

Alcohol Intoxication Increases Allopregnanolone Levels in Female Adolescent Humans

JM Torres¹ and E Ortega^{*,1}

¹Department of Biochemistry and Molecular Biology, Institute of Neurosciences, School of Medicine, University of Granada, Granada, Spain

Teenage drinking is a cause of growing concern in industrialized countries, where almost 35% of alcohol drinkers are under 16 years old. Increased anxiety, irritability, and depression among adolescents may induce them to seek the anxiolytic and rewarding properties of alcohol. We studied the effects of acute alcohol intoxication (AAI) on the plasma levels of progesterone and allopregnanolone in female adolescents. Blood samples were drawn from female adolescents who arrived at the emergency department. One study group was formed by those who arrived with evident behavioral symptoms of AAI and the other by those arriving for mild trauma (contusions, sprains) after no consumption of alcohol (controls). Our results demonstrate that AAI significantly increases serum progesterone and allopregnanolone levels in both follicular and luteal phases of the ovarian cycle. Since alcohol and allopregnanolone positively modulate gamma-aminobutyric acid type A (GABA_A) receptors, allopregnanolone may play a major role in the anxiolytic and rewarding effects of alcohol, either directly or by influencing the sensitivity of GABA_A-receptors to alcohol.

Neuropsychopharmacology (2003) **28**, 1207–1209, advance online publication, 2 April 2003; doi:10.1038/sj.npp.1300170

Keywords: allopregnanolone; progesterone; alcohol intoxication; adolescents; female; human

INTRODUCTION

Alcohol abuse among adolescents is a widespread and growing threat to the health of young people in industrialized countries (Bailey and Valery, 1993), where almost 35% of habitual drinkers are under 16 years old (Shope *et al*, 1994). A growing number of young adolescents drink alcoholic beverages every weekend.

Our group has reported the deleterious effects of alcohol abuse on the reproductive and growth axis of adolescents (Frias *et al*, 2000a,b). An important question to be addressed is why increasing numbers of adolescents consume alcohol, despite the adverse and probably long-lasting effects of this drug on their behavior and physiology.

Owing to the pace of life in modern industrialized society, among other factors, adolescents may feel that their affective needs are not fully met, leading to the development of symptoms of anxiety, irritability, and depression. This could in part explain why these young people seek the anxiolytic and rewarding properties of alcohol, a socially permitted drug.

Alcohol positively modulates gamma-aminobutyric acid type A (GABA_A) receptors and produces sedative and

anxiolytic effects. There is evidence for interaction between alcohol and neurosteroids at GABA_A-receptors (Grobin *et al*, 1998). The neurosteroid allopregnanolone is one of the most potent endogenous positive modulators of GABA_A-receptors and has sedative, hypnotic, anticonvulsant, and anxiolytic effects (Lambert *et al*, 1995). In animals, it has been suggested that allopregnanolone produced in response to systemic ethanol administration could contribute to several of the effects of ethanol that are associated with the modulation of GABA_A-receptors (Morrow *et al*, 2001a). Neurosteroids have been shown to be rewarding in rodents (Finn *et al*, 1997). Therefore, neurosteroids may contribute to the anxiolytic and rewarding effects of alcohol.

Many animal studies have investigated the effect of alcohol on neurosteroid concentrations (Barbaccia *et al*, 1999). However, we know of no studies that have demonstrated the effect of alcohol on neurosteroids in adolescent humans, possibly because of the difficulty to recruit appropriate study subjects. Studies involving the controlled administration of alcohol are evidently ruled out in adolescents on ethical grounds.

The present study aimed to determine allopregnanolone levels in female adolescent humans in situations of acute alcohol intoxication (AAI).

MATERIALS AND METHODS

A study was conducted of 35 white female adolescents (13–17 years old) of variable weight, height, and educational level who arrived at the emergency department of our

*Correspondence: Dr E Ortega, Department of Biochemistry and Molecular Biology, Facultad de Medicina, Universidad de Granada, Avda. de Madrid s/n, 18012 Granada, Spain, Tel: +34 58 243519, Fax: +34 58 249015, E-mail: esortega@ugr.es

Received 22 July 2002; revised 24 October 2002; accepted 13 January 2003

Online publication: 22 January 2003 at <http://www.acnp.org/citations/Npp012203469>

hospital between 12.00 and 3.30 am. The subjects were assigned to one of the two study groups. One group (AAI, $n = 20$) was formed by those arriving at the emergency department with evident behavioral symptoms of AAI (slurred speech, unstable walking); after remission of these symptoms, all of them reported that they habitually became drunk every weekend. The second group (controls, $n = 15$) comprised young women who arrived at the department for mild trauma (contusions, sprains) and reported no alcohol consumption. All subjects were interviewed regarding their current ovarian phase and any drug treatments, in the case of AAI after the remission of their symptoms. No subjects were receiving medication at the time of the study or suffered any apparent endocrine disorder. All participants, or their parents, gave their informed consent for participation in the study, which was carried out in accordance with the Helsinki Declaration of 1975.

Blood samples were extracted from all subjects on arrival at the emergency area. Serum samples were frozen and stored at -20°C . Assessment of progesterone concentrations was performed by radioimmune assay (RIA), using commercially available Sorin Biomedica kits (Vercelli, Italy) without modifications. Allopregnanolone was measured by RIA as previously described (Torres *et al*, 2001), using an antiserum kindly provided by Roussel-Uclaf. The plasma samples were extracted with diethyl ether (Genazzani *et al*, 1998) and the extracts were purified according to Corpechot *et al* (1993). Since the ovary is an important source of circulating progesterone and allopregnanolone in humans (Rapkin *et al*, 1997; Genazzani *et al*, 2002), the study included women in follicular (AAI, $n = 10$; controls, $n = 7$) or luteal (AAI, $n = 10$; controls, $n = 8$) phases of their ovarian cycle. Their consumption of alcohol was confirmed by the measurement of blood alcohol concentrations, using a gas-chromatographic head-space method described previously by our group (Frias *et al*, 2002).

Statistically significant differences between the groups were analyzed by a two-way ANOVA. Since the Levene test for equality of variance showed significance, logarithmic transformation was performed, meeting the assumption of homogeneity of variance. The Bonferroni method was used in this study. The SPSS version 9.0 for Windows software package was used in the statistical analysis. Results are expressed as mean \pm SEM.

RESULTS

Serum levels of allopregnanolone and progesterone in the AAI and control groups, respectively, are shown in Figures 1 and 2. Two-way ANOVA of the allopregnanolone results showed significantly higher allopregnanolone levels in the AAI group *vs* controls ($F_{1,31} = 75.33$, $P < 0.001$) and significantly higher allopregnanolone levels in luteal *vs* follicular phase ($F_{1,31} = 47.36$, $P < 0.001$). Application of the Bonferroni method showed that the AAI females had significantly higher allopregnanolone levels *vs* controls in both follicular and luteal phase ($P < 0.01$). With respect to progesterone values, significantly higher levels were found in AAI women *vs* controls ($F_{1,31} = 59.14$, $P < 0.001$), and progesterone levels were significantly higher in luteal than follicular phase ($F_{1,31} = 770.07$, $P < 0.001$). Application of

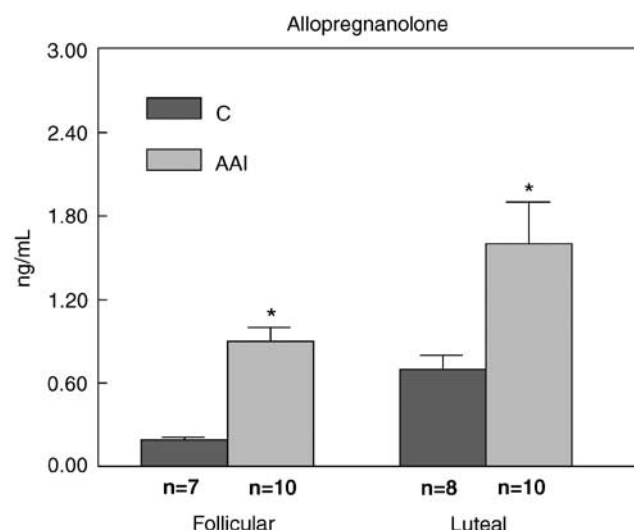


Figure 1 Serum allopregnanolone in AAI and controls (C). The data are expressed as mean \pm SEM. The Bonferroni method showed statistical differences in AAI *vs* C $P < 0.01$ in both follicular and luteal phases of the ovarian cycle.

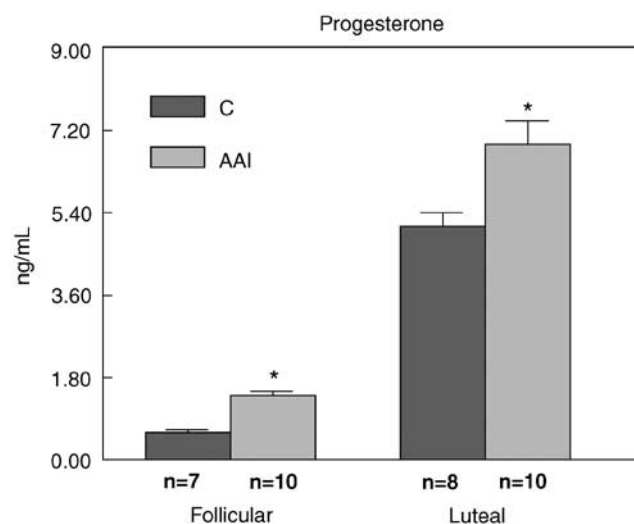


Figure 2 Serum progesterone in AAI and controls (C). The data are expressed as mean \pm SEM. The Bonferroni method showed statistical differences in AAI *vs* C $P < 0.01$ in both follicular and luteal phases of the ovarian cycle.

the Bonferroni method showed that progesterone levels were significantly higher in AAI women *vs* controls in both follicular and luteal phase ($P < 0.01$).

There was no significant difference in the blood alcohol level of the AAI adolescents between follicular and luteal phases (108 ± 4 *vs* 99 ± 3.5 mg/dl, respectively).

DISCUSSION

According to the present results, AAI raises the plasma concentrations of progesterone and allopregnanolone in adolescent females during both the luteal and follicular

phases of their ovarian cycle. These findings are consistent with a previous report that alcohol injection increased plasma concentrations of allopregnanolone in rats (Barbaccia *et al*, 1999). Although the effects of ethanol on the hepatic metabolism of steroids should be taken into account, our data may indicate that alcohol ingestion promotes neurosteroidogenesis in peripheral organs. A previous study reported that exogenously administered allopregnanolone attenuated the stress-induced release of corticotropin-releasing factor (CRF), adrenocorticotropin hormone (ACTH), and corticosterone and blunted the anxiogenic effect of CRF. Our group earlier reported that AAI produces a high increase in pituitary–adrenal axis hormones in female adolescents (Frias *et al*, 2000a). Circulating allopregnanolone is increased in humans and rats after CRF and ACTH administration (Genazzani *et al*, 1998; Torres *et al*, 2001). Allopregnanolone may therefore represent a novel feedback of the hypothalamic–pituitary–adrenal axis, as previously proposed (Purdy *et al*, 1991).

The ovary and adrenal cortex are sources of circulating allopregnanolone. Although we could not know the contribution of each source, we observed an increase in serum allopregnanolone in our AAI females in both follicular and luteal phase. Earlier reports demonstrated that circulating allopregnanolone crosses the blood–brain barrier (Morrow *et al*, 2001b) and may exert its anxiolytic, analgesic, anticonflictive, and rewarding effects in the CNS through allosteric modulation of GABA_A-receptors.

The increase in allopregnanolone in response to AAI in female adolescents may have major biological repercussions. Allopregnanolone may increase the anxiolytic or rewarding effects of this socially permitted drug either directly, or by increasing the sensitivity of GABA receptors to alcohol.

ACKNOWLEDGMENTS

We thank Roussel-Uclaf for their generous gift of allopregnanolone reagent kits. We thank R Davies for revising the English text, and M Quintana and R Arcas for their technical assistance. The authors are indebted to the Clinico University and Trauma Hospitals of Granada for their help. This work was funded in part by DGICYT PM97-0177 and by the Andalusia Regional Government through its 'Endocrinology and Metabolism' research project.

REFERENCES

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

- Barbaccia ML, Affricano D, Trabucchi M, Purdy RH, Colombo G, Agabio R *et al* (1999). Ethanol markedly increases 'GABAergic' neurosteroids in alcohol-preferring rats. *Eur J Pharmacol* 384: R1–R2.
- Corpechot C, Young J, Calvel M, Wehrey C, Veeeltz N, Touyer G *et al* (1993). Neurosteroids 3 α Hydroxy-5 α Pregnan-20-one and its precursors in the brain, plasma and steroidogenic gland of male and female rats. *Endocrinology* 133: 1003–1009.
- Finn DA, Phillip TJ, Okorn DM, Chester JA, Cunningham CL (1997). Rewarding effects of the neuroactive steroid 3 α -hydroxy-5 α -pregnan-20-one in mice. *Pharmacol Biochem Behav* 56: 261–264.
- Frias J, Rodriguez R, Torres JM, Ruiz E, Ortega E (2000a). Effects of acute alcohol intoxication on pituitary–gonadal axis, pituitary–adrenal axis hormones β -endorphin and prolactin in human adolescents of both sexes. *Life Sci* 67: 1081–1086.
- Frias J, Torres JM, Miranda MT, Ruiz E, Ortega E (2002). Effects of acute alcohol intoxication on pituitary–gonadal axis, pituitary–adrenal axis hormones β -endorphin and prolactin in human adults of both sexes. *Alcohol Alcoholism* 37/2: 169–173.
- Frias J, Torres JM, Rodriguez R, Ruiz E, Ortega E (2000b). Effects of acute alcohol intoxication on growth axis in human adolescents of both sexes. *Life Sci* 67: 2691–2697.
- Genazzani AD, Luisi M, Malavasi B, Stucchi C, Stefano L, Casarosa E *et al* (2002). Pulsatile secretory characteristics of allopregnanolone, a neuroactive steroids, during the menstrual cycle and in amenorrheic subjects. *Eur J Endocrinol* 146: 347–356.
- Genazzani AR, Petraglia F, Bernardi F, Casarosa E, Salvestrone C, Tonetti A *et al* (1998). Circulating levels of allopregnanolone in humans: gender, age and endocrine influences. *J Clin Endocr Metab* 83: 2099–2103.
- Grobin AC, Matthees DB, Devaud LL, Morrow AL (1998). The role of GABA_A receptors in the acute and chronic effects of ethanol. *Psychopharmacology* 139: 2–19.
- Lambert JJ, Belelli D, Hill-Venning C, Peters JA (1995). Neurosteroids and GABA receptors function. *Trend Pharmacol Sci* 16: 295–303.
- Morrow AL, VanDoren MJ, Fleming R, Penland S (2001b). Ethanol and neurosteroid interactions in the brain. *Int Rev Neurobiol* 46: 349–377.
- Morrow AL, VanDoren MJ, Penland SN, Matthew DB (2001a). The role of GABAergic neuroactive steroids in ethanol action, tolerance and dependence. *Brain Res Brain Res Rev* 37: 98–109.
- Purdy RH, Morrow L, Moor PH, Paul SM (1991). Stress-induced elevation of aminobutyric acid type A receptor active steroids in the brain. *Proc Natl Acad Sci USA* 88: 4553–4557.
- Rapkin AJ, Morgan M, Goldman L, Brann DW, Simone D, Mahesh VB (1997). Progesterone Metabolite allopregnanolone in women with premenstrual syndrome. *Obstet Gynecol* 90: 709–714.
- 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. *Alcoholism* 18: 726–733.
- Torres JM, Ruiz E, Ortega E (2001). Effects of CRH and ACTH administration on plasma and brain neurosteroid levels. *Neurochem Res* 26: 555–558.