime of chronic intermittent consumption, similar to that followed by young adolescents consuming alcoholic beverages every weekend. Therefore, we have used a validated model of binge-like ethanol exposure in adolescent rats that is similar to reports from human adolescents of binge-like (Broadwater et al. 2011). Moreover, we have chosen a high dose of alcohol (4 g/kg) because adolescent rats may metabolize ethanol faster than adults (Morris et al. 2010), and they are less sensitive than adults to a number of ethanol's effects such as the anxiolytic (Morris et al. 2010).

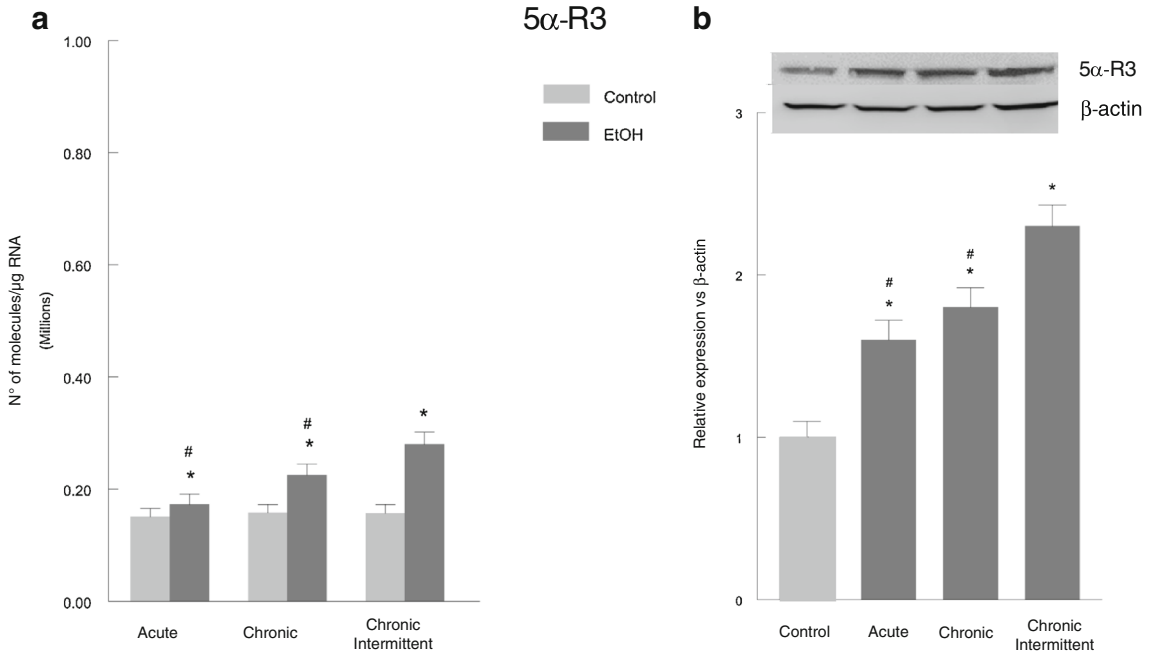

Fig. 3 Effects of acute, chronic, and chronic intermittent alcohol administration on mRNA (**a**) and protein levels (**b**) of steroid 5α-reductase type 3 (5α-R3) in prefrontal cortex of adolescent male rats. *at least $p<0.05$ vs. their controls. #at least $p<0.05$ vs. chronic intermittent-treated group

The results of our experiment show that the three different ethanol administration regimes studied, i.e., acute, chronic, and chronic intermittent, produce an increase in mRNA and protein levels of 5α-R1, 5α-R2, and 5α-R3 in the prefrontal cortex of adolescent male rats. Therefore, even though the activity of theses isozymes has not been measured in this study, our results point out to a possible increase in cortical AlloP levels after the three ethanol administration regimes. One major finding was that the increase in both mRNA and protein levels of 5α-R isozymes was significantly higher after chronic intermittent than after acute or chronic administration of ethanol. However, we can emphasize that the blood ethanol levels at the time of sacrifice (unfortunately not measured in this work) might be different in the three experimental groups depending on the ethanol administration regime (Silvers et al. 2003).

