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Effects of estradiol treatment on voluntary and forced alcohol consumption in male rats

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Abstract

Estrogens have been related to alcohol as a dependent variable, but scarcely as a causal variable, that affects the alcohol consumption. The scope of the present work was to study the effect of estrogens on both the amount and the pattern of alcohol consumption. Male Wistar rats were individually exposed to forced alcohol consumption (FAC) and voluntary alcohol consumption (VAC) in each of the following four periods: precastration (PreC), postcastration (PosC) or post-sham castration, estradiol (E) treatment (5 μg of estradiol benzoate/day/rat) and postestradiol (PosE). Estrogenic treatment reduced significantly the alcohol consumption with respect to the PreC and PosE periods in castrated (C) males during VAC. E treatment showed the lowest value of alcohol intake in FAC, but differences were significant only with respect to PreC regardless of the male gonadal condition. E treatment decreased food intake regardless of the male gonadal condition in both FAC and VAC. Castration and E treatment modified differentially the patterns of alcohol consumption depending on the volitive characteristics of alcohol intake. Castration reduced the size of the licking rates without affecting the number of drinking bouts in FAC. This pattern was maintained in the E and PosE periods of C males. Castration did not affect the pattern of alcohol consumption in VAC, but estrogen reduced both the bout size and the number of bouts during the day, which gave an additional support to the inhibitory effect of estrogens on VAC. Results are discussed in terms of a possible inhibitory action of estrogens on the opioid system, which possibly reduces the rewarding properties of alcohol.

Keywords: Estrogens; Alcohol consumption; Patterns of alcohol intake; Estradiol; Male rats

1. Introduction

There is evidence that sex differences in alcohol consumption emerge after puberty (Lancaster et al., 1996; Rachamin et al., 1980), which seems to be due to an activational action of hormones after this period (Mishra et al., 1989; Rachamin et al., 1980; Sutker et al., 1987; Van Thiel et al., 1988). It has been described that castration produces an increase in the alcohol metabolic rate and in the alcohol dehydrogenase (ADH) activity and that subsequent hormonal restitution with testosterone returns these variables to precastration (PreC) values in the rat (Rachamin et al., 1980). This action of testosterone seems to be mediated at least partially by its metabolite dihydrotestosterone (DHT), which reduces the enzymatic action of the ADH and therefore delays the alcohol clearance (Mezey et al., 1986, 1998; Vaubourdolle et al., 1991). There is evidence that DHT treatment reduces the voluntary alcohol consumption (VAC) possibly as consequence of its action upon alcohol metabolism (Almeida et al., 1998). It is well known that besides the DHT, estrogen is a very important metabolite of testosterone in males, however, estrogens affecting alcohol consumption in males have been scarcely studied. On the contrary, alcohol affecting the estrogenic levels has been extensively studied. Therefore, it has been described an increase in estrogenic levels after alcohol treatment, in women (Mendelson et al., 1988), in men (Couwenbergs, 1988) and in male rats (Esquifino et al., 1989), and a positive correlation between estrogens and alcohol intake in women has been documented (Gavaler and Van Thiel, 1992; Gavaler et al., 1991; Muti et al., 1998). There is a general consensus that estrogens increase the ADH activity (Qulali and Crabb, 1992; Qulali et al., 1991; Rachamin et al., 1980; Teschke and Heymann, 1982) and the alcohol metabolic rate (Rachamin et al., 1980) in the rat, contrary to the effect described for DHT. Therefore,

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whether DHT decreases the alcohol consumption and has the opposite effect that the estrogens on the alcohol metabolism, we could suppose that the estrogens should increase the alcohol intake. The few studies in this respect describe that, at least initially, estrogen decreases VAC in ovariectomized females (Almeida et al., 1998; Sandberg and Stewart, 1982; Sandberg et al., 1982). On the other hand, the few studies that, as far as we know, has been concerned in the estrogenic effect on alcohol consumption in males show controversial results. It has been described as an increase in voluntary alcohol intake under estrogenic treatment in mice (Hilakivi, 1996) and a reduction in alcohol consumption under acute treatment with estrogens in selected rats that show high preference for alcohol (Messiha, 1981). Differences in the species, in the control groups and in the preference to alcohol in the subjects utilized could be the cause of these controversial results. On the other hand, these studies assessed only the amount of alcohol consumption. Although this measure gives valuable information about how much subjects drink, it is also valuable to know the pattern of alcohol intake, that is to say, how the consumption is distributed during the day. We have described that the amount of alcohol consumed and the pattern of alcohol intake can be modified each independently of the other (Juárez and Barrios De Tomasi, 1999). Therefore, it is possible that estrogens could affect differentially the amount and the pattern of alcohol consumption.

Taking into account that there is sufficient evidence that estrogens can play an important role in the alcohol intake, though the available data are controversial, the scope of the present work was to study the effect of the estrogen treatment on the amount and the pattern of alcohol consumption in males rats. It has been described that forced alcohol consumption (FAC), in contrast with VAC, can affect differentially the alcohol intake in rats (Adams et al., 1991; Juárez and Barrios De Tomasi, 1999), therefore, estrogen treatment was assessed during forced and voluntary access to alcohol.

2. Method

Wistar male rats were obtained from a colony bred in the Institute of Neurociencias, Universidad de Guadalajara. Subjects were maintained on a 12–12-h light–dark cycle, lights on at 8:00 AM, and food pellets were available ad libitum. Temperature, feeding and light–dark cycle were maintained identical in the course of the study. Sixteen males belong to different litters were maintained in groups of four individuals each from weaning to 82 days of age and placed in individual cages at 83 days of age. At 90 days of age, these males were individually exposed to different drinking and experimental conditions, which will be described in the following sections. The general experimental design and treatment schedule is showed in the Table 1.

With the purpose of studying the patterns of alcohol consumption during the day and its possible modification during the different experimental periods, the subjects were periodically tested in cages attached to a contact lickometer system (ENV-250, MED Associates, USA). This system accurately records the rate of licking of sipper tubes attached to bottles containing liquid (Juárez and Barrios De Tomasi, 1999). The contact signal from sipper tubes (licking frequency) is sent to a computer by an interface (DIG-726, MED Associates) specially designed for these purposes. Licking frequency is accumulated and recorded each 10 min throughout the day. Therefore, 144 possible values per day and per subject could be obtained. The total intake of the available liquids and food was measured each 24 h in all the experimental conditions. Body weight was recorded peri-

Table 1
Experiment design and treatment schedule

Age in days	16 Males in individual cages	Period
83–89	Habituation to individual home cages	
90–111	FAC (four of these days in LST)	PreC1 (Days 90–100), PreC2 (Days 101–111)
112–133	VAC (four of these days in LST)	PreC1 (Days 112–122), PreC2 (Days 123–133)
134–136	Castration to eight males and sham castration to eight males	
137–146	Postsurgical recovery (exposure to water only)	
147–150	VAC (one of these days in LST)	PosC
151–154	FAC (one of these days in LST)	PosC
155–158	VAC	PosC
159–166	5 μg of EB/day/male (8 days)	
	VAC from Day 159 to Day 162 (one of these days in LST)	E
	FAC from day 163 to 166 (one of these days in LST)	E
167–174	VAC	PosE (Days 171–174)
175–178	FAC	PosE

Forced (FAC) and voluntary (VAC) alcohol consumption; lickometer system test (LST); estradiol benzoate (EB); precastration (PreC); postcastration (PosC); estradiol treatment (E) and postestradiol (PosE).

odically. Males had not any experience in the intake of any other liquid besides tap water before to start the study.

2.1. FAC in the PreC period

At 90 days of age and after 1 week of habituation to individual home cages, the 16 males were continuously exposed to a solution containing 6% ethanol (99.8%, Merck) v/v, water and 2 g of sucrose/100 ml (EtOH 6%) for 22 days. This solution was the only available liquid in the home cages. All subjects were individually tested on the lickometer system each 4–6 days for 24 h in this period. Therefore, the pattern of FAC of each male was assessed in four of these 22 days in the PreC period. The amount of alcohol intake was measured daily within the same hour.

2.2. VAC in the PreC period

After 22 days of continuous access to forced alcohol intake, one bottle containing a solution of water plus 2 g of sucrose/100 ml (vehicle) was added to each cage, therefore, free choice to EtOH 6% and vehicle was permitted continuously for 22 days. All males were individually tested on the lickometer system each 4–6 days for 24 h. Therefore, the pattern of VAC of each male was assessed four of these 22 days in this PreC condition.

2.3. Castration and sham castration

Immediately after the previous condition, eight of the 16 males were surgically castrated (C) under pentobarbital anesthesia (35 mg/kg). At the same time, the remaining eight males were sham-castrated (ShC), performing the same anesthetic and surgical procedure as that in the C group, but gonads were maintained intact in the ShC group. A period of 9–10 days of recuperation was permitted after the surgical procedure. Tap water was the only available liquid during this period.

2.4. VAC and FAC in the postcastration (PosC) period

Taking into account that the exposure of only water in the postsurgical period could influence in the consumption of the subsequent alcohol exposure, VAC was assessed after the postsurgical period and after a period of forced alcohol intake. Therefore, males were exposed to VAC for 4 days, later on, to FAC for 4 days and, finally, to VAC for 4 additional days. The analysis to assess possible differences between these two periods of VAC showed that there were no differences in the alcohol consumption between them, therefore, the two VAC periods were averaged for analysis purposes. The exposure of liquids in the forced and voluntary conditions had the same characteristics described in the PreC period. In this PosC period, the males were tested on the lickometer system in the third or fourth day of the first period of VAC and in the third or fourth day of the only period of FAC (1 day in each condition).

2.5. VAC and FAC in the estradiol (E) treatment

Immediately after the previous period, 5 μg of estradiol benzoate/subject/day was administered subcutaneously for 8 days to both groups of males (C and ShC). Both groups were exposed to VAC during the first 4 days of this treatment and later on to forced intake during the last 4 days. In a previous study using the same doses of E during the same period (8 days), we did not find differences in alcohol consumption either between the 8 days or between the average of the first 4 days with the average of the last 4 days of treatment. Considering the apparent absence of significant changes in alcohol intake during the course of the estrogenic treatment, we chose the VAC exposure followed by FAC. All males were tested on the lickometer for 24 h in the third or fourth day of each condition of alcohol consumption (voluntary and forced) in this period of E treatment.

2.6. VAC and FAC in the postestradiol (PosE) period

The same schedule of alcohol exposure used during the estrogen treatment was analyzed after the E treatment (PosE period), that is to say, 4 days of voluntary alcohol exposure and 4 days of forced alcohol exposure. Males were exposed to VAC immediately after the E treatment, but we analyzed the PosE period starting off in the fifth day after the last injection of E to permit the clearance of the exogenous hormone. As in the previous period, the males were tested in the lickometer for 24 h in each condition of alcohol consumption (voluntary and forced).

2.7. Lickometer system test

On the lickometer test, males were individually placed in the cages of the lickometer system for 24 h, starting off at 2:00 PM. The frequency of licks to drinking spouts recorded each 10 min was used to analyze the distribution (throughout the day) and the size of the drinking bouts (licking rate/10 min) in each male and in each experimental condition. Considering the changes in activity depending on the daily light–dark cycle, the hours of each day were divided and analyzed in two phases: light phase from 8:00 AM to 7:59 PM, and dark phase from 8:00 PM to 7:59 AM. We analyzed different intervals of licking frequency (21–150, 151–300, 301–450, 451–600, 601–750 and higher than 750 licks/10 min) to assess the size of each drinking bout/10 min.

2.8. Statistical analyses

The length of the PreC period as much in the forced as in the voluntary consumption (22 days each) was

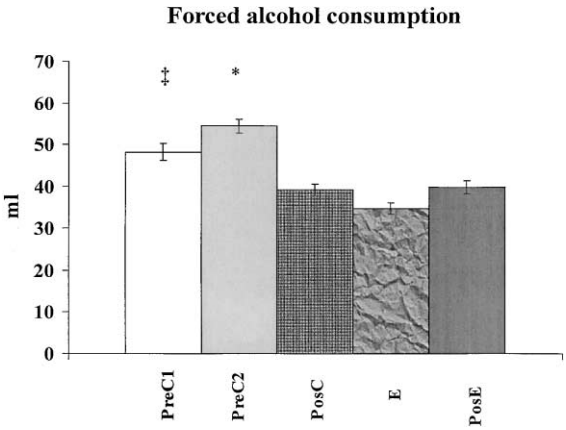

Fig. 1. AC (ml) regardless of the male gonadal condition in the different experimental periods: precastration 1 (PreC1), precastration 2 (PreC2), postcastration (PosC), estrogen treatment (E) and post-estrogen treatment (PosE). Bars show means (±S.E.M.). * Significantly higher than PreC1, PosC, E and PosE. ‡ Significantly higher than PosC, E and PosE.

selected to permit a sufficient familiarization with the taste and the conditions of liquid availability (Juárez and Barrios De Tomasi, 1999). Considering the possible occurrence of tolerance to alcohol due to the length of this period, VAC and FAC periods were divided in two periods each (11 days per period). Therefore, two periods (PreC1 and PreC2) for the forced condition and two periods (PreC1 and PreC2) for the voluntary condition were obtained and analyzed separately.

