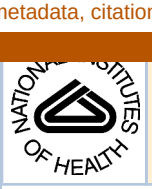

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Estrogen-dependent enhancement of NO production in the nucleus tractus solitarius contributes to ethanol-induced hypotension in conscious female rats

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Abstract

Background—Our previous pharmacological and cellular studies showed that peripheral (cardiac and vascular) NOS-derived NO is implicated in the estrogen-dependent hypotensive action of ethanol in female rats. The objective of this study was to test the hypothesis that enhanced NO production in the nucleus tractus solitarius (NTS) is implicated in the estrogen-dependent hypotensive action of ethanol.

Methods—To achieve this goal, we utilized in vivo electrochemistry to measure real time changes in neuronal NO to investigate the acute effects of intragastric ethanol (0, 0.5 or 1 g/kg) on NO in NTS neurons, blood pressure (BP) and heart rate (HR) in conscious female rats in the absence (ovariectomized, OVX, rats) or presence of estrogen.

Results—In sham operated (SO) rats, ethanol elicited dose-related increase in NTS NO and reduction in BP. These neurochemical and blood pressure effects of ethanol were absent in OVX rats. Whether the neurochemical effect of ethanol and the associated hypotension are dependent on *rapid* estrogen signaling was investigated. In OVX rats pretreated, 30 min earlier, with estrogen (E_2, 1μg/kg), intragastric ethanol (1 g/kg) increased NTS NO and reduced BP and these responses were comparable to those obtained in SO rats.

Conclusions—The present findings suggest that increased production of NO in NTS neurons contributes to ethanol-evoked hypotension in female rats. Further, ethanol enhancement of neuronal NO production in the brainstem is dependent on *rapid* estrogen signaling.

Keywords

Electrochemistry; NO; NTS; Ethanol; LPS; conscious Rat

Ethanol exerts complex cardiovascular actions, and its acute administration causes increases (El-Mas & Abdel-Rahman, 1992), decreases (Kawano *et al.*, 1992), or no change (Abdel-Rahman *et al.*, 1987a) in blood pressure. The mechanism by which ethanol increases blood pressure may be related to activation of sympathetic activity, as shown by the rise in plasma norepinephrine (Ireland *et al.*, 1984), and attenuation of the baroreflexes (El-Mas & Abdel-Rahman, 1992). On the other hand, ethanol causes direct myocardial depression (Zhang *et al.*, 2004), vasodilation (Turlapaty *et al.*, 1979), and α-adrenoceptor blockade (Abdel-Rahman *et al.*, 1987b); these effects may counterbalance the pressor response and result in no change in blood pressure or even hypotension. Our previous studies showed that the hemodynamic

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effects of ethanol are sexually dimorphic as intragastric ethanol elicits hypotension in female, but not in age-matched male, rats (El-Mas & Abdel-Rahman, 1999b). This hypotensive action of ethanol is estrogen dependent, because it is absent in ovariectomized rats, and is restored after estrogen replacement (El-Mas & Abdel-Rahman, 1999a). More recent studies have shown that pretreatment with NOS inhibitors virtually abolish the hypotensive action of ethanol in female rats (El-Mas *et al.*, 2006; El-Mas *et al.*, 2007). However, although the hypotensive effect of ethanol in female rats is well documented and is estrogen and NO-dependent, the current evidence is based on findings that implicated peripheral (mainly myocardial) NOS-NO signaling in the hypotensive response; a possible contribution of central NO to the hypotensive response has not been investigated.

The NTS plays a major role in the autonomic control of blood pressure. Previous studies showed that NOS isoforms are present in NTS and other brainstem neurons that project to it (Ohta *et al.*, 1993), and that increased production of NO in the NTS leads to sympatho-inhibition (Scislo *et al.*, 2005) and hypotension (Wu *et al.*, 2002). Evidence suggests that immediate early gene c-jun might regulate NOS expression (Qian *et al.*, 2007). Interestingly, ethanol enhances the expression of c-jun, in the NTS (Wang & Abdel-Rahman, 2004) and increases central NOS activity (Al-Rejaie & Dar, 2006). Reported findings also demonstrate the ability of estrogen to enhance NOS-derived NO via genomic as well as rapid signaling mechanisms (Kim & Bender, 2005). These reported findings raise the interesting possibility that NO in the NTS might be involved in ethanol-evoked hypotension in female rats.