According to our results, acute administration of ethanol increased 5α-R1 expression in cortex of adult rats (Kim et al. 2003). This finding is consistent with the increase in cortical AlloP levels after acute ethanol injection in male rats (Barbaccia et al. 1999; VanDoren et al. 2000; Cook et al. 2014). In contrast to our results, it has been demonstrated that chronic ethanol consumption significantly decreased cortical 5α-R1 activity in both withdrawal seizure-prone (WSP) and withdrawal seizure-resistant (WSR) male mice (Tanchuck et al. 2009), data in line with the decreased cortical AlloP levels found in these mice (Tanchuck et al. 2009) and in male rats (Janis et al. 1998). Differences in animal species, initial dosage of the drug, route of administration, schedule and ways of exposition to ethanol, brain area assessed, and age of the animal should be kept in mind to explain these discrepancies. With respect to the chronic intermittent ethanol (CIE) administration, Cagetti et al. (2004) found in hippocampus of adult male rats decreased levels of both AlloP and 5α-R1 mRNA, data in contrast to our present results with respect to 5α-R1. However, according to our findings, other authors demonstrated that 2 days after CIE exposure of adolescent male rats, a challenge of ethanol increased hippocampal and cortical AlloP levels (Silvers et al. 2006).

Because 5α-R1 is the main isozyme in the biosynthesis of AlloP (Paul and Purdy 1992), our results suggest that AlloP levels might be presumably higher in the chronic intermittent regime (which mimics the ethanol intake pattern followed by adolescent humans) than in the others. Several reasons may explain how this increase in AlloP may lead to higher rates in the consumption of alcohol by adolescents. AlloP modulates some effects of ethanol (e.g., anxiolytic, analgesic, anticonflictive, and rewarding) through allosteric modulation of $GABA_A$-R (VanDoren et al. 2000; Sanna et al. 2004). Exogenous administration of AlloP dose-dependently modulated ethanol intake in rodents (Ford et al. 2005a). Alcohol also facilitates social relationships in adolescents (Kuntsche et al. 2005). The pharmacological blockade of ethanol acquisition might involve a reduction in ethanol's perceived effects that normally would promote continued consumption (Gessa et al. 2005). Taking all together, these data could in part explain the reoccurrence of young people to alcohol intake on weekends (Torres and Ortega 2003a, 2004).

It is well documented that prolonged or repeated ethanol intake leads to the development of tolerance to this drug (Morrow et al. 2001). Tolerance to ethanol may involve a loss of ethanol-induced increase of AlloP and, consequently, a reduction in ethanol-related effects (Morrow et al. 2001), which may lead to increased consumption of alcohol (Boyd et al. 2010). Some studies have demonstrated decreased AlloP levels in ethanol-dependent male rats and in ethanol-withdrawing mice (Morrow et al. 2001), and such negative effects might cause reoccurrence of alcohol use (Koob 2003; Heilig et al. 2010). Alterations in $GABA_A$-R expression and function, in part mediated by AlloP, may also contribute to development of ethanol tolerance and dependence (Morrow et al. 2001; Kumar et al. 2009). Ethanol-dependent rats are sensitized to the anticonvulsant effects of AlloP and have enhanced $GABA_A$-R sensitivity to AlloP animals (Devaud et al. 1996, 1998), likely due to the reduction of ethanol-induced increase in AlloP (Morrow et al. 2006). It is well established that chronic ethanol exposure may lead to tolerance and dependence to ethanol (Morrow et al. 2001). However, Silvers et al. (2006) also demonstrated that after CIE exposure of adolescent rats, a challenge of ethanol administered 14 days after removed from the CIE treatment produced a decrease on hippocampal AlloP levels, i.e., intermittent ethanol exposure produces first an increase (as we mentioned above) and then a decrease on AlloP levels. Therefore, CIE exposure produced long-lasting effects and functional tolerance in adolescent rats.

Our results showed that 5α-R2 mRNA and protein levels were significantly increased after the three regimes of ethanol administration, being this increase higher after CIE administration. Therefore, although the activity of this isozyme has not been measured in this study, the high expression found for 5α-R2 indicates that this isoform might also contribute along with 5α-R1 to the biosynthesis of cortical AlloP (Matsui et al. 2002; Torres and Ortega 2003b). Even though the main function of 5α-R2 in the brain is to convert testosterone (T) to dihydrotestosterone (DHT) (Poletti et al. 1998; Torres and Ortega 2003b), in several conditions, 5α-R2 may participate in the biosynthesis of 3α,5α-reduced neuroesteroids (Sánchez et al. 2009) contributing to the rewarding effects of alcohol. This is consistent with previous works addressing the effects of finasteride (FIN), a specific inhibitor of 5α-R2 isozyme, on alcohol consumption. Thus, pretreatment with FIN reduced the ethanol-induced increase in cortical AlloP levels and blocked the anti-convulsant effect of ethanol (VanDoren et al. 2000; Sanna et al. 2004). Some authors have shown that FIN reduces voluntary alcohol intake in rodents (Ford et al. 2005b; Ramaker et al. 2011) and prevents the development of alcohol preference in mice (Ford et al. 2008). In humans, FIN

attenuates subjective responses to self-administered alcohol in moderate drinkers (Pierucci-Lagha et al. 2005) and decreases alcohol consumption among former male users of FIN with persistent sexual side effects (Irwig 2013). It has also been demonstrated that V89L SRD5A2 polymorphism significantly reduced 5α-R2 enzyme activity in human prostate cancer tissue (Makridakis et al. 2004). Interestingly, V89L also resulted in diminished craving levels during alcohol withdrawal in male alcohol addicts, although exerted no influence on the alcohol dependence (Lenz et al. 2012).

To our knowledge, this is the first study showing an association between 5α-R3 isozyme and alcohol administration. Nevertheless, the increase found in 5α-R3 mRNA levels in the three different alcohol regimes was lower than those observed in 5α-R1 and 5α-R2 isozymes when compared to their respective control groups. Although 5α-R3 has been also proposed as a partner in the AlloP biosynthesis (Titus et al. 2013), its contribution to the alcohol-mediated effects would be less than 5α-R1 and 5α-R2 isozymes. Little is known about 5α-R3 function in the brain. In the last years, it has been reported that 5α-R3 plays an important role in protein glycosylation (Cantagrel et al. 2010) and alterations in this process are associated with some brain pathologies. Thus, mutations in the SRD5A3 gene have been identified in patients with cerebellar ataxia and congenital eye malformations (Morava et al. 2010). Chronic alcohol consumption alters protein glycosylation (Waszkiewicz et al. 2012). In fact, the carbohydrate-deficient transferrin (CDT) is used as a biomarker for chronic alcohol intake (Golka and Wiese 2004). The increase observed in the expression of 5α-R3 after alcohol injection probably allows glycosylation of other target proteins.

Even though AlloP levels have not been measured in this study, our results contributes the first evidence that ethanol modifies in PFC of adolescent male rats the expression of the three 5α-Rs, the key enzyme in the biosynthesis of AlloP, being this effect higher after CIE exposure, which mimics the ethanol intake pattern followed by adolescent humans. Although our biochemical and molecular studies were performed in rats, the results presented here might be consistent with studies in humans, where excessive alcohol intake in adolescent may predict alcohol dependence in the adulthood.

Acknowledgments This study was funded in part by the European Fund of Regional Development (FEDER-BFU2008-05340) and the Andalusian Regional Government (Endocrinology and Metabolism Group).

Conflict of interest The authors do not have direct financial conflicts of interest.

References

Bailey SL, Rachal JV (1993) Dimensions of adolescent problem drinking. J Stud Alcohol 54:555–565

Barbaccia ML, Affricano D, Trabucchi M, Purdy RH, Colombo G, Agabio R, Gessa GL (1999) Ethanol markedly increases "GABAergic" neurosteroids in alcohol-preferring rats. Eur J Pharmacol 384:R1–R2

Bonomo YA, Bowes G, Coffey C, Carlin JB, Patton GC (2004) Teenage drinking and the onset of alcohol dependence: a cohort study over seven years. Addiction 99:1520–1528

Bradford MN (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. Anal Biochem 72:248–254

Boyd KN, Kumar S, O'Buckley TK, Morrow AL (2010) Chronic ethanol exposure produces tolerance to elevations in neuroactive steroids: mechanisms and reversal by exogenous ACTH. J Neurochem 115: 142–152

Broadwater M, Varlinskaya EI, Spear LP (2011) Chronic intermittent ethanol exposure in early adolescent and adult male rats: effects on tolerance, social behavior, and ethanol intake. Alcohol Clin Exp Res 35:1392–1403

Cagetti E, Pinna G, Guidotti A, Baicy K, Olsen RW (2004) Chronic intermittent ethanol (CIE) administration in rats decreases levels of neurosteroids in hippocampus, accompanied by altered behavioral responses to neurosteroids and memory function. Neuropharmacology 46:570–579

Cantagrel V, Lefeber DJ, Ng BG et al (2010) SRD5A3 is required for converting polyprenol to dolichol and is mutated in a congenital glycosylation disorder. Cell 142:203–217

Castro B, Sánchez P, Torres JM, Ortega E (2013) Effects of adult exposure to bisphenol A on genes involved in the physiopathology of rat prefrontal cortex. PLoS One 8:e73584

Cook JB, Dumitru AM, O'Buckley TK, Morrow AL (2014) Ethanol administration produces divergent changes in GABAergic neuroactive steroid immunohistochemistry in the rat brain. Alcohol Clin Exp Res 38:90–99

Devaud LL, Fritschy JM, Morrow AL (1998) Influence of gender on chronic ethanol-induced alterations in GABAA receptors in rats. Brain Res 15:222–230

Devaud LL, Purdy RH, Finn DA, Morrow AL (1996) Sensitization of gamma-aminobutyric acidA receptors to neuroactive steroids in rats during ethanol withdrawal. J Pharmacol Exp Ther 278:510–517

Eva C, Mele P, Collura D, Nai A, Pisu MG, Serra M, Biggio G (2008) Modulation of neuropeptide Y and Y1 receptor expression in the amygdala by fluctuations in the brain content of neuroactive steroids during ethanol drinking discontinuation in Y1R/LacZ transgenic mice. J Neurochem 104:1043–1054

Finn DA, Sinnott RS, Ford MM, Long SL, Tanchuck MA, Phillips TJ (2004) Sex differences in the effect of ethanol injection and consumption on brain allopregnanolone levels in C57BL/6 mice. Neuroscience 123:813–819

Ford MM, Nickel JD, Phillips TJ, Finn DA (2005a) Neurosteroid modulators of GABAA receptors differentially modulate ethanol intake patterns in male C57BL/6 J mice. Alcohol Clin Exp Res 29:1630–1640

Ford MM, Nickel JD, Finn DA (2005b) Treatment with and withdrawal from finasteride alter ethanol intake patterns in male C57BL/6 J mice: potential role of endogenous neurosteroids? Alcohol 37:23–33

Ford MM, Yoneyama N, Strong MN, Fretwell A, Tanchuck M, Finn DA (2008) Inhibition of 5alpha-reduced steroid biosynthesis impedes acquisition of ethanol drinking in male C57BL/6 J mice. Alcohol Clin Exp Res 32:1408–1416

Fröjd S, Ranta K, Kaltiala-Heino R, Marttunen M (2011) Associations of social phobia and general anxiety with alcohol and drug use in a community sample of adolescents. Alcohol Alcohol 46:192–199

Fronhoffs S, Totzke G, Stier S, Wernert N, Rothe M et al (2002) A method for the rapid construction of cRNA standard curves in quantitative real-time reverse transcription polymerase chain reaction. Mol Cell Probes 16:99–110

Gessa GL, Serra S, Vacca G, Carai MA, Colombo G (2005) Suppressing effect of the cannabinoid CB1 receptor antagonist, SR147778, on alcohol intake and motivational properties of alcohol in alcohol-preferring sP rats. Alcohol Alcohol 40:46–53

Golka K, Wiese A (2004) Carbohydrate-deficient transferrin (CDT)–a biomarker for long-term alcohol consumption. J Toxicol Environ Health B Crit Rev 7:319–337

Grobin AC, Matthews DB, Devaud LL, Morrow AL (1998) The role of GABA(A) receptors in the acute and chronic effects of ethanol. Psychopharmacology 139:2–19

Heilig M, Egli M, Crabbe JC, Becker HC (2010) Acute withdrawal, protracted abstinence and negative affect in alcoholism: are they linked? Addict Biol 15:169–184

Irwig MS (2013) Decreased alcohol consumption among former male users of finasteride with persistent sexual side effects: a preliminary report. Alcohol Clin Exp Res 37:1823–1826

Janis GC, Devaud LL, Mitsuyama H, Morrow AL (1998) Effects of chronic ethanol consumption and withdrawal on the neuroactive steroid 3alpha-hydroxy-5alpha-pregnan-20-one in male and female rats. Alcohol Clin Exp Res 22:2055–2061

Kim HJ, Ha M, Park CH, Park SJ, Youn SM, Kang SS, Cho GJ, Choi WS (2003) StAR and steroidogenic enzyme transcriptional regulation in the rat brain: effects of acute alcohol administration. Brain Res Mol Brain Res 115:39–49

Koob GF (2003) Alcoholism: allostasis and beyond. Alcohol Clin Exp Res 27:232–243

Kumar S, Porcu P, Werner DF, Matthews DB, Diaz-Granados JL, Helfand RS, Morrow AL (2009) The role of GABA(A) receptors in the acute and chronic effects of ethanol: a decade of progress. Psychopharmacology (Berl) 205:529–564

Kuntsche E, Knibbe R, Gmel G, Engels R (2005) Why do young people drink? A review of drinking motives. Clin Psychol Rev 2:841–861

Lenz B, Schöpp E, Müller CP, Bleich S, Hillemacher T, Kornhuber J (2012) Association of V89L SRD5A2 polymorphism with craving and serum leptin levels in male alcohol addicts. Psychopharmacology 224:421–429

Makridakis N, Akalu A, Reichardt JK (2004) Identification and characterization of somatic steroid 5alpha-reductase (SRD5A2) mutations in human prostate cancer tissue. Oncogene 23:7399–7405

Matsui D, Sakari M, Sato T, Murayama A, Takada I, Kim M, Takeyama K, Kato S (2002) Transcriptional regulation of the mouse steroid 5-alpha-reductase type II gene by progesterone in brain. Nucleic Acids Res 30:1387–1393

Mellon SH, Griffin LD, Compagnone NA (2001) Biosynthesis and action of neurosteroids. Brain Res Brain Res Rev 37:3–12

Morava E, Wevers RA, Cantagrel V, Hoefsloot LH, Al-Gazali L et al (2010) A novel cerebello-ocular syndrome with abnormal glycosylation due to abnormalities in dolichol metabolism. Brain 133:3210–3220

Morris SA, Kelso ML, Liput DJ, Marshall SA, Nixon K (2010) Similar withdrawal severity in adolescents and adults in a rat model of alcohol dependence. Alcohol 44:89–98

Morrow AL, Janis GC, VanDoren MJ, Matthews DB, Samson HH, Janak PH, Grant KA (1999) Neurosteroids mediate pharmacological effects of ethanol: a new mechanism of ethanol action? Alcohol Clin Exp Res 23:1933–1940

Morrow AL, Porcu P, Boyd KN, Grant KA (2006) Hypothalamic-pituitary-adrenal axis modulation of GABAergic neuroactive steroids influences ethanol sensitivity and drinking behavior. Dialogues Clin Neurosci 8:463–477

Morrow AL, VanDoren MJ, Penland SN, Matthews DB (2001) The role of GABAergic neuroactive steroids in ethanol action, tolerance and dependence. Brain Res Brain Res Rev 37:98–109

Paul SM, Purdy RH (1992) Neuroactive steroids. FASEB J 6:2311–2322

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, 2nd edn. Academic, New York

Pereira G, Wood L, Foster S, Haggar F (2013) Access to alcohol outlets, alcohol consumption and mental health. PLoS One 8:e53461

Pierucci-Lagha A, Covault J, Feinn R, Nellissery M, Hernandez-Avila C, Oncken C, Morrow AL, Kranzler HR (2005) GABRA2 alleles moderate the subjective effects of alcohol, which are attenuated by finasteride. Neuropsychopharmacology 30:1193–1203

Poletti A, Negri-Cesi P, Rabuffetti M, Colciago A, Celotti F, Martini L (1998) Transient expression of the 5alpha-reductase type 2 isozyme in the rat brain in late fetal and early postnatal life. Endocrinology 139:2171–2178

Quoilin C, Didone V, Tirelli E, Quertemont E (2013) Higher long-lasting ethanol sensitization after adolescent ethanol exposure in mice. Psychopharmacology Dec 7 (in press)

Ramaker MJ, Ford MM, Fretwell AM, Finn DA (2011) Alteration of ethanol drinking in mice via modulation of the GABA(A) receptor with ganaxolone, finasteride, and gaboxadol. Alcohol Clin Exp Res 35: 1994–2007

Sánchez P, Torres JM, Olmo A, O'Valle F, Ortega E (2009) Effects of environmental stress on mRNA and protein expression levels of steroid 5alpha-Reductase isozymes in adult rat brain. Horm Behav 56:348–353

Sánchez P, Torres JM, Ortega E (2005) Effects of dihydrotestosterone on brain mRNA levels of steroid 5alpha-reductase isozymes in early postnatal life of rat. Neurochem Res 30:577–581

Sanna E, Talani G, Busonero F, Pisu MG, Purdy RH, Serra M, Biggio G (2004) Brain steroidogenesis mediates ethanol modulation of GABAA receptor activity in rat hippocampus. J Neurosci 24:6521–6530

Shope JT, Copeland LA, Dielman TE (1994) Measurement of alcohol use and misuse in a cohort of students followed from grade 6 through grade 12. Alcohol Clin Exp Res 18:726–733

Silvers JM, Tokunaga S, Mittleman G, Matthews DB (2003) Chronic intermittent injections of high-dose ethanol during adolescence produce metabolic, hypnotic, and cognitive tolerance in rats. Alcohol Clin Exp Res 27:1606–1612

Silvers JM, Tokunaga S, Mittleman G, O'Buckley T, Morrow AL, Matthews DB (2006) Chronic intermittent ethanol exposure during adolescence reduces the effect of ethanol challenge on hippocampal allopregnanolone levels and Morris water maze task performance. Alcohol 39:151–158

Tanchuck MA, Long SL, Ford MM, Hashimoto J, Crabbe JC, Roselli CE, Wiren KM, Finn DA (2009) Selected line difference in the effects of ethanol dependence and withdrawal on allopregnanolone levels and 5alpha-reductase enzyme activity and expression. Alcohol Clin Exp Res 33:2077–2087

Titus MA, Li Y, Kozyreva OG, Maher V, Godoy A, Smith GJ, Mohler JL (2013) 5α-reductase type 3 enzyme in benign and malignant prostate. Prostate. doi:10.1002/pros.22745

Torres JM, Ortega E (2003a) Alcohol intoxication increases allopregnanolone levels in female adolescent humans. Neuropsychopharmacology 28: 1207–1209

Torres JM, Ortega E (2003b) Differential regulation of steroid 5alpha-reductase isozymes expression by androgens in the adult rat brain. FASEB J 17:1428–1433

Torres JM, Ortega E (2004) Alcohol intoxication increases allopregnanolone levels in male adolescent humans. Psychopharmacology 172:352–355

Torres JM, Ortega E (2006) Steroid 5alpha-reductase isozymes in the adult female rat brain: central role of dihydrotestosterone. J Mol Endocrinol 36:239–245

VanDoren MJ, Matthews DB, Janis GC, Grobin AC, Devaud LL, Morrow AL (2000) Neuroactive steroid 3α-hydroxy-5α-pregnan-20-one modulates electrophysiological and behavioral actions of ethanol. J Neurosci 20:1982–1989

Waszkiewicz N, Szajda SD, Zalewska A, Szulc A, Kępka A, Minarowska A, Wojewódzka-Żelezniakowicz M, Konarzewska B, Chojnowska S, Ladny JR, Zwierz K (2012) Alcohol abuse and glycol conjugate metabolism. Folia Histochem Cytobiol 50:1–11

Copyright of Psychopharmacology is the property of Springer Science & Business Media B.V. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.