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Ovariectomy abolishes ethanol-induced impairment of baroreflex control of heart rate in conscious rats

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Abstract

Our previous studies have shown that ethanol attenuates baroreflex control of heart rate in male rats. The present study investigated whether this effect of ethanol is gender-related, and whether it involves hormonal factors. The effect of intragastric administration of ethanol or equal volume of water on baroreflex-mediated decreases in heart rate in response to increments in blood pressure evoked by phenylephrine were evaluated in conscious age-matched male and female Sprague–Dawley rats as well as in ovariectomized rats. Baroreflex curves relating changes in blood pressure and associated heart rate responses were constructed, and the slopes of the regression lines were taken as a measure of baroreflex sensitivity. Phenylephrine (1–16 μg kg^{-1}, i.v.) elicited dose-dependent pressor responses that were similar in all groups of rats. However, the associated reflex bradycardic responses depended on the rat preparation and the dose of ethanol employed. In water-treated (control) animals, significantly ($P < 0.05$) lesser reflex bradycardic responses were observed in female compared with male rats (baroreflex sensitivity, -1.21 ± 0.12 vs. -1.67 ± 0.12 beats min^{-1} mmHg^{-1}). Ovariectomy resulted in a further reduction in baroreflex sensitivity (-0.82 ± 0.06 beats min^{-1} mmHg^{-1}), suggesting a favorable role for ovarian hormones in baroreflex modulation. In male rats, ethanol (0.25, 0.5, or 1 g kg^{-1}, intragastric) elicited dose-related decreases in reflex bradycardic responses. The reduction in the regression coefficient obtained by the two higher doses (0.5 and 1 g kg^{-1}) of ethanol was statistically significant compared with control values. The ability of ethanol to reduce baroreflex sensitivity appears to be gender-independent as it was similarly demonstrated in intact female rats. In contrast, ethanol had no effect on reflex bradycardic responses in ovariectomized rats at any of the doses tested. The data suggest that ethanol reduces baroreflex control of heart rate irrespective of the rat gender. Further, the lack of an effect of ethanol on baroreflex sensitivity in ovariectomized rats may suggest a role for ovarian hormones in ethanol-evoked baroreflex attenuation.

Keywords: Ethanol; Baroreflex sensitivity; Ovariectomy; Gender

1. Introduction

Ethanol has been shown in our previous studies (Abdel-Rahman et al., 1987a; El-Mas and Abdel-Rahman, 1992, 1993b; Russ et al., 1991) and in others (Varga and Kunos, 1990, 1992) to attenuate baroreflex-mediated heart rate responses in humans and experimental animals. Our own findings suggest that the effect of ethanol on baroreflex sensitivity involves selective impairment of aortic baroreceptor function (El-Mas and Abdel-Rahman, 1992, 1993a). It has been suggested that impairment of arterial baroreceptors may play a contributory role in the development of ethanol-induced hypertension (Abdel-Rahman and Wooles, 1987; Russ et al., 1991). Reported findings indicate that ethanol impairs baroreflex sensitivity after oral (Abdel-Rahman et al., 1987a) and parenteral administration (El-Mas and Abdel-Rahman, 1992, 1993b; Abdel-Rahman et al., 1985; Zhang et al., 1988), as well as after microinjection into discrete brain areas that control baroreflexes (El-Mas and Abdel-Rahman, 1993b; Mao and Abdel-Rahman, 1995; Varga and Kunos, 1990, 1992). The latter findings suggest a central site of action for ethanol on baroreflex sensitivity. However, the exact mechanism by which ethanol attenuates the baroreflex sensitivity is not known. Recent reports from our laboratory have shown that selective blockade by ethanol of NMDA receptors in the nucleus tractus solitarius (El-Mas and Abdel-Rahman, 1993b) and rostral ventrolateral medulla (Mao and Abdel-Rahman, 1995) contributes to ethanol-induced attenuation

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of baroreflex sensitivity. Varga and Kunos (1990, 1992) reported that the inhibitory effect of ethanol on baroreflex sensitivity involves, at least in part, enhancement of the action of endogenous γ-aminobutyric acid in the nucleus tractus solitarius. Further, Sun and Reis (1992) have shown that ethanol-evoked impairment of baroreflex sensitivity is associated with enhancement and attenuation of γ-aminobutyric acid and L-glutamate evoked neuronal responses, respectively, in the rostral ventrolateral medulla.

It is notable that in previous experimental studies that dealt with the effect of ethanol on baroreflex sensitivity, ethanol was given by the intravenous route (El-Mas and Abdel-Rahman, 1992, 1993a; Sun and Reis, 1992; Varga and Kunos, 1992) or microinjected into specific brain areas (El-Mas and Abdel-Rahman, 1993b; Sun and Reis, 1992; Varga and Kunos, 1990, 1992). Whether similar findings can be replicated after intragastric administration of ethanol, which simulates human consumption, has not been investigated. Furthermore, all of the reported studies were undertaken in male rats (El-Mas and Abdel-Rahman, 1992; Russ et al., 1991; Varga and Kunos, 1990, 1992). Even when both genders were employed in a previous clinical study from our laboratory (Abdel-Rahman et al., 1987a), the data from both genders were pooled because the small sample size of each gender did not permit determination of whether or not the effect of ethanol on baroreflex sensitivity was sexually dimorphic. The importance of gender as a determinant factor of autonomic cardiovascular control has been clinically documented. In a previous study (Abdel-Rahman et al., 1994), we showed that young women exhibit smaller heart rate responses to similar increments in blood pressure elicited by bolus administration of phenylephrine compared with men, a finding that suggests sex-related differences in baroreflex function. A similar clinical finding has also been obtained in older individuals (Huikuri et al., 1996). Furthermore, these clinical findings have been replicated in young rats in a preliminary study (Abdel-Rahman, 1994b). The notion that women drink less frequently and consume lesser amounts of alcohol compared with men has hampered research in this area. Nonetheless, the epidemiological findings that long-term alcohol consumption leads to hypertension in both genders (Arkwright et al., 1982) warrant studies on the effects of alcohol on the cardiovascular function and reflexes in the female population.

The present study addressed two questions pertinent to the interaction of ethanol with cardiac reflexes. First, whether young female rats (11–12 weeks) are as susceptible as male rats to the deleterious effect of ethanol on baroreflex control of heart rate. The second objective was to investigate the effect of ovariectomy on ethanol-induced attenuation of baroreflex responsiveness to determine whether it involves hormonal factors. It has been shown that hormone replacement therapy produces favorable effects on the cardiovascular autonomic regulation in postmenopausal women (Huikuri et al., 1996) and oral contraceptive users exhibit greater heart rate responses during behavioral stressors (Girdler et al., 1990). It is, therefore, interesting to determine whether alteration of hormonal balance in ovariectomized rats, as a model for surgical menopause (Alper and Schmitz, 1996), may influence the action of ethanol on baroreflex sensitivity. To this end, the effect of intragastric administration of different doses (0.25, 0.5, and 1 g kg^{-1}) of ethanol or equal volume of water on baroreflex-mediated decreases in heart rate in response to increments in blood pressure evoked by bolus doses of phenylephrine were evaluated in conscious freely moving male, female and ovariectomized Spraque–Dawley rats. Baroreflex curves relating changes in blood pressure and reciprocal changes in heart rate were constructed, and the slopes (regression coefficients) were taken as a measure of baroreflex sensitivity. These studies were undertaken in conscious rats to avoid the confounding effects of anesthesia on the measured responses (El-Mas and Abdel-Rahman, 1992; Watkins and Maixner, 1991).

2. Materials and methods

2.1. Preparation of the rats

Age matched (11–12 weeks) male and female Sprague–Dawley rats (Charles River, Raleigh, NC) were used in the present study. For measurement of blood pressure, the method described in our previous studies (El-Mas and Abdel-Rahman, 1992, 1993a) was adopted. Briefly, the rats were anesthetized by methohexital (50 mg kg^{-1} i.p.). Catheters (Polyethylene 50) were placed in the abdominal aorta and vena cava via the femoral artery and vein for measurement of blood pressure and i.v. administration of drugs, respectively. The catheters were inserted about 5 cm into the femoral vessels and secured in place with sutures. The arterial catheter was connected to a Gould–Statham pressure transducer (Oxnard, CA) and blood pressure was displayed on a Grass polygraph (model 7D, Grass Inst., Quincy, MA). Heart rate was computed from blood pressure waveforms by a Grass tachograph and was displayed on another channel of the polygraph. Intragastric catheterization was performed by inserting a polyethylene 50 tubing into the stomach through a nostril (Choikwon et al., 1990). This technique allows intragastric administration of drugs in freely moving rats (Choikwon et al., 1990).

Finally, the catheters were tunnelled subcutaneously and exteriorized at the back of the neck between the scapulae. The catheters were flushed with heparin (200 U ml^{-1}) and plugged by stainless steel pins. Incisions were closed with surgical clips and swabbed with povidone–iodine solution. Each rat received a subcutaneous injection of the analgesic buprenorphine hydrochloride (Buprenex; 0.3 μg rat^{-1}) and an intramuscular injection of 60 000 U

of penicillin G benzathine and penicillin G procaine in an aqueous suspension (Durapen) and was housed in a separate cage. The experiment started 48 h later. Experiments were performed in strict accordance with institutional animal care and use guidelines.

2.2. Ovariectomy

Bilateral ovariectomy was performed on 9–10-week-old female rats as described (Alper and Schmitz, 1996) two weeks before intravascular cannulation. A single 2–3 cm incision was made in the back skin and underlying muscle. The ovaries were isolated, tied-off with sterile suture, and removed. The muscle and skin were sutured, and the rats were allowed approximately 2 weeks to recover prior to intravascular cannulation. Sham operation involved exposure of the ovaries without isolation. Postoperative procedure was followed as described above.

2.3. Blood ethanol concentration

A volume of 0.1 ml of blood was drawn through the arterial cannula 10 min after ethanol administration. The ethanol content of the samples was measured using the enzymatic method described by Bernt and Gutmann (1974), and as in our previous studies (El-Mas and Abdel-Rahman, 1992).

2.4. Protocol and experimental groups

On the day of the experiment, the arterial catheter was connected to a pressure transducer for measurement of blood pressure and heart rate as mentioned above. A period of at least 30 min was allowed at the beginning of the experiment for stabilization of blood pressure and heart rate. A total of 45 rats (Table 1) were used in these experiments to investigate the effect of intragastric administration of ethanol (0.25, 0.5 or 1 g kg^{-1}) or equal volume of water on baroreflex-mediated bradycardia in male, intact female and ovariectomized rats. Generally, each rat in a particular group received two different treatments on two consecutive days (48 and 72 h after surgery). Previous studies from our laboratory have shown that the effects of acutely administered ethanol are both reversible and reproducible (Abdel-Rahman, 1989). Baroreflex testing was performed 10 min after intragastric administration of ethanol or water. Equal volumes (1 ml/100 g body weight) of ethanol (0.25, 0.5 or 1 g kg^{-1}) or water were administered intragastrically. Ethanol diluted in water as 3.25%, 6.5% and 13% was used for the doses 0.25, 0.5 and 1 g kg^{-1}, respectively.

Table 1
Baseline values of mean arterial pressure (MAP, mmHg) and heart rate (HR, beats min^{-1}) and blood ethanol concentration (mg %) measured 10 min after intragastric ethanol administration

Group	*n*	MAP	HR	Blood ethanol
Males				
Water	8	121±3	393±10	0
Ethanol (0.25 g kg^{-1})	6	126±6	398±14	20±3
Ethanol (0.5 g kg^{-1})	7	126±4	395±8	45±7
Ethanol (1 g kg^{-1})	8	121±5	411±12	118±13
Females				
Water	7	119±4	433±9	0
Ethanol (0.25 g kg^{-1})	6	119±5	433±13	17±2
Ethanol (0.5 g kg^{-1})	9	116±3	440±10	38±8
Ethanol (1 g kg^{-1})	8	121±4	458±13	106±8
Ovariectomized				
Water	6	116±3	461±9	0
Ethanol (0.25 g kg^{-1})	7	127±4	429±13	12±3
Ethanol (0.5 g kg^{-1})	8	118±4	443±16	35±7
Ethanol (1 g kg^{-1})	9	118±5	457±14	103±10

Values are mean ± S.E.M.

2.4.1. Baroreflex testing

A dose–response curve of the responses of blood pressure and heart rate to phenylephrine was constructed in all rats by i.v. injection of randomized doses of phenylephrine hydrochloride (1, 2, 4, 8, 16 μg kg^{-1}) at 5-min intervals. Phenylephrine was dissolved in saline, and the injection volume was kept constant at 0.05 ml/100 g body weight with a flush volume of approximately 0.1 ml saline. The mean arterial pressure (diastolic plus one-third pulse pressure) and heart rate values before and after phenylephrine administration were measured, and the peak changes in both variables (mean arterial pressure and heart rate) were used for construction of the baroreflex curves.

2.5. Drugs

Methohexital sodium (Brevital, Eli Lilly, Indianapolis, IN), phenylephrine hydrochloride (Sigma), povidone–iodine solution (Norton, Rockford, IL), Durapen (Vedco, Overland Park, KS), ethanol (Midwest Grain Products, Weston, MO).

2.6. Statistical analysis

Values are expressed as mean ± S.E.M. The relationship between increases in mean arterial pressure and associated decreases in heart rate was assessed by regression analysis for individual animals as described in our previous studies (El-Mas and Abdel-Rahman, 1992, 1993a). Only data points in the linear range of the MAP–HR curve were used for regression analysis. The regression coefficient (slope of the regression line) expressed as beats min^{-1} $mmHg^{-1}$ was taken as an index of baroreflex sensitivity (Korner et al., 1972; El-Mas and Abdel-Rahman, 1992, 1993a). Analysis of variance (ANOVA) followed by a Newman–Keuls post-hoc analysis was used for multiple comparisons. The Student's *t*-test was used in the

analysis of paired and unpaired data with the level of significance set at $P < 0.05$.

3. Results

The baseline values of mean arterial pressure and heart rate of all groups of rats are shown in Table 1. The mean arterial pressure values were similar in all groups of rats, whereas heart rate values were significantly ($P < 0.05$) higher in female (sham, 441 ± 9 beats min^{-1}; ovariectomized, 447 ± 10 beats min^{-1}) compared with male (399 ± 8 beats min^{-1}) rats (Table 1). Intragastric administration of ethanol (0.25, 0.5 or 1 g kg^{-1}) or water (1.3 ml kg^{-1}) had no significant effect on baseline blood pressure and heart rate during the testing period (data not shown). The blood ethanol concentrations (mg %) measured 10 min after intragastric administration of ethanol correlated well with doses of ethanol employed and were similar in male, female and ovariectomized rats (Table 1).

3.1. Effect of ethanol on baroreceptor reflex control of heart rate in male and female rats

This experiment investigated whether the ability of ethanol to attenuate baroreflex function is sexually dimorphic. Pressor responses to intravenous bolus administration of phenylephrine in conscious freely moving male and female rats receiving intragastric ethanol (0.25, 0.5 or 1 g kg^{-1}) or equal volume of water are shown in Table 2. Phenylephrine (1–16 μg kg^{-1}) elicited dose-dependent pressor responses of similar magnitudes in male and female rats, indicating that peripherally-mediated pressor responses are not influenced by gender (Table 2). Intragastric administration of ethanol had no effect on phenylephrine-evoked pressor responses in both genders except for slight but significant ($P < 0.05$) decreases elicited by the lower dose of ethanol (Table 2).

Table 2
Effect of intragastric administration of ethanol on phenylephrine-evoked increases in mean arterial pressure in conscious male, sham-operated female and ovariectomized rats

Group	Phenylephrine (μg kg^{-1}, i.v.)				
	1	2	4	8	16
Males					
Water	21±2	31±3	43±4	58±3	69±2
Ethanol (0.25 g kg^{-1})	13±2[a]	18±3[a]	34±4	51±4	70±3
Ethanol (0.5 g kg^{-1})	16±3	29±3	41±3	57±3	65±2
Ethanol (1 g kg^{-1})	15±3	27±3	40±4	54±3	67±2
Females					
Water	16±4	29±4	41±2	58±2	61±5
Ethanol (0.25 g kg^{-1})	11±2	16±2[a]	33±4	51±5	57±5
Ethanol (0.5 g kg^{-1})	12±2	21±3	32±3	44±3	62±3
Ethanol (1 g kg^{-1})	17±3	24±2	37±3	57±2	65±3
Ovariectomized					
Water	19±3	29±3	36±4	56±3	66±3
Ethanol (0.25 g kg^{-1})	14±2	19±3[a]	38±4	44±5	57±5
Ethanol (0.5 g kg^{-1})	14±2	24±2	35±3	53±5	63±5
Ethanol (1 g kg^{-1})	13±3	20±3	34±4	50±4	61±6

Values are mean ± S.E.M.
[a] $P < 0.05$ vs. respective control values.

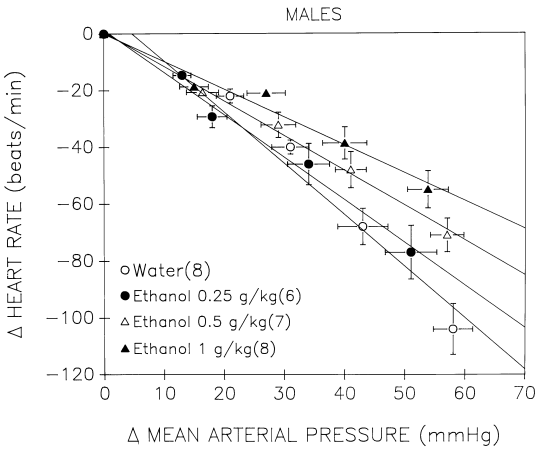

Fig. 1. Effect of intragastric administration of ethanol (0.25, 0.5 or 1 g kg^{-1}) or equal volume of water on baroreflex curves relating decreases in heart rate to increments in mean arterial pressure evoked by phenylephrine in conscious unrestrained male Sprague–Dawley rats. Values are expressed as mean ± S.E.M., and number of rats in each group is shown in parentheses.

Baroreflex curves relating decreases in heart rate responses to phenylephrine-induced increases in mean arterial pressure in conscious freely moving male and female rats are shown in Figs. 1 and 2, respectively. Comparison of baroreflex curves generated in control (water-treated) male and female rats revealed a lesser steep regression line in case of females; i.e., for a comparable rise in mean arterial pressure there was a smaller bradycardic response

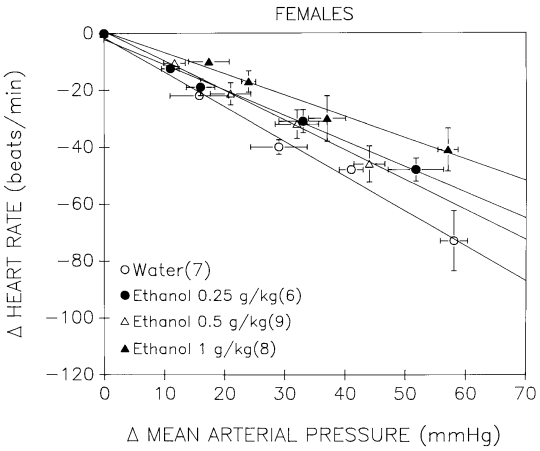

Fig. 2. Effect of intragastric administration of ethanol (0.25, 0.5 or 1 g kg^{-1}) or equal volume of water on baroreflex curves relating decreases in heart rate to increments in mean arterial pressure evoked by phenylephrine in conscious unrestrained female Sprague–Dawley rats. Values are expressed as mean ± S.E.M., and number of rats in each group is shown in parentheses.

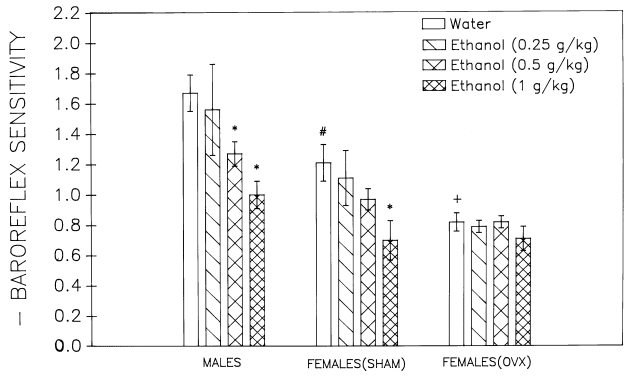

Fig. 3. Regression coefficient (baroreflex sensitivity; beats min^{-1} $mmHg^{-1}$) in conscious unrestrained male, sham-operated female rats, and ovariectomized (OVX) rats. Values are expressed as mean ± S.E.M. *, # and + $P < 0.05$ vs. water-treated, male and sham-operated values, respectively.

in female compared with male rats (Figs. 1 and 2). The slope of the linear regression line, which represented the baroreflex sensitivity, was significantly ($P < 0.05$) smaller in female compared with male rats (-1.21 ± 0.12 vs. -1.67 ± 0.12 beats min^{-1} $mmHg^{-1}$; Fig. 3). The correlation coefficients of the regression lines were highly significant ($P < 0.001$) and ranged from 0.90 to 0.99.

Intragastric administration of ethanol (0.25, 0.5 or 1 g kg^{-1}) in male rats caused dose-related upward shifts in the baroreflex curves compared with control rats (Fig. 1). The slopes of the regression lines were decreased by ethanol in a dose-dependent manner. The slopes after the two higher doses (0.5 and 1 g kg^{-1}) of ethanol (-1.27 ± 0.08 and -1.00 ± 0.09 beats min^{-1} $mmHg^{-1}$) were significantly ($P < 0.05$) reduced compared with control values (-1.67 ± 0.12 beats min^{-1} $mmHg^{-1}$) (Fig. 3). Similarly, intragastric administration of ethanol (0.25, 0.5 or 1 g kg^{-1}) to

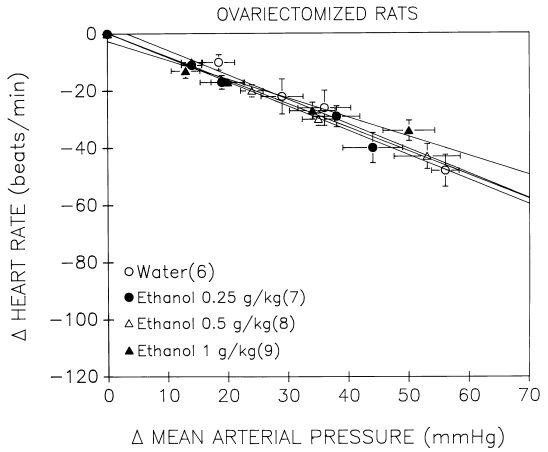

Fig. 4. Effect of intragastric administration of ethanol (0.25, 0.5 or 1 g kg^{-1}) or equal volume of water on baroreflex curves relating decreases in heart rate to increments in mean arterial pressure evoked by phenylephrine in conscious unrestrained ovariectomized Sprague–Dawley rats. Values are expressed as mean ± S.E.M., and number of rats in each group is shown in parentheses.

conscious sham-operated female rats elicited dose-related reductions in reflex bradycardic responses to phenylephrine-evoked increases in mean arterial pressure as indicated by the upward shift in the baroreflex curves (Fig. 2). The slope of the baroreflex curve was also dose-dependently reduced by ethanol compared with control values, and the difference was statistically significant ($P < 0.05$) with the higher dose of ethanol (Fig. 3); the slopes amounted to -0.70 ± 0.13 and -1.21 ± 0.12 beats min^{-1} $mmHg^{-1}$ in rats receiving ethanol (1 g kg^{-1}) and water, respectively. Comparison of the baroreflex sensitivity values showed that reductions caused by ethanol (0.25, 0.5 or 1 g kg^{-1}) in reflex bradycardia were similar in male (7%, 24% and 40%, respectively) and female (8%, 20% and 42%, respectively) rats.

3.2. Effect of ovariectomy on ethanol-induced impairment of baroreflex control of heart rate

Whether ovarian hormones modulate the attenuating effect of ethanol on baroreflex control of heart rate was investigated by evaluating the action of ethanol on baroreflex sensitivity in ovariectomized rats. Ovariectomy had no effect on peripherally mediated pressor responses to phenylephrine (Table 2), but significantly ($P < 0.05$) attenuated the associated baroreflex-mediated bradycardic responses compared with sham-operated rats. As shown in Fig. 3, ovariectomized rats exhibited a significantly ($P < 0.05$) smaller baroreflex sensitivity compared with sham-operated rats (-0.82 ± 0.06 vs. -1.21 ± 0.12 beats min^{-1} $mmHg^{-1}$). Intragastric administration of ethanol (0.25, 0.5 or 1 g kg^{-1}) had no significant effect on baroreflex curves at any of the doses tested (Fig. 4). The slopes of the regression lines relating decreases in heart rate to phenylephrine-evoked increases in mean arterial pressure were similar in ethanol and water-treated ovariectomized rats (Fig. 3). The baroreflex sensitivity in ethanol (1 g kg^{-1}) and water-treated ovariectomized rats amounted to -0.71 ± 0.08 and -0.82 ± 0.06 beats min^{-1} $mmHg^{-1}$, respectively (Fig. 3).

4. Discussion

The present study is the first to report on the acute effect of ethanol, administered intragastrically, on the baroreflex sensitivity in conscious freely moving rats. The present study presents two important findings that pertain to the acute effects of ethanol on baroreflex sensitivity in sexually mature young female rats in the presence and absence of ovarian hormones. First, female rats are as susceptible as male rats to the deleterious effect of ethanol on baroreflex control of heart rate. Second, the depressant effect of ethanol on baroreflex control of heart rate was not demonstrated in ovariectomized rats, a finding that may

suggest a role for ovarian hormones in ethanol-evoked attenuation of baroreflex function in sexually mature female rats.

Ethanol has been shown in several studies including our own to attenuate baroreflex heart rate responses in male rats (El-Mas and Abdel-Rahman, 1992; Russ et al., 1991; Varga and Kunos, 1990, 1992). However, the question whether this effect of ethanol is sexually dimorphic has not been addressed. This may be important, particularly in view of accumulated evidence that suggests sex-related differences in baroreflex function (Abdel-Rahman, 1994b; Abdel-Rahman et al., 1994; Huikuri et al., 1996). Furthermore, recent reports suggest that estrogen facilitates cardiac reflexes (Alper and Schmitz, 1996; Huikuri et al., 1996). Therefore, the possibility must be considered that the effect of ethanol on baroreflex control of heart rate may be influenced by the female sex hormones. In this study, we sought evidence to determine whether the ability of ethanol to attenuate baroreflex function may be gender-related, and may be influenced by ovarian hormones. The latter was addressed by investigating the effects of ethanol on baroreflex sensitivity of ovariectomized rats, a model for surgical menopause (Alper and Schmitz, 1996). Also, it was important to determine whether the effects of ethanol were dose-related. Baroreflex curves relating changes in blood pressure evoked by phenylephrine and reciprocal changes in heart rate were constructed, and the slopes (baroreflex sensitivity) were taken as a measure of baroreflex sensitivity (Korner et al., 1972). Experiments were undertaken in conscious unrestrained rats to avoid the confounding effects of anesthesia on the measured responses (El-Mas and Abdel-Rahman, 1992; Watkins and Maixner, 1991).

The present study showed that baseline baroreflex sensitivity, as a measure of the capacity of baroreceptors to buffer abrupt changes in blood pressure, is significantly lesser in conscious freely moving female compared with age-matched male rats. This finding supports our previous observations (Abdel-Rahman, 1994b; Abdel-Rahman et al., 1994) and others (Huikuri et al., 1996) that suggested gender-related differences in baroreceptor reflex function. The reason for sex differences in baroreflex sensitivity, and whether it involves neural, hormonal or developmental factors, is not clear. In a preliminary study from our laboratory, we found that female rats exhibit a weaker vagal response to baroreceptor activation compared with male rats (Abdel-Rahman, 1994b). The presence of an attenuated baroreflex function in female rats may suggest a downregulating effect for ovarian hormones on the baroreflex control of heart rate. Nonetheless, the present finding that ovariectomy caused further reduction in baroreflex sensitivity highlights a favorable role for ovarian hormones on baroreflex function. It is notable, however, that recent reports including our own suggest a differential effect of estrogen (facilitation; El-Mas and Abdel-Rahman, 1998) and progesterone (inhibition; Masilamani and Heesch, 1997) on baroreflex gain. A lower baroreflex responsiveness in female compared with male rats may, therefore, be explained in view of the opposite effects of estrogen and progesterone on baroreflex sensitivity. Further, the present study measured baroreflex sensitivity in randomly cycling female rats. Variations in plasma estrogen levels during different phases of the cycle (Freeman, 1988) raise the possibility that baroreflex sensitivity may not be the same throughout the cycle. Preliminary findings from our laboratory showed that the baroreflex sensitivity of female rats reached its peak during proestrus, characterized by having highest plasma estrogen levels, and was similar to that of male rats (unpublished data).

The physiological interaction between sex hormones and the neuropeptide arginine vasopressin is another factor that may explain, at least in part, the sex-related differences in baroreflex sensitivity. Several studies have shown that the central expression of vasopressin mRNA, plasma vasopressin levels, and the daily urinary excretion of vasopressin are all higher in male than in female rats (Crofton et al., 1985; Wang and De Vries, 1995). A higher vasopressin activity in male rats may be due to the ability of testosterone to facilitate the biosynthesis of vasopressin (Crofton et al., 1985). On the other hand, ovarian hormones exert opposite effects on vasopressin release (enhancement by estrogen and inhibition by progesterone) (Forsling et al., 1982). Given that vasopressin sensitizes the central baroreflex neurons to afferent input and augments the baroreflex control of heart rate (Peuler et al., 1990; Zhang et al., 1992), the greater buffering capacity of arterial baroreceptors in male rats may conceivably be related to the elevated vasopressin activity. Similarly, the reduction in baroreflex sensitivity in ovariectomized rats, whose central vasopressin activity is reduced (Peysner and Forsling, 1990), may be a result of the loss of estrogen facilitation of vasopressin release.

The significantly lower basal baroreflex sensitivity in female compared with male rats raised the interesting possibility that the inhibitory action of ethanol on baroreflex sensitivity may be reduced or absent in female rats. In agreement with our previous findings (Abdel-Rahman, 1994a; El-Mas and Abdel-Rahman, 1992, 1993a) and others (Varga and Kunos, 1990, 1992), the current study showed that ethanol significantly attenuated the baroreflex sensitivity in a dose-related manner in male rats. Nonetheless, the present findings are the first that demonstrated the dose-related impairment of baroreflex sensitivity following intragastric administration of ethanol in conscious male rats. In these previous studies, ethanol was administered parenterally (El-Mas and Abdel-Rahman, 1992, 1993a; Sun and Reis, 1992; Varga and Kunos, 1992), or microinjected into brainstem areas that process baroreceptor information (El-Mas and Abdel-Rahman, 1993b; Sun and Reis, 1992; Varga and Kunos, 1990, 1992).

Results of the present study showed that the ability of ethanol to attenuate baroreflex sensitivity is gender-inde-

pendent. In spite of a significantly lower baseline baroreflex sensitivity in female compared with male rats, ethanol still produced a dose-related attenuation of baroreflex sensitivity in female rats. The decrease in baroreflex sensitivity evoked by the higher dose (1 g kg^{-1}) of ethanol reached the level of statistical significance, compared with control (water-treated) rats, in both rat genders whereas the decrease by the middle dose (0.5 g kg^{-1}) of ethanol was significant only in male rats. It is notable that in previous studies significant attenuation of baroreflex function was a consistent finding after the 1 g kg^{-1} dose of ethanol, whereas the effect of the 0.5 g kg^{-1} dose was variable (Abdel-Rahman et al., 1987b; El-Mas and Abdel-Rahman, 1992; Sun and Reis, 1992; Varga and Kunos, 1992). Taken together, findings of the present study suggest that female rats are as susceptible as male rats to the deleterious effect of ethanol on baroreflex control of heart rate. It is notable that when the effects of ethanol were expressed as percent of baseline values, the decreases in baroreflex sensitivity elicited by ethanol in both genders were similar.

The hypothesis that the ability of ethanol to reduce baroreflex responsiveness involves hormonal factors was investigated in the present study. The results showed that in contrast to sham operation, ovariectomy abolished the depressant effect of ethanol on baroreflex-mediated bradycardia in conscious unrestrained rats. The lack of effect of ethanol on baroreflex sensitivity in ovariectomized rats provides the first experimental evidence that supports a role for ovarian hormones in the interaction of ethanol with the autonomic control of cardiac reflexes. The lack of the effect of ethanol on baroreflex sensitivity in ovariectomized rats cannot be accounted for by differences in blood ethanol concentrations or by hemodynamic consequences of ovariectomy. Blood ethanol concentrations, as well as baseline blood pressure and heart rate in sham-operated and ovariectomized rats, were not significantly different. It may be argued that the lack of an action of ethanol on baroreflex sensitivity in ovariectomized rats may be accounted for by a significantly lower basal (pre-ethanol) baroreflex sensitivity in these rats as compared with sham-operated rats. This issue may be addressed by findings of the present study, as well as by our previous findings. First, in the present study, the higher dose (1 g kg^{-1}) of ethanol produced similar attenuation of baroreflex sensitivity in intact male and female rats in spite of a significantly lower basal baroreflex sensitivity in female rats. Second, our previous findings have supported the notion that the basal level of baroreflex sensitivity has no impact on the inhibitory influence of ethanol on baroreflex sensitivity (Abdel-Rahman, 1994a; El-Mas and Abdel-Rahman, 1992, 1993a). Ethanol attenuated baroreflex sensitivity in carotid barodenervated rats (El-Mas and Abdel-Rahman, 1993a), but not in spontaneously hypertensive rats (Abdel-Rahman, 1994a) or aortic barodenervated rats (El-Mas and Abdel-Rahman, 1992), although the three rat models exhibit similar baseline baroreflex sensitivity values (Abdel-Rahman, 1994a; El-Mas and Abdel-Rahman, 1992, 1993a). Finally, ethanol significantly depressed the baroreflex sensitivity in anesthetized rats (Abdel-Rahman et al., 1987b; El-Mas and Abdel-Rahman, 1993b; Varga and Kunos, 1990, 1992), whose baseline baroreflex sensitivity is similar to that of ovariectomized rats in the present study.

The mechanism by which ovariectomy abolished the depressant effect of ethanol on baroreflex sensitivity is not clear. One possible explanation is that ovariectomy may alter central baroreceptor pathways that are involved in the depressant effect of ethanol on baroreflexes. This view may be supported by the present finding that ovariectomy caused a significant reduction in baroreflex sensitivity, suggesting a facilitatory role for ovarian hormones in the reflex control of heart rate. In effect, estrogen receptor mRNA-containing neurons have been identified in the nucleus tractus solitarius and caudal ventrolateral medulla (Simerly et al., 1990), brainstem areas that are involved in the central processing of baroreceptor information (Chalmers and Pilowsky, 1991; Lawrence and Jarrott, 1996) as well as in the depressant effect of ethanol on baroreflex sensitivity (El-Mas and Abdel-Rahman, 1993b; Varga and Kunos, 1990, 1992). Moreover, estrogen has been shown to facilitate and inhibit central glutamatergic (Wong and Moss, 1992) and γ-aminobutyric acid (GABA) (Kelly et al., 1992) neurotransmission, respectively. Both types of neurotransmissions are essential modulatory pathways in central baroreflex function (Lawrence and Jarrott, 1996). These latter findings seem important because our own findings (El-Mas and Abdel-Rahman, 1993b; Mao and Abdel-Rahman, 1995) and others (Sun and Reis, 1992; Varga and Kunos, 1990, 1992) have highlighted the importance of ethanol-evoked inhibition of glutamatergic neurotransmission and facilitation of GABA-ergic neurotransmission in its depressant effect on baroreflex sensitivity. Alternately, an ovariectomy-mediated alteration in central vasopressin activity may be responsible for the lack of ethanol-evoked inhibition of baroreflex sensitivity in ovariectomized rats. As discussed above, ovariectomy reduces vasopressin release (Peysner and Forsling, 1990), which is known to enhance baroreflex function (Peuler et al., 1990; Zhang et al., 1992). Interestingly, a recent study from our laboratory showed that ethanol attenuates the facilitatory action of vasopressin on baroreflex sensitivity (Mao and Abdel-Rahman, 1996). Taken together, these findings may suggest an important role for ovarian hormones in modulating the effect of ethanol on central baroreceptor pathways that mediate the depressant effect of ethanol on baroreflex sensitivity.

To summarize, the present study presents evidence to suggest that the ability of ethanol to attenuate baroreflex control of heart rate is not gender-related. Ethanol elicited significant decreases in baroreflex sensitivity that were similar in age-matched conscious male and female rats. Further, the lack of effect of ethanol on baroreflex sensitiv-

ity in ovariectomized rats may support a role for ovarian hormones in the mediation of ethanol-evoked attenuation of baroreflex function. The mechanism by which ovarian hormones influence the depressant effect of ethanol on baroreflex sensitivity needs to be investigated.

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