In the present study, we tested the hypothesis that increased production of NO in the NTS may contribute to the estrogen-dependent hypotension elicited by ethanol in female rats. To achieve this goal, we investigated the effects of intragastric ethanol on NTS NO and blood pressure in conscious female rats in the presence or absence of estrogen. We measured real time changes in NO by in vivo electrochemistry, which enabled us to simultaneously investigate the effect of systemic ethanol on neuronal NTS and blood pressure. The neurochemical (NTS NO) and blood pressure effects of moderate amounts of ethanol (0.5 or 1 g/kg) were investigated in ovariectomized (OVX) rats, a model of surgical menopause (Riveiro *et al.*, 2001), and in age-matched sham-operated (SO) rats. Given the dependence of ethanol elicited hypotension on the estrogen, the studies were undertaken during the proestrus phase, which exhibit highest plasma estrogen level (Marcondes *et al.*, 2001). To ascertain whether *rapid* estrogen effects underlie ethanol-evoked enhancement of NO production in NTS neurons and the associated hypotension, the neurochemical and blood pressure effects of ethanol were investigated in OVX rats pretreated, 30 min earlier, with E_2 (1μg/kg). The studies were conducted in conscious rats to circumvent possible confounding effects of anesthesia on the measured variables.

Methods

Animals

The animals used in this study were Sprague Dawley female rats, 12–13 weeks old, obtained from Charles River (Raleigh, NC). All animals were maintained in an AAALAC-accredited animal housing facility. Food and water were continuously available to the animals. All surgical procedures were approved by the Institutional Animal Care and Use Committee.

Surgery

Intra-cranial cannulation—Rats were anesthetized with ketamine (90 mg/kg)-xylazine (10 mg/kg) mixture and supplemented as needed, then placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). Intra-cranial cannulation was performed as in our previous studies (Li *et al.*, 2005a, 2005b). Four small holes were carefully drilled in the skull to the level of the dura mater. Two screws were fixed into two of the holes; a 21.5 gauge guide cannula (Small

Parts Inc., Miami, FL) and a AgCl reference electrode were inserted into the other two holes, with the tip of guide cannula placed 2 mm above the NTS (Li *et al.*, 2005a, 2005b). Dental cement was used to secure the guide cannula and reference electrode. A protective cover, made out of a 1 ml Becton Dickinson syringe, was placed over the guide cannula and was secured with dental cement. Each rat received buprenorphine hydrochloride (i.p.) (Buprenex; 30μg/kg) and benzathine/penicillin G procaine in an aqueous suspension (s.c.) (Durapen, 50,000U/kg) postoperatively, and housed in a separate cage.

Vascular catheterization—Three days after intra-cranial cannulation, the rats were anesthetized as described above. Arterial and venous catheters (PE-50) filled with heparinized saline (100 units/ml) were advanced into the abdominal aorta and vena cave via the left femoral artery and vein, respectively. The distal ends of the catheters were plugged with stainless steel pins and guided subcutaneously to the back of the neck and exteriorized. Five days were allowed for recovery from intracranial surgery and 2 days were allowed after vascular surgery before the studies were performed.

Ovariectomy—Seven days before cannulation, ovariectomy was performed. For bilateral ovariectomy (OVX), the ovaries were isolated, tied off with sterile suture, and removed as described in our reported studies (El-Mas & Abdel-Rahman, 2000). The sham operation was performed by exposing the ovaries without isolation.

Nasogastric catheterization—Intragastric catheterization was performed on the day before the experiment by inserting a 20-cm polyethylene-50 tubing into the stomach through a nostril (El-Mas *et al.*, 2006). The tubing was bent by heat 6 cm from one end to a 45° angle to fit into the nose. The longer (14-cm) portion of the tubing was guided through the esophagus into the stomach. This technique allows intragastric administration of drugs in freely moving rats.

Preparation and calibration of the carbon fiber electrodes—The carbon fiber electrodes were prepared as reported (Gerhardt, 1995; Li et al., 2005a) and then coated with a two-step coating process prior to use; first with nafion and then with o-phenylenediamine (o-PD). Nafion coating performed by dip-coating in 5% nafion (Aldrich, Milwaukee, WI), dried at 85°C for 5 min repeated 7–10 times, which enhances its selectivity for monoamine neurotransmitters over anionic species such as ascorbic acid (AA) and DOPAC. Then the probes were electrochemically coated in 5 mM o-phenylenediamine dihydrochloride (1,2-benzenediamine, