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Effects of estradiol valerate on voluntary alcohol consumption, β-endorphin content and neuronal population in hypothalamic arcuate nucleus

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Abstract

The main goal of the present experiment was to study the voluntary consumption of alcohol before and after a single injection of estradiol valerate (EV); another goal was to assess β-endorphin (β-EP) neurons and β-EP peptide in hypothalamic arcuate nucleus 10 weeks after the injection of EV.

Wistar female rats were injected either with a single 2.0 mg/rat injection of EV or with 0.2 ml of corn oil/rat (vehicle group). Two weeks before the injection and 10 weeks after it, every other day both groups were exposed to a free-choice alcohol drinking procedure. In weeks 4 and 5, the post-injection-consumption of alcohol was higher in the EV group than the vehicle group. In the EV group, food intake decreased and coincided with body weight lost in week 1 of post-EV injection. EV treated females showed significantly lower number of β-EP neurons than control group (reduction of 51.22%); however, β-EP content was similar in both groups, and they did not differ in the number of TSH and LHRH neurons. The present results suggest a positive relationship between high alcohol consumption and possible initial deficiency of β-endorphin content. The transient increase in alcohol consumption suggests a possible compensatory secretory effect of the surviving β-endorphinergic neurons, particularly when they are chronically stimulated with alcohol.

Keywords: Alcohol; β-endorphins; Ethanol; Estrogens; Estradiol valerate; Cytotoxicity; Alcohol abuse; Arcuate nucleus

1. Introduction

It has been shown that alcohol intake increases the levels of endogenous β-endorphin (β-EP) (De Waele and Gianoulakis, 1994; Gianoulakis, 1990, 1998). This finding is important because β-EP neurons play an important role in the rewarding effects of alcohol (Gianoulakis, 1996; Gianoulakis et al., 1996). β-endorphin levels, however, are reduced in alcoholic subjects compared with their controls (Genazzani et al., 1982), generating the hypothesis that a β-EP deficiency is intimately related to alcohol abuse. In addition, β-EP heterocigotous knock-out mice, which show decreased β-EP expression, consume more alcohol in comparison to wild-type mice (Grisel et al., 1999), supporting the possible mediation of alcohol consumption by the β-EP system activity.

On the other hand, the administration of a single injection of 2 mg of estradiol valerate (EV) produces a significant reduction of β-endorphin neurons in the hypothalamic arcuate nucleus of normally cycling female rats (Brawer et al., 1993; Desjardins et al., 1993). This neuronal cytotoxicity is accompanied of a reduction in the hypothalamic β-EP levels (Brawer et al., 1993; Desjardins et al., 1990, 1992, 1993), but the reduction of this peptide is not consistently supported by data (e.g., Reid et al., 2002, 2003; Marinelli et al., 2003). To explore the relationship between opioid system and alcohol intake, Reid et al. (2002) conducted a series of experiments assessing the effect of estradiol valerate on alcohol consumption. They found that when the rats were continuously exposed to a sweetened alcohol solution, before and after EV injection, the consumption of alcohol was significantly reduced by the EV injection. In contrast, when the rats were exposed to alcohol one month after the EV injection, the consumption of alcohol increased significantly with respect to

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that showed by the placebo group. In another study, it was supported that exposing rats for the first time to alcohol 15 days after the EV treatment is a period sufficiently enough for rats to show an increasing intake of a sweetened solution of alcohol (Reid et al., 2003). Consistently with Reid's et al. results, Marinelli et al. (2003) reported that the consumption of alcohol significantly increased when the rats were exposed to alcohol 9 weeks after the EV injection (Although, in this study a sweetened solution of alcohol was not used).

It follows that to use a model of cytotoxicity on β-EP neurons by EV, at least two aspects need to be considered: 1) the effect of the estrogens released from the EV injection on alcohol consumption, and 2) the time course of the cytotoxicity process involving several central and peripheral physiological changes culminating with the lost of β-EP neurons (Brawer et al., 1978, 1980, 1986, 1993; Desjardins et al., 1990, 1993; Lara et al., 1993; Barria et al., 1993; Dissen et al., 2000; Schipper et al., 1990, 1991). With respect to the first aspect, it has been shown that the daily administration of estradiol produces an initial decrease in the consumption of alcohol in female (e.g., Sandberg and Stewart, 1982; Sandberg et al., 1982) and in male rats (Juárez et al., 2002, 2005); afterward, a return to the base line levels of alcohol intake is observed two weeks later (Sandberg and Stewart, 1982; Sandberg et al., 1982) or a significant increase in alcohol intake is found when the animals are exposed to alcohol one week after a treatment with estrogens (Juárez et al., 2005).