Two-way ANOVAs [Sham-Castrated × Experimental Periods (PreC1, PreC2, PosC, E and PosE)] were used to analyze the food intake and the amount of alcohol and vehicle consumption. Two-way ANOVAs [Phases (light–dark) × Experimental Periods (PreC1, PreC2, PosC, E and PosE)] were used to analyze the frequency of accesses to drinking spouts for each liquid and for each group of males separately. Two-way ANOVAs [Phases (light–dark) × Intervals of Licking Rate (21–150, 151–300, 301–450, 451–600, 601–750 and more than 750 licks/10 min] were used to analyze the distribution and the size of licking bouts for each period and for each group of males separately.

Tukey's *t* test was used to compare pairs when ANOVA main effects or interactions were significant. A *P* value of $<.05$ was considered statistically significant.

3. Results

3.1. Alcohol consumption in forced condition

Analyses of the amount of alcohol consumption in the different periods during the forced condition showed the following results. Significant differences between experimental periods [$F(4,56)=36.09$, $P<.0001$] regardless of the male gonadal condition (C or ShC) were observed. This result indicated that the alcohol consumption in the two periods previous to castration (PreC1 and PreC2) was higher than in PosC, E and PosE periods. At the same time, alcohol consumption was lower in PreC1 than in PreC2 (Fig. 1). Neither there were differences between male groups nor interaction between the analyzed factors.

3.2. Alcohol consumption in voluntary condition

Analyses of the amount of alcohol consumption during the voluntary condition showed the following results: Significant differences between periods [$F(4,56)=17.93$, $P<.0001$] regardless of the male gonadal condition were observed. This data indicated higher alcohol intake during PreC1 with respect to all the other periods and higher alcohol consumption in PreC2 than in the E period. There were no significant differences between C and ShC males regardless of the experimental periods, but interaction between these two analyzed factors was significant [$F(4,56)=3.98$, $P=.0065$]. This interaction showed that the alcohol consumption was steadily decreasing from PreC1 to PosE period in the ShC group and the analysis of differences between periods indicated higher alcohol consumption in PreC1 than in PosC, E and PosE periods and higher alcohol intake in PreC2 than in E and PosE (Fig. 2). On the other hand, the C males showed a steady decrease in the alcohol consumption from PreC1 to E treatment, but a significant recovery in the alcohol intake was observed during the PosE period (Fig. 2). The analysis of differences between periods showed that the alcohol consumption was significantly lower in the E treatment than in PreC1, PreC2 and PosE and the differences between PreC1, PreC2, PosC and PosE were not significant in this group of C males.

Analysis of the amount of vehicle consumption, when free choice to alcohol and vehicle was available, did not showed any significant difference.

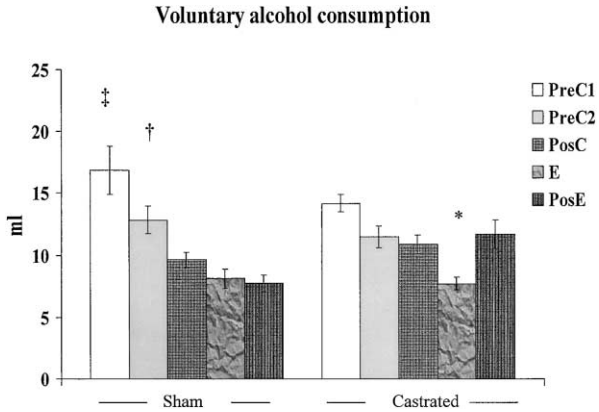

Fig. 2. VAC (ml) in ShC and C males in the different experimental periods: precastration 1 (PreC1), precastration 2 (PreC2), postcastration (PosC), estrogen treatment (E) and post-estrogen treatment (PosE). Bars show means (±S.E.M.). ‡ Significantly higher than PosC, E and PosE. * Significantly lower than PreC1, PreC2 and PosE. † Significantly higher than E and PosE.

3.3. Food intake during FAC and VAC

Analyses of food intake (two-way ANOVA Sham-Castrated × Experimental Periods) showed differences between periods when alcohol was the only available liquid [$F(4,56) = 7.77$, $P = .0001$] and when free-choice to alcohol and vehicle was available [$F(4,56) = 11.38$, $P < .0001$]. This data indicated lower food intake during the E treatment than all the other periods regardless of the male gonadal condition (Fig. 3A–B). There were no differences between the male groups (ShC and C).

Taking into account that both food intake and alcohol consumption decreased during the E treatment, we analyzed the correlation between the total liquid consumption and the total food intake for each subject per day and for each period. There was no significant correlation between these two variables, neither in FAC nor in VAC.

3.4. Body weight

Body weight was recorded throughout the study and how it was expected trended to increase with age. ANOVA (Sham-Castrated × Ages) showed that differences in body weight were significant between ages [$F(8,112) = 269.31$, $P < .0001$]. In most cases, the higher the age, the higher the body weight, however, the only exception was the body weight at the final stage of the E treatment, which was significantly lower than the previous measure recorded 1 week before the beginning of the E treatment. Interaction between age and male gonadal condition was also significant [$F(8,112) = 2.86$, $P = .006$] and indicated that the body weight was recovered and increased significantly 2 weeks after the E treatment in the sham group while body weight was significantly increased until approximately 4 weeks after the final stage of the E treatment in the C males.

3.5. Patterns of alcohol consumption (test in the lickometer system)

3.5.1. Forced condition

Frequency of accesses (licks) to alcohol drinking spouts was analyzed for the light and the dark phase in the different periods. Two-way ANOVAs (Light–Dark × Periods) performed separately for each group showed significantly higher frequency of accesses to alcohol in the dark than in the light phase regardless of the period in the sham-castrated [$F(1,63) = 116.68$, $P < .0001$] and in the castrated males [$F(1,63) = 45.39$, $P < .0001$]. Differences between experimental periods regardless of the phase were significant only in castrated males [$F(4,63) = 3.75$, $P = .008$]. These differences indicated higher frequency of accesses in PreC1 than in PosC and E treatment.

We analyzed the size of each drinking bout (licking rate/10 min) and its distribution throughout the day in the different periods. Taking into account that the daily pattern of alcohol consumption cannot be averaged with other days and with the purpose to simplify the data analyses, the pattern of alcohol consumption in the PreC period was assessed considering the last of the 4 days of test in the lickometer system. Two-way ANOVAs considering phase (light–dark) and interval of licking rate (20–150, 151–300, 301–450, 451–600, 601–750 and >750 licks/10 min) was performed separately for each experimental period and for each group of males. These analyses showed the following results: Frequency of bouts was significantly higher during the dark than in the light phase in all the experimental

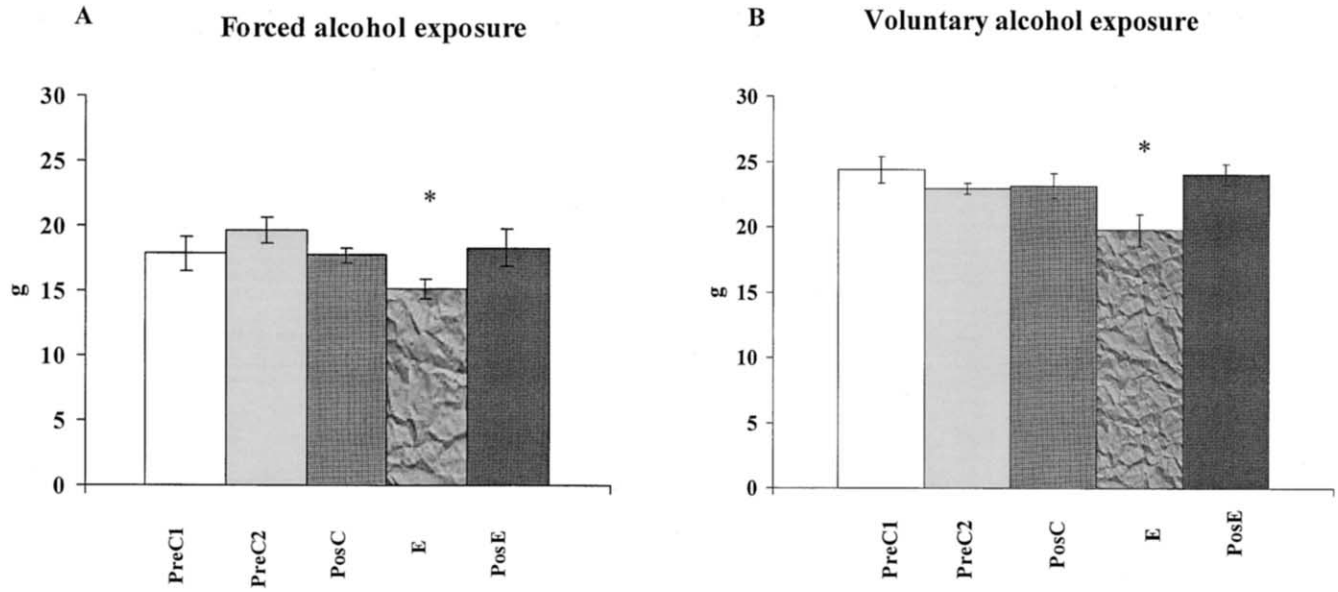

Fig. 3. Food intake (g) regardless of the male gonadal condition in forced (A) and voluntary (B) alcohol exposure. Each graphic shows the differences between means (±S.E.M.) of the different experimental periods: precastration 1 (PreC1), precastration 2 (PreC2), postcastration (PosC), estrogen treatment (E) and post-estrogen treatment (PosE). * Significantly lower than PreC1, PreC2, PosC and PosE.

periods and in both groups of males, except in the E treatment of ShC group, where the difference between phases was not significant. Differences between licking rates regardless of the phase indicated that the rate of 20–150 licks/10 min was the more frequent rate of alcohol consumption in all periods and in both groups. The specific differences between licking rates are described separately for each male group in the next sections.