Considering that a single EV injection releases estrogens for more than 12 days, but probably less than 20 (Reid et al., 2003), it may be possible that the consumption of alcohol would be affected during this period of the estrogen action. At the same time, the physiological changes that will promote the cytotoxicity process on arcuate hypothalamic nucleus are established during this period of estrogen releasing after the EV injection. Although this neuronal damage is evident two months after the administration of EV, the central changes promoting the cytotoxicity process on arcuate hypothalamic nucleus are detectable as early as one month after the EV injection (Brawer et al., 1978).

Thus, based on the evidence that alcohol consumption can be differentially affected by the time course of an estrogen treatment (Juárez et al., 2005), and that changes in the opioid system can affect the consumption of alcohol (Gianoulakis, 2001, 2004), the present study assessed the voluntary consumption of alcohol before a single injection of EV and 10 weeks following it, when the cytotoxic process occurs. In addition, at the end of the study β-EP neurons and β-EP peptide content in arcuate nucleus were estimated.

At the present time, however, a difference in the arcuate nucleus β-EP content between female rats treated with EV and their control females is controversial. Some studies support a significant difference between the two groups when rats are not exposed to ethanol (Desjardins et al., 1990, 1992). But others maintain that such a difference does not exist in ethanol naïve rats (Marinelli et al., 2003), and ethanol exposed animals (Reid et al., 2002; Marinelli et al., 2003). Because in these studies the animals were sacrificed 24 h after being exposed to alcohol, and considering that alcohol produces a release of β-endorphins, the chronic and residual effects of a recent consumption of alcohol may affect the hypothalamic levels of this peptide. Thus, to minimize the possible effects of the residual levels of alcohol on β-endorphin content, in the present study the rats were sacrificed 6 days after the consumption of alcohol was interrupted.

2. Methods

2.1. Subjects

Twenty two Wistar female rats, obtained from a colony bred in the Institute of Neurociencias (Universidad de Guadalajara), participated as subjects. Animals were maintained on a 12–12 h light–dark cycle, lights on at 8:00 AM, in a room with a constant temperature (22°–24°) where food and water were continuously available. When the rats were 78 days of age, they were housed in individual cages.

2.2. Induction to voluntary consumption of alcohol

At 80 days of age, all rats were exposed to a period of alcohol induction according to a free-choice drinking procedure where two bottles were placed in each cage, one of them containing tap water, and the other one containing a solution of water plus ethanol (MERCK, Darmstadt, Germany). Initially, the concentration of alcohol was set at 2% vol/vol; then, it was increased in 2% steps every other day up to a concentration of 10% vol/vol. After the alcohol induction period was completed, the 10% alcohol concentration was maintained and subjects were exposed to the free-choice procedure every other day for 24 h. The following two weeks were used to establish the base line of alcohol and food consumption before the hormonal treatment. The days that alcohol was not offered, only one bottle containing water was presented and when alcohol was offered, the position of alcohol–water bottles was changed daily to avoid place preference. Body weight, consumption of alcohol, food, and water were measured after each 24 h when alcohol was available. Immediately after these measures were taken, alcohol and water bottles were refilled with fresh liquid, and a fixed measure of food was replaced.

The rats were hierarchically ordered considering their alcohol intake base line and alternately assigned to two groups of 11 females each. This procedure was done with the purpose of having similar values of alcohol intake in each group before the hormonal treatment.

2.3. Hormonal and vehicle treatment

Forty-eight hours after the alcohol intake base line period was concluded, one group of 11 females was injected with 2.0 mg im of estradiol valerate (Primogyn Depot, SHERING) per rat as unique dose. A second group of 11 females was injected with 0.2 ml im of corn oil/rat as unique dose. Twenty-four hours after the injection, the free-choice alcohol drinking procedure reinstated for the following 10 weeks in both groups. Body weight and consumption of alcohol, water and food were measured every 24 h in the days of alcohol exposure.

2.4. Immunocytochemistry

Ten rats (five per group) were sacrificed 6 days after alcohol was interrupted in week 10 post-EV injection. The animals were deeply anesthetized and perfused transcardially with 100 ml 0.1 m PBS (pH 7.4) and 300 ml PBS containing 4% paraformaldehyde. Brains were removed and immersed for 24 h at 4 °C in the same fixative solution, and cryoprotected overnight at 4 °C in PBS containing 30% sucrose. The brain tissue was then embedded in Tissue-Tek compound (OCT, Miles, Inc., Elkhart, IN) and frozen. Consecutive sections (25 μm thick) prepared on cryostat were mounted onto gelatin-coated slides for light microscopic immunocytochemistry. Endogenous peroxidase activity was inhibited in hydrated section with 20% methanol and 1% H_2O_2 for 20 min. Tissue was rinsed in 0.1 M PBS containing 0.1% bovine albumin, and 0.1% triton X-100 (pH 7.4). This solution was used for all incubations and intermediate washes. For β-end immunolabeling, tissue was incubated overnight at 4 °C with rabbit anti-rat β-endorphin (Peninsula Laboratories, Belmont, CA) diluted 1:1000, and then incubated for 2 h at 20 °C with a biotinylated goat anti-rabbit IgG (Vector) diluted 1:200. For TH neurons identification, a polyclonal rabbit anti-rat TH (Santa Cruz Biotechnology, Inc.) diluted 1:500 was used, followed by the same linking antibody. Both reactions were incubated in avidin–biotin complex (Vector) and revealed with DAB. For GnRH neurons, tissue was incubated overnight at 4 °C with mouse anti-GnRH (Chemicon international, Inc.) diluted 1:100, and then incubated for 2 h at 20 °C with a biotinylated horse anti-mouse IgG (Vector) diluted 1:200. The reaction was incubated in avidin–biotin complex (Vector) and revealed with 3′3 DAB.

The sequence of 25 μm sections was used alternately for analyses of β-endorphin neurons (β-EP), Gonadotropin-releasing hormone (GnRH) and Tyrosine hydroxylase (TH). Five windows of 364 μm^2 in each of eight sections per rat were used to photograph the β-EP neurons coming into focus and a double blind quantification of neurons was made. Therefore a total of 200 windows of 364 μm^2 each were used to quantify β-EP neurons. The same procedure was used to quantify GnRH and TH neurons, but in this case only 100 windows of 364 μm^2 each were used in each neuronal lineage.

2.5. Enzymatic immunoassay of β-endorphins

Ten rats (five per group) were sacrificed 6 days after alcohol was interrupted in week 10 post-EV injection. The animals were deeply anesthetized and perfused transcardially with 100 ml 0.1 m PBS (pH 7.4), their brains were quickly removed and the left hypothalamic arcuate nucleus was dissected. Tissues were immediately placed in 500 ml of ice-cold 0.2 N HCl, and then boiled for 5 min. Subsequently tissues were stored at −70 °C until samples were thawed in ice water and sonicated. Aliquots were reserved for protein estimation using the Bradford procedure (Bradford, 1976). The remaining homogenized mixture was centrifuged (14,000 rpm for 7 min at 48 °C) and the supernatants were transferred to clean sample tubes and stored at −70 °C in preparation for the enzymatic immunoassay (EIA). The competitive EIA uses a rat polyclonal antibody (commercial kit S-1170, Peninsula Laboratories, Belmont, CA), which is highly specific for rat β-EP. Assay sensitivity is 0.03 ng/ml, and the results are expressed as ng β-EP/mg protein.

2.6. Statistical analyses

Alcohol, water, food intake, and body weight were analyzed by a two way mixed ANOVA (treatment (EV and vehicle) × weeks), while Tukey's test for post hoc comparisons was used. Student's *t* test was performed to analyze separately the number of β-EP, TSH and GnRh neurons. β-EP peptide content in the hypothalamic arcuate nucleus was also analyzed by a Student's *t* test. In all cases, the level of significance was set at $p \leq 0.05$.

Animal care and use, as well as, procedures involving animals were performed in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

3. Results

3.1. Alcohol intake

Alcohol consumption was calculated in g/kg and analyzed using the average per week per rat (i.e. the mean of each block of three days starting off the EV injection). The alcohol intake base line (BL) was analyzed based on the average of the six days (two weeks) of this period. Two way mixed ANOVA (treatment × weeks) showed differences between weeks ($F(10,200)=6.78$, $p<0.0001$) regardless of treatment. Alcohol intake tended to increase with time. Post hoc analyses showed that BL intake was lower than that obtained in weeks 4, 5, 7, 8, 9 and 10. Intake at

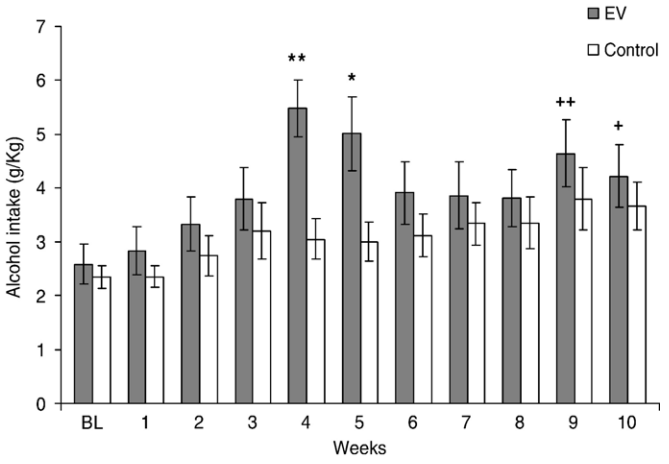

Fig. 1. Alcohol consumption (g/kg) in the base line (BL) and during the ten weeks after the estradiol valerate (EV) and vehicle (Control) injection. Bars show the mean± SE. **BL and weeks 1–10 of the control group ($p=0.01$); BL and weeks 1–2 ($p=0.01$), 3 and 8 ($p=0.05$) of the EV group. *BL and weeks 1–6 ($p=0.01$), 7 and 8 ($p=0.05$) of the control group; BL and weeks 1–2 ($p=0.05$) of the EV group. $^{++}$BL and weeks 1–2 of the control group ($p=0.01$); BL ($p=0.01$) and week 1 ($p=0.05$) of the EV group. $^{+}$BL and week 1 of the control group ($p=0.01$).

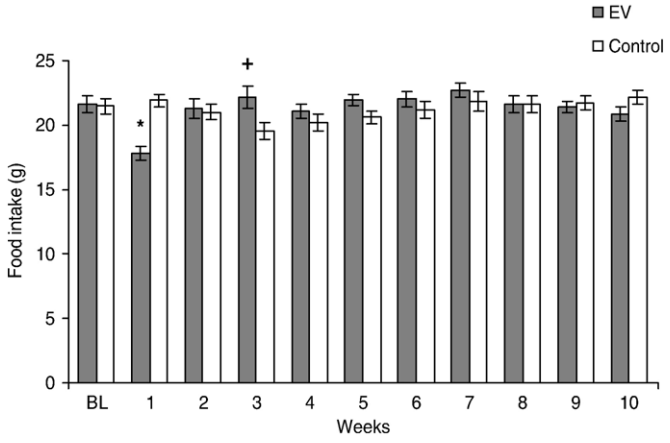

Fig. 2. Food intake (g) in the base line (BL) and during the ten weeks after the estradiol valerate (EV) and vehicle (Control) injection. Bars show the mean ± SE. *BL and weeks 1–2, 5–10 ($p=0.01$) and week 4 ($p=0.05$) of the control group; BL and weeks 2–10 of the EV group ($p=0.01$). $^{+}$Week 3 of the control group ($p=0.05$).

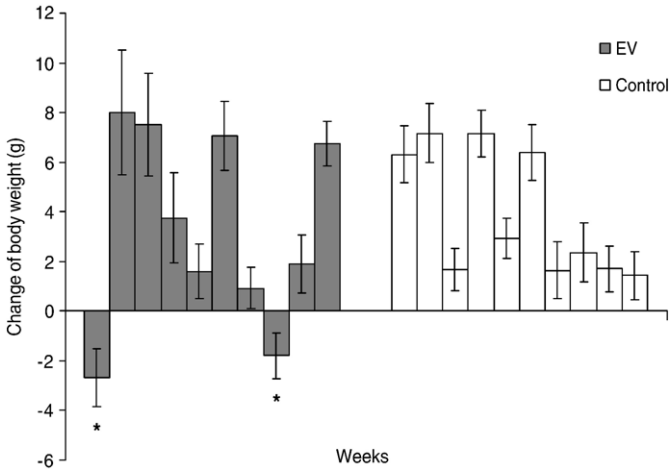

Fig. 3. Change in body weight (g) was calculated and represented by the differences between the body weight in each week and the body weight of its immediate previous week. Bars show the mean ± SE. *Weeks 1, 2, 4 and 6 of the control group ($p=0.01$); weeks 2, 3, 6 and 10 of the EV group ($p=0.01$).

week 1 was lower than intake at weeks 4, 5, 9 and 10; and intake at week 2 was lower than intake at weeks 4 and 9.

The interaction between factors (treatment × weeks) was also significant ($F(10,200)=2.21$, $p=0.018$). Post hoc analyses indicated that in weeks 4 and 5 alcohol consumption in the EV group was higher than that obtained in any week of the control group, including the base line. The only non-significant differences occurred between week 5 of the EV group, and weeks 9 and 10 of the control group (Fig. 1). Additionally, intake at week 10 of the EV group was higher than that showed by the control group in BL and week 1. When intragroup comparisons were performed, alcohol intake by the EV group during weeks 4 and 5 was significantly higher than that obtained during the BL period and weeks 1 and 2 following the EV treatment. Moreover, the EV group alcohol intake during week 4 was significantly higher than that of weeks 3 and 8; also, intake at week 9 was higher than that obtained during BL and week 1 (Fig. 1). In the control group, there were no significant differences in alcohol intake between weeks.

3.2. Water intake

Two way mixed ANOVA (treatment × weeks) was used to analyze water intake; there were no significant differences between treatments. However, a main effect was observed for the weeks factor ($F(10,200)=3.72$, $p=0.0001$). Post hoc analyses indicated higher water intake at week 2 compared to that obtained at weeks 4, 9, and 10 post-injection, regardless of treatment. Additionally, water intake during BL was higher than that at weeks 4 and 9 post-injection.

3.3. Food intake

Mean food intake during the three days of alcohol exposure was calculated in grams, per week/rat. A two way mixed ANOVA (treatment × weeks) showed differences between weeks ($F(10,200)=3.56$, $p=0.0002$) regardless of treatment. For week 1, post hoc analyses showed lower food intake than that for BL and weeks 6 to 10 post-injection. Food intake was lower during week 4 than during week 7. For the EV group, the interaction between treatment and weeks was also significant ($F(10,200)=6.5$, $p<0.0001$), indicating lower food intake during week 1 post-EV injection compared to all the previous

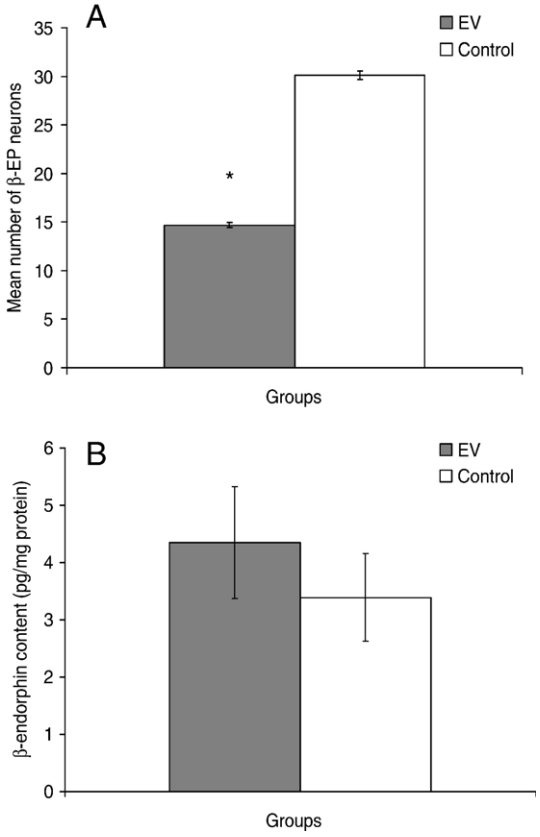

Fig. 4. Mean number of β-endorphin (β-EP) neurons (A) and β-EP content in hypothalamic arcuate nucleus (B). Bars show the mean ± SE of estradiol valerate (EV) and vehicle (control) injected females. *Control group ($p<0.0001$).

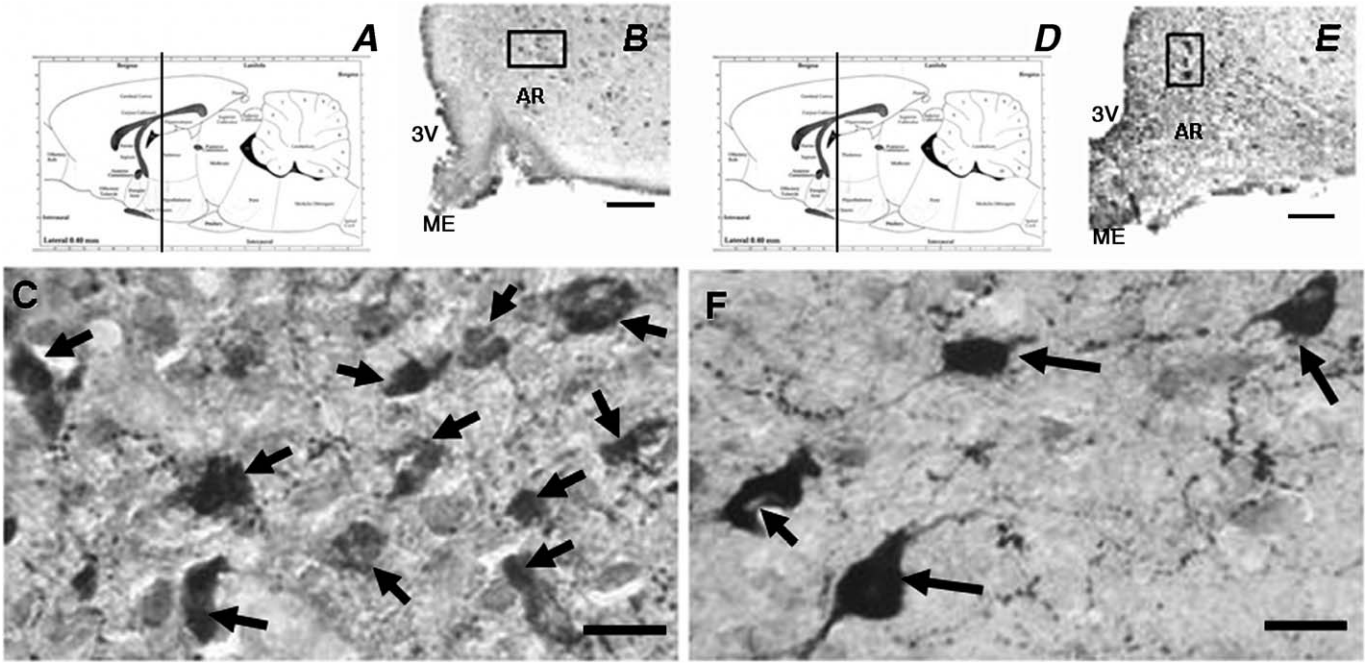

Fig. 5. Immunocytochemistry localization of β-EP neurons in the arcuate nucleus. Vehicle group: A) Scheme of stereotaxic coordinates; B) Low magnification of arcuate nucleus; C) High magnification of panel B. Group EV: D) Scheme of stereotaxic coordinates. E) Low magnification of arcuate nucleus. F) High magnification of panel E. Immunoreactivity β-EP neurons are indicated with arrows in C) and F). B and E: Bar = 100 μm. C and F: Bar = 20 μm. AR: arcuate nucleus, ME: median eminence, 3V: third ventricle.

and subsequent weeks of both groups (except week 3 of the control group [see Fig. 2]). In contrast, for the control group no significant differences in food intake were observed between weeks. For week 3, food intake was higher for the EV group than for the control group.

3.4. Body weight

Considering that body weight increases over time, difference between the body weight in each week and the body weight of its immediately previous week was calculated per rat and analyzed by a two way mixed ANOVA (treatment × week). Differences in the week main effect were significant ($F(9,180) = 7.02$, $p < 0.0001$), indicating that body weight increased each week compared to the immediately previous week, regardless of treatment. The interaction of treatment × week was also significant ($F(9,180) = 5.5$, $p < 0.0001$), indicating a loss of body weight at weeks 1 and 8 after the EV injection. Additionally, for the EV treated group the values of body weight in weeks 1 and 8 were significantly lower than those of weeks 2, 3, 6, and 10. In turn, the EV-group-body weight values in weeks 1 and 8 were lower than those of weeks 1, 2, 4 and 6 of the control group (Fig. 3). In the vehicle group, all values of body weight indicated gains, and differences between weeks were not significant in this group.

3.5. β-EP neurons and β-EP content

To analyze the number of β-EP neurons and the β-EP content in the hypothalamic arcuate nucleus, a Student's *t* test was performed. Subjects treated with EV showed significantly ($t(198) = 31.132$, $p < 0.0001$) lower number of β-EP neurons than females in the vehicle group (Fig. 4A). Fig. 5 shows an example of the proportion of immunoreactive β-EP neurons in each group. Nevertheless a 51.22% reduction in β-EP neurons was obtained in the EV group in respect of the vehicle group; there were no significant differences in β-EP content between groups (Fig. 4B).

3.6. TH and GnRH neurons

A Student's *t* test was performed to analyze TH and GnRH neurons. There were not differences between EV and vehicle-treated subjects in GnRH or in TH number of neurons (Fig. 6).

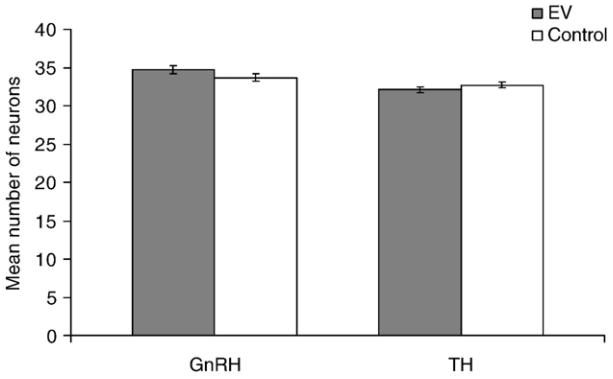

Fig. 6. Mean number of GnRH and TH neurons in hypothalamic arcuate nucleus. Bars show the mean ± SE of estradiol valerate (EV) and vehicle (control) injected females.

4. Discussion

Compared with females treated with vehicle, female rats treated with a single dose of estradiol valerate showed a significant increase in alcohol consumption during weeks 4 and 5 after hormone administration. This finding is consistent with the idea that alcohol consumption increases in the period when, as suggested by Reid et al. (2003), the exogenous hormone has completely disappeared from the organism. Although in previous studies the effects of the EV on alcohol consumption have been assessed (e.g., Reid et al., 2002, 2003; Marinelli et al., 2003), discrepancies regarding the observed effects and methodological procedures employed have been described. For example, Reid et al. (2002) assessed several periods of alcohol exposure before and after a single dose of EV and found that when female rats were exposed to alcohol before and after the EV injection, alcohol intake was reduced significantly with respect to the control group. Although, in their study the time course of alcohol exposure was similar to that employed in the present study, the outcomes are different. The discrepancies, however, may be due to methodological differences; in the study of Reid et al. (2002) alcohol was offered for 2 h at day and saccharin was added to an alcohol solution while in the present study, alcohol was available 24 h at day and sweetener was not added to the alcoholic solution. It is well known that sweetness works as a reinforcer in maintaining instrumental behavior, and that when added to water rats drink significantly more (Juárez et al., 2002). The importance of using a sweetened solution becomes evident when rats previously treated with EV are exposed to an alcoholic solution that initially contained 0.25% saccharin and then it is reduced to 0% (Reid et al., 2003). The present experiment showed reduction in alcohol consumption that paralleled reduction in sweetener concentration, suggesting that alcohol intake after EV administration may be affected by a stimulus with intrinsic reinforcing properties.

In another experiment, Reid et al. (2002) found that 1 month after a single dose of EV, females exposed to alcohol significantly increased consumption with respect to both the placebo group and a group exposed to alcohol two months after the EV injection. This finding is interesting because, although the alcohol exposure strategy was different in Reid's study from that of the present study, 4 weeks after the EV injection alcohol intake was similar in both studies. More recently, Reid et al. (2003) reported that exposing rats for the first time to alcohol 15 days after the EV injection is sufficient to observe significant increments in the alcohol consumption compared to the placebo group. Reid et al. (2002) did not find significant differences in alcohol consumption between females treated with EV 2 months before having a chance to drink alcohol and a placebo group; however, Marinelli et al. (2003) describe that when the first exposure to alcohol occurs 9 weeks after a single dose of EV, alcohol intake is increased in the weeks 10 and 11 after the EV treatment as much as in Wistar as in Lewis strain female rats; however, in this last study, sweet was not added to the alcoholic beverage.

The first month after EV administration seems to be critical, because at the end of the fourth week it is possible to observe several changes in the astrocytes in the hypothalamic arcuate nucleus. In this sense, dense lipid pools and large bundles of glial filaments are observed in rats sacrificed 1 month after the EV injection (Brawer et al., 1978, 1980), and there is evidence that this cytotoxic process selectively affects β-endorphinergic neurons (Desjardins et al., 1992, 1993; Brawer et al., 1993). Considering this, the results of the present study concerning the increase in alcohol consumption in weeks 4 and 5 after the EV injection can be explained as follows: the deficit in the number of secretory β-EP neurons detected after 1 month of the EV treatment implies a deficit in the content of the β-endorphin peptide, and this deficit may produce opioid receptor up-regulation (Desjardins et al., 1990). If we accept that alcohol intake stimulates the β-EP secretion, alcohol would be more rewarding under conditions of higher opioid receptor availability than under normal conditions. This explanation is consistent with a mechanism associated to the β-endorphins deficit observed in chronic alcoholics (Genazzani et al., 1982), suggesting that high volume in alcohol intake involves neurophysiologic mechanisms related to opioid peptides and its receptors (Gianoulakis, 1996, 2001, 2004; Genazzani et al., 1982). This rationale could also explain the increase in alcohol intake one month or 10 weeks after the EV injection reported by Reid et al. (2002) and Marinelli et al. (2003).

The question raised in the present study of why alcohol consumption increases significantly in weeks 4 and 5, and then declines starting week 6 after the EV injection, remain unanswered. A possible explanation is that the deficit of β-endorphins produced by the decrease in the number of β-endorphinergic neurons is not maintained permanently, and the survived neurons increase the synthesis of this peptide as a compensatory mechanism with the consequent opioid receptor down-regulation. In this condition the lower availability of receptors would reduce the rewarding effects of alcohol intake and its consumption would decrease again. This speculation would be supported by the absence of significant differences in the β-endorphin content between EV-treated and control females when the animals were sacrificed 10 weeks after treatment in the present study. This absence of differences in the hypothalamic β-EP content between EV-treated and control subjects, has been described in rats with voluntary alcohol consumption and in rats without alcohol exposure (Marinelli et al., 2003). However, after 8 weeks of EV a significant decrease in β-EP levels was obtained between females treated with EV and controls no exposed to alcohol (Desjardins et al., 1990, 1992, 1993).

Since in the majority of studies using alcohol exposure the animals are sacrificed 24 h after alcohol is interrupted, and considering that alcohol produces release of β-endorphins; in the present study the animals were sacrificed 6 days after the alcohol was interrupted with the purpose to minimize the possible effects of the recent and residual levels of alcohol on β-EP content, which could influence the hypothalamic content of this peptide. However, the differences in β-EP levels between EV and vehicle group were not significant, coinciding with the findings of Marinelli et al. (2003). Moreover, this author found a non-significant increase in β-EP levels in the EV-treated group with respect to the control group as observed in the present study.

The specificity of the cytotoxicity on β-endorphinergic neurons by EV treatment (Desjardins et al., 1993) was supported

in the present study, since the density of this cellular lineage was significantly lower in female rats treated with EV than in females treated with vehicle. By contrast, the differences in the cellular density of TSH and LHRH neurons were not significant between these groups.

Food intake decrease in the first week after the EV injection coincided with a decrease in body weight compared to the previous week. This anorexic effect of the EV treatment has been described previously in several studies using estradiol treatment in females (Varma et al., 1999; Butera et al., 1996, 1990; Dagnault et al., 1993; Donohoe et al., 1984; Donohoe and Stevens, 1982; Sandberg et al., 1982) and in males (Juárez et al., 2005). This decrease in food intake seems to be mediated by an estrogen action on the hypothalamus (Butera et al., 1990, 1996; Donohoe and Stevens, 1982) possibly involving an increase in the colecistokinine satiety effects (Butera et al., 1996). This anorexic effect was reverted in the second week after injection of the EV treatment, suggesting that this anorexic effect is exclusive of the initial period post-estrogen treatment and later the food regulation system responds in defense of the body weight set point. This recovery in food intake coincides with the recovery in the body weight in the second week after the EV injection. The decrease in body weight in week 8 coincides with a decrease in food intake and possibly is related to chronic alcohol consumption; however, we do not have any biological argument supporting this interpretation.

The present results suggest a positive relationship between high alcohol consumption and possible initial deficiency of β-EP content produced by the cytotoxic effect of a single injection of estradiol valerate on β-endorphinergic neurons. At the same time, the present findings suggest that the transient increase of alcohol consumption possibly indicates a compensatory secretory activity of the surviving β-endorphinergic neurons, particularly when they are chronically stimulated with alcohol; however, the support of this rationale would require future investigations.

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