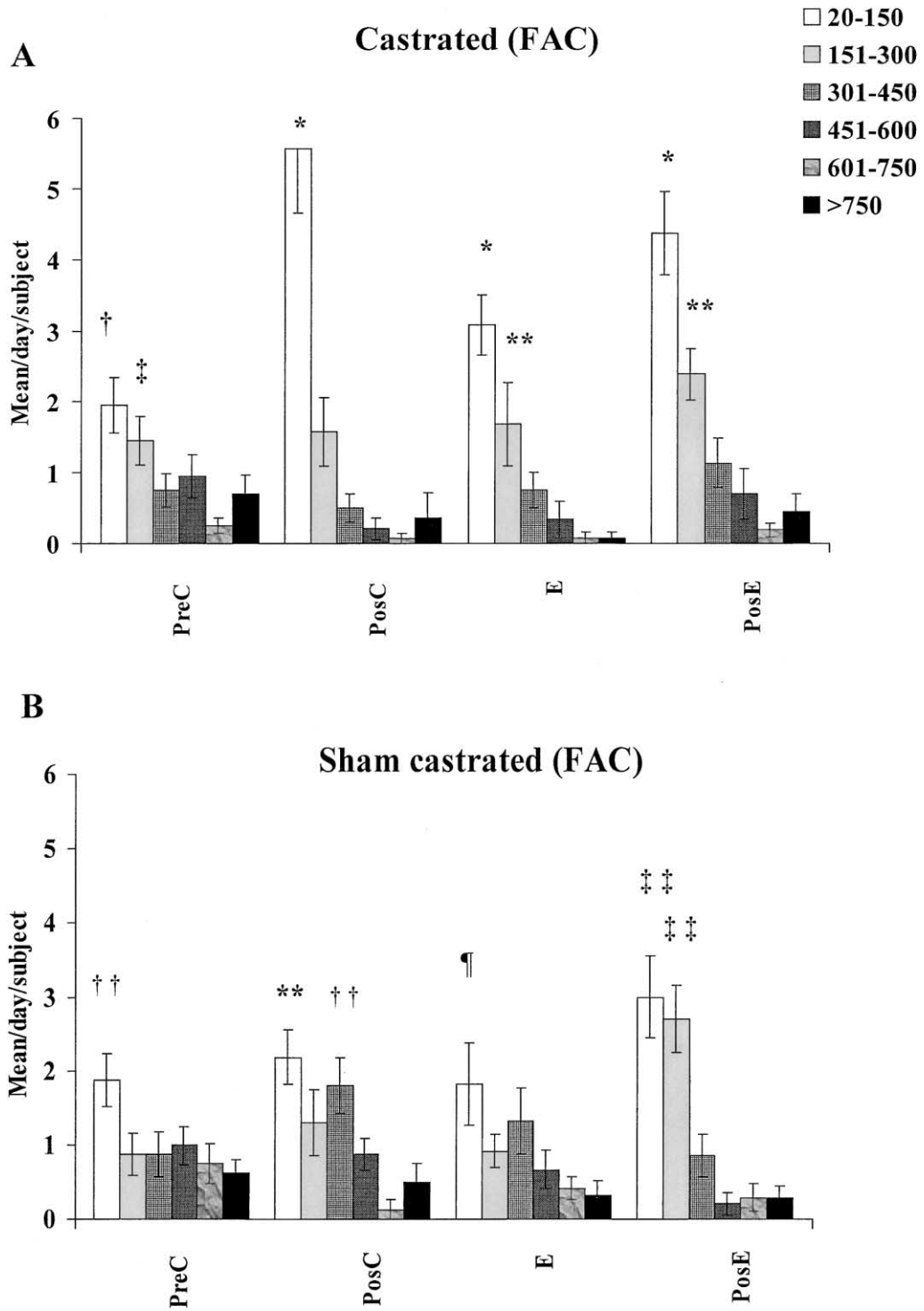

Fig. 4. Differences between licking rates in each experimental period of C (A) and ShC (B) males in forced alcohol consumption (FAC). Bars show the means/day/subject (±S.E.M.) of the frequency of each licking rate in the periods: precastration 1 (PreC1), precastration 2 (PreC2), postcastration (PosC), estrogen treatment (E) and post-estrogen treatment (PosE). * Significantly higher than 151–300, 301–450, 451–600, 601–750 and >751. ‡‡ Significantly higher than 301–450, 451–600, 601–750 and >751. ** Significantly higher than 451–600, 601–750 and >751. † Significantly higher than 301–450, 601–750 and >751. †† Significantly higher than 601–750 and >751. ‡ Significantly higher than 601–750. ¶ Significantly higher than rates >751.

3.5.1.1. C group. The rate of 20–150 licks/10 min was significantly more frequent than the rates of 301–450 and over 600 licks/10 min in the PreC period (Fig. 4A), [$F(5,77)=4.8$, $P=.001$]. This rate of 20–150 was also more frequent than all the other licking rates in PosC [$F(5,66)=25.38$, $P<.0001$], E treatment [$F(5,55)=14.05$, $P<.0001$] and PosE [$F(5-77)=20.5$, $P<.0001$] (Fig. 4A). At the same time, the rate of 151–300 was significantly more frequent than the rate of 601–750 in the PreC period and more frequent than the rates over 451 licks in the

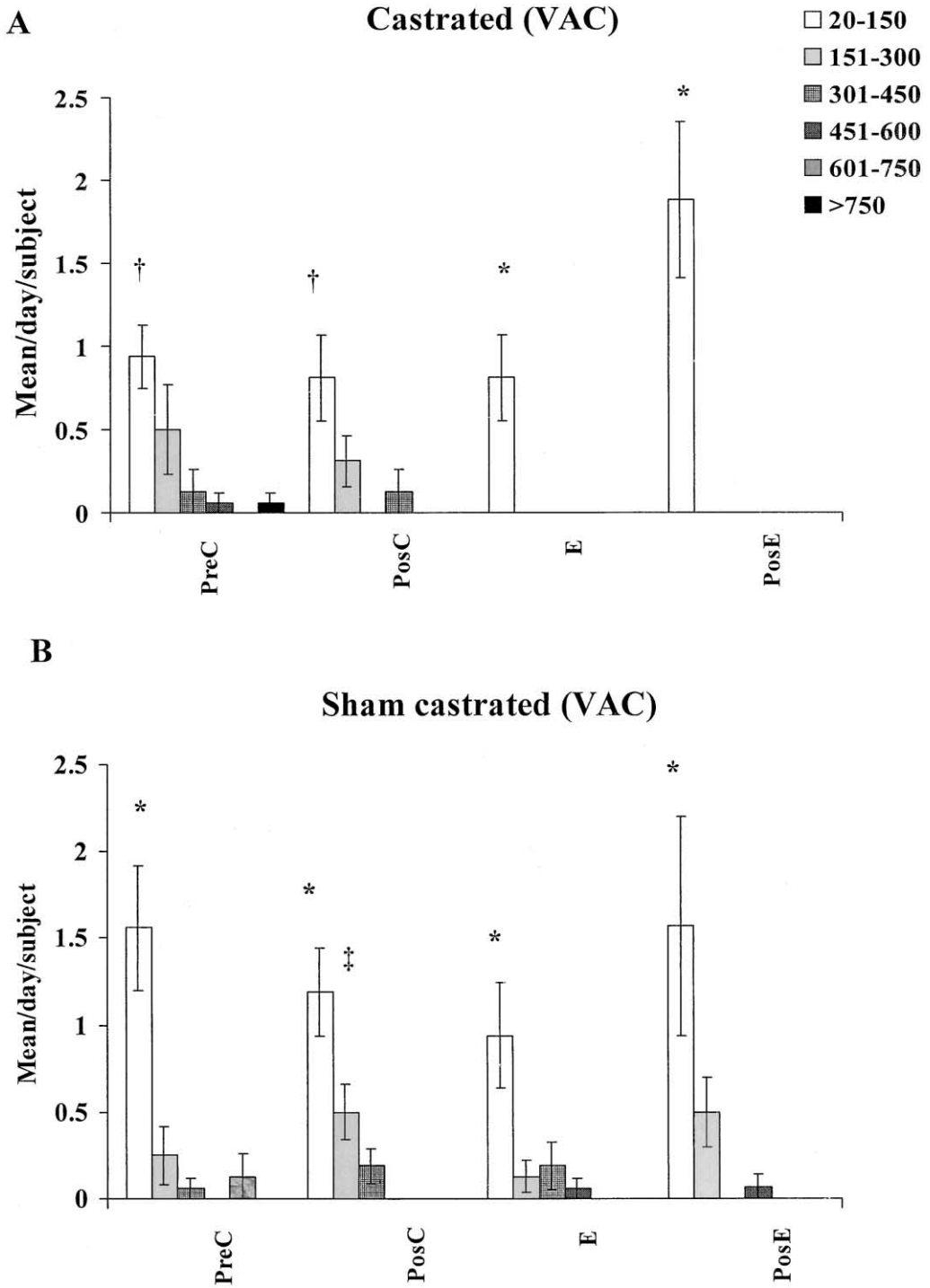

Fig. 5. Differences between licking rates in each experimental period of C (A) and ShC (B) males in voluntary alcohol consumption (VAC). Bars show the means/day/subject (±S.E.M.) of the frequency of each licking rate in the periods: precastration 1 (PreC1), precastration 2 (PreC2), postcastration (PosC), estrogen treatment (E) and post-estrogen treatment (PosE). * Significantly higher than 151–300, 301–450, 451–600, 601–750 and >751. † Significantly higher than 301–450, 451–600, 601–750 and >751. ‡ Significantly higher than 451–600, 601–750 and >751.

E treatment and in the PosE period (Fig. 4A). It is noteworthy that castration reduced the size of the licking rates without affecting the number of drinking bouts. On the other hand, the PosE period showed the same significant differences between rates that were observed in the E period, which indicated a very similar pattern of alcohol consumption, but the frequency of drinking bouts trended to be higher in the PosE than in the E period in all licking rates.

3.5.1.2. ShC group. The rate of 20–150 was also the more frequent rate in all the periods studied in the ShC group, however, the differences with the other licking rates were less evident than those observed in the C group. That is to say, the rate of 20–150 was significantly more frequent than the rates over 600 licks/10 min in the PreC period (Fig. 4B) [$F(5,77)=2.89$, $P=.019$] and more frequent than the rates over 450 licks in the PosC period [$F(5,77)=6.43$, $P<.0001$]. Frequency of bouts trended to decrease in most licking rates during the E treatment, but the only significant difference [$F(5,55)=2.79$, $P=.026$] indicated that the rate of 20–150 was more frequent than the rates over 750 licks (Fig. 4B). The greatest differences between licking rates in the sham group were observed during the PosE period, where the rates between 20 and 300 licks/10 min were more frequent than the rates over 300 licks [$F(5,66)=14.71$, $P>.0001$]. Finally, the rate of 301–450 was more frequent than the rates over 600 licks in the PosC period. It is important to point out that given the significant differences between licking rates in the different periods, the frequency of bouts per day did not show significant differences between periods neither in sham nor in C males.

3.5.2. Voluntary condition

ANOVAs (Light–Dark × Periods) of the total frequency of licking behavior to alcohol drinking spouts during the voluntary alcohol condition did not show any significant difference. On the other hand, the ANOVAs of the size and distribution of drinking bouts (Phase × Interval of Licking Rate) showed the following results.

3.5.2.1. C group. The frequency of drinking bouts was higher in the dark than in the light phase only in the PreC period [$F(1,77)=4.37$, $P=.04$]. The interval of 20–150 licks/10 min was the more frequent rate in all the experimental periods and it was the only rate observed in the E and PosE periods (Fig. 5A). This rate of 20–150 was significantly more frequent than the rates over 300 licks/10 min in the PreC and PosC periods. The lowest frequency of bouts occurred during the E treatment and although the rate of 20–150 was the only rate observed in the E and PosE periods, the mean frequency of bouts during PosE was more than twofold the frequency observed during the E period (Fig. 5A).

3.5.2.2. ShC group. The frequency of drinking bouts was significantly higher in the dark than in the light phase only in the PosC period [$F(1,77)=4.49$, $P=.037$]. The rate of 20–150 licks/10 min was also the more frequent licking rate in all periods of the ShC group. At least three different licking rates occurred in each period, and the patterns of licking behavior were more similar between the different periods in this group of males compared with the C group (Fig. 5B).

4. Discussion

The effect of E treatment on the alcohol consumption apparently depended on two factors, the exposure to FAC or VAC and the male gonadal condition. The lowest values of alcohol consumption occurred during the E treatment in the forced alcohol exposure, however, the differences between different experimental periods regardless of the male group showed that the alcohol intake during the E treatment was significantly lower only with respect to the two periods of PreC. These data indicate that E did not have an evident effect on the amount of alcohol consumption in the forced alcohol exposure. This result probably was due to the fact that castration trended to decrease also the alcohol consumption, and an additional reduction would be difficult to obtain taking into account that the only source of liquids was the alcohol solution in this forced condition. On the other hand, the effect of E treatment on the alcohol consumption was more evident during the voluntary alcohol exposure. This effect was different depending on the male gonadal condition. In the ShC group, alcohol consumption decreased progressively from PreC1 to PosE, however, there were no significant differences between the periods of PosC, E and PosE. Castration did not significantly reduced the alcohol intake during the voluntary condition, but the E treatment significantly decreased the alcohol consumption with respect to all the other periods with the exception of the PosC period in the group of C males. We can suppose that PosC and PosE share similar biological characteristics (castration without exogenous estrogens). Therefore, although the low levels of alcohol consumption in the E treatment did not show significant differences with respect to the PosC period, significant differences with respect to the PosE period were shown, which suggests an inhibitory effect of E on the alcohol consumption. Differences between periods were not significant in the vehicle consumption during voluntary exposure, however, vehicle intake was increasing from PreC1 to PosC at the same time that alcohol consumption was decreasing in the same periods in the ShC males. Therefore, it is possible that at least in the three initial periods, the increasing preference for vehicle consumption had some influence in the progressive decrease of voluntary alcohol intake observed in the ShC group. The absence of significant differences in the amount of vehicle consumption indicates that the decrease in alcohol consumption during the E period of C males cannot be explained by a compensatory mechanism in the choice of liquids to maintain either the caloric supply or the daily liquid requirements.

The question is why estrogens decrease the alcohol consumption? It has been described that estrogens increase the ADH activity (Qulali and Crabb, 1992; Qulali et al., 1991; Rachamin et al., 1980; Teschke and Heymann, 1982) and the alcohol metabolic rate (Rachamin et al., 1980) in the rat. Therefore, an increase in the alcohol consumption could be expected, since if alcohol elimination is faster, it would be necessary to drink more to maintain a given blood alcohol level in time. However, the rapid elimination of alcohol does not necessarily should increase the alcohol consumption, because the degree of liking to alcohol can play an important role in its consumption, mainly when the intake is voluntary. The scarce studies that analyze the effects of estrogens on alcohol consumption in males show controversial results. It has been described that estrogens increase the alcohol consumption in male mice (Hilakivi, 1996), however, this effect was significant with respect to males treated with tamoxifen (antiestrogen), but not with respect to a placebo group. On the other hand, a decrease in the alcohol consumption of selected rats with high preference for alcohol has been documented (Messiha, 1981). It is possible that differences in the species used and the utilization of subjects with different preference for alcohol could determine the discrepancies between these studies. The present results agree with those obtained in the Messiha (1981) study and suggest that the inhibitory effect of estrogens on alcohol consumption can occur regardless of the characteristics of preference to alcohol in the rat. A possible explanation to the effects of E treatment in the present study is based on the following data. It has been described that estrogens produce an important decrease in endogenous opioids, particularly of beta-endorphins (Desjardins et al., 1993; Lapchak, 1991; Schipper et al., 1994), possibly because alcohol consumption was less rewarding during the E treatment, producing a decrease in the alcohol intake. This possible mechanism could be analogous to that attributed to the effect of opioid antagonists on the alcohol consumption, that is to say, it has been described that the opioid antagonist, naloxone, decreases the alcohol consumption in rats (DeWitte, 1984; Froehlich et al., 1990; Hubbell et al., 1986; Marfaing-Jallat et al., 1983). This effect has been attributed to a decrease in the rewarding properties of the alcohol.

Food intake and body weight were also decreased by E treatment in ShC and C males. Food intake showed the lowest values during the E treatment in both groups of males and in both conditions of alcohol exposure. This decrease in food intake by estrogen treatment is not surprising because this effect has been amply described in female rats (Butera et al., 1990, 1996; Dagnault et al., 1993; Donohoe and Stevens, 1982; Donohoe et al., 1984; Sandberg et al., 1982; Varma et al., 1999). This effect seems to be mediated by an action of estrogen on hypothalamus (Butera et al., 1990, 1996; Donohoe and Stevens, 1982), involving possibly a potentiation of the satiety effect of cholecystokinin (Butera et al., 1996). Considering that these studies have been done in females, the effect of estrogen on food intake is extended to males in the present study.

The frequency of accesses to alcohol was higher during the dark than in the light phase in forced alcohol exposure, which is not surprising because the greater activity in rodents occurs during the dark. However, the typical cyclic pattern of higher frequency of liquid access during the dark phase was absent in all periods and in both groups of males during VAC. This result can be interpreted in two ways: the low preference for alcohol in this condition probably produced an indiscriminate distribution of alcohol consumption during the day, or, the subjects purposely trend to distribute homogeneously their alcohol intake throughout the day as consequence of the low preference for alcohol. Whatever it may be the explanation, remains to be studied. On the contrary, vehicle consumption showed the typical cyclic pattern in this voluntary condition as it has been previously described (Juárez and Barrios De Tomasi, 1999).

The main effect of castration on the pattern of FAC was a significant reduction in the size of licking rates without significantly affecting the number of drinking bouts. This pattern was maintained in the E and PosE periods of C males during this forced exposure, however, a light decrease in the frequency of bouts was observed during the E treatment. On the other hand, E treatment decreased both the size of licking rates and the frequency of drinking bouts during VAC in C males, which gives an additional support to the inhibitory effect of E on alcohol consumption. VAC was increased again in the PosE period after the apparent inhibitory effect of the estrogens. This recovery in alcohol consumption during PosE occurred by an increase in the number of bouts, but the bout size was similar to that in the E period of this group of C males. These results suggest that E treatment can affect the pattern of alcohol intake modifying the size and/or the frequency of drinking bouts. The specific modification can apparently depend on the forced or free-choice availability of alcohol. It has been described that the level of alcohol intoxication achieved in each individual can be influenced by the size, frequency and duration of drinking bouts, which integrally produce the pattern of alcohol intake in each subject (Gill et al., 1996). Therefore, what specific characteristics of the alcohol pattern is affected by any pharmacological treatment can be very valuable to study the underlying mechanisms that regulate or modify alcohol consumption. However, the study of patterns of alcohol consumption has been very scarcely studied at the present time.

The present results suggest that estrogen treatment produces an inhibitory effect on alcohol consumption, which could depend on an inhibitory action of estrogens on the opioid system. This hypothesis is currently studied in our laboratory. Estrogen treatment also produced an inhibitory effect on food intake and reduction in body weight. Although these effects have been amply described in the literature with respect to females, both effects are extended to males in the present study. Besides the effects on the

amount of alcohol intake, present results suggest that castration and estrogen treatment can modify differentially the patterns of alcohol consumption depending on the volitive characteristics of its intake.

Considering the scarcity of data concerning the hormonal action on the alcohol consumption in males, future investigations are necessary to understand the mechanisms involved in the physiological and pharmacological interaction between sexual hormones and alcohol consumption.

References

Adams ML, Little PJ, Bell B, Cicero TJ. Alcohol affects rat testicular interstitial fluid volume and testicular secretion of testosterone and beta-endorphin. J Pharmacol Exp Ther 1991;258:1008–14.

Almeida OFX, Shoaib M, Deicke J, Fischer D, Darwish MH, Patchev VK. Gender differences in ethanol preference and ingestion in rats. The role of gonadal steroid environment. J Clin Invest 1998;101:2677–85.

Butera PC, Beikirch RJ, Willard DM. Changes in ingestive behaviors and body weight following intracranial application of 17-alpha-estradiol. Physiol Behav 1990;47:1291–3.

Butera PC, Xiong M, Davis RJ, Platania SP. Central implants of dilute estradiol enhance the satiety effect of CCK-8. Behav Neurosci 1996; 110:823–30.

Couwenbergs CJ. Acute effects of drinking beer or wine on the steroid hormones of healthy men. J Steroid Biochem 1988;31:467–73.

Dagnault A, Ouerghi D, Richard D. Treatment with alpha-helical-CRF(9–41) prevents the anorectic effect of 17-beta-estradiol. Brain Res Bull 1993;32:689–92.

Desjardins GC, Brawer JR, Beaudet A. Estradiol is selectively neurotoxic to hypothalamic beta-endorphin neurons. Endocrinology 1993; 132:86–93.

DeWitte P. Naloxone reduces alcohol intake in a free-choice procedure even when both drinking bottles contain saccharin sodium or quinine substances. Neuropsychobiology 1984;12:73–7.

Donohoe TP, Stevens R. Modulation of food intake by hypothalamic implants of estradiol benzoate, estrone, estriol and CI-628 in female rats. Pharmacol, Biochem Behav 1982;16:93–9.

Donohoe TP, Stevens R, Johnson NJ, Barker S. Effects of stereoisomers of estradiol on food intake, body weight and hoarding behavior in female rats. Physiol Behav 1984;32:589–92.

Esquifino A, Mateos A, Agrasal C, Martin I, Canovas JM, Fermoso J. Time-dependent effects of alcohol on the hypotalamic–hypophyseal–testicular function in the rat. Alcohol: Clin Exp Res 1989; 13:219–23.

Froehlich JC, Harts J, Lumeng L, Li TK. Naloxone attenuates voluntary ethanol intake in rats selectively bred for high ethanol preference. Pharmacol, Biochem Behav 1990;35:385–90.

Gavaler JS, Van Thiel DH. Hormonal status of postmenopausal women with alcohol-induced cirrhosis: further findings and a review of the literature. Hepatology 1992;16:312–9.

Gavaler JS, Love K, Van Thiel D, Farholt S, Gluud C, Monteiro E. An international study of the relationship between alcohol consumption and postmenopausal estradiol levels. Alcohol Alcohol Suppl 1991; 1:327–30.

Gill K, Amit Z, Smith BR. Alcohol as a food: a commentary on Richter. Physiol Behav 1996;60:1485–90.

Hilakivi CL. Role of estradiol in alcohol intake and alcohol-related behaviors. J Stud Alcohol 1996;57:162–70.

Hubbell CL, Czirr SA, Hunter GA, Beaman CM, LeCann NC, Reid LD. Consumption of ethanol solution is potentiated by morphine and attenuated by naloxone persistently across repeated daily administrations. Alcohol 1986;3:39–54.

Juárez J, Barrios De Tomasi E. Sex differences in alcohol drinking patterns during forced and voluntary consumption in rats. Alcohol 1999;19:15–22.

Lancaster FE, Brown TD, Coker KL, Elliott JA, Wren SB. Sex differences in alcohol preference and drinking patterns emerge during the early postpubertal period in Sprague–Dawley rats. Alcohol: Clin Exp Res 1996;20:1043–9.

Lapchak PA. Effect of estradiol treatment on beta-endorphin content and release in the female rat hypothalamus. Brain Res 1991;554:198–202.

Marfaing-Jallat P, Miceli D, Le Magnen J. Decrease in ethanol consumption by naloxone in naive and dependent rats. Pharmacol, Biochem Behav 1983;185:537–9.

Mendelson JH, Lukas SE, Nello NK, Amass L, Ellingboe J, Skupny A. Acute alcohol effects on plasma estradiol levels in women. Psychopharmacology 1988;94:464–7.

Messiha FS. Steroidal actions and voluntary drinking of ethanol by male and female rats. Prog Biochem Pharmacol 1981;18:205–15.

Mezey E, Potter JJ, Diehl AM. Depression of alcohol dehydrogenase activity in rat hepatocyte culture by dihydrotestosterone. Biochem Pharmacol 1986;35:335–9.

Mezey E, Rennie Tankersley L, Potter JJ. Effect of dihydrotestosterone on turnover of alcohol dehydrogenase in rat hepatocyte culture. Hepatology 1998;27:185–90.

Mishra L, Sharma S, Potter JJ, Mezey E. More rapid elimination of alcohol in women as compared to their male siblings. Alcoholism 1989;13:752–4.

Muti P, Trevisan M, Micheli A, Krogh V, Bolelli G, Sciajno R. Alcohol consumption and total estradiol in premenopausal women. Cancer Epidemiol Biomarkers 1998;7:189–93.

Qulali M, Crabb DW. Estradiol regulates class I alcohol dehydrogenase gene expression in renal medulla of male rats by post-transcriptional mechanism. Arch Biochem Biophys 1992;297:277–84.

Qulali M, Ross RA, Crabb DW. Estradiol induces class I alcohol dehydrogenase activity and mRNA in kidney of female rats. Arch Biochem Biophys 1991;288:406–13.

Rachamin G, Macdonald JA, Wahid S, Clapp JJ, Khanna JM, Israel Y. Modulation of alcohol dehydrogenase and ethanol metabolism by sex hormones in the spontaneously hypertensive rat. Effect of chronic ethanol administration. Biochem J 1980;186:483–90.

Sandberg D, Stewart J. Effects of estradiol benzoate and MER-25 on ethanol consumption in the ovariectomized rat. J Comp Physiol Psychol 1982;96:635–48.

Sandberg D, David S, Stewart J. Effects of estradiol benzoate on the pattern of eating and ethanol consumption. Physiol Behav 1982;29:61–5.

Schipper HM, Desjardins GC, Beaudet A, Brawer JR. The 21-aminoesteroid antioxidant, U74389F, prevents estradiol-induced depletion of hypothalamic beta-endorphin in adult female rat. Brain Res 1994; 652:161–3.

Sutker PB, Goist KC, Allain AN, Bugg F. Acute alcohol intoxication: sex comparisons on pharmacokinetic and mood measures. Alcoholism 1987;11:507–12.

Teschke R, Heymann K. Effect of sex hormones on the activities of hepatic alcohol-metabolizing enzyme in male. Enzyme 1982;28:268–77.

Van Thiel DH, Tarter RE, Rosenblum E, Gavaler JS. Ethanol its metabolism and gonadal effects: does sex make a difference? Adv Alcohol Subst Abuse 1988;7:131–69.

Varma M, Chai JK, Meguid MM, Laviano A, Gleason JR, Yang ZJ. Effect of estradiol and progesterone on daily rhythm in food intake and feeding patterns in Fischer rats. Physiol Behav 1999;68:99–107.

Vaubourdolle M, Guechot J, Chazouilleres O, Poupon RE, Giboudeau J. Effect of dihydrotestosterone on the rate of ethanol elimination in healthy men. Alcohol: Clin Exp Res 1991;15:238–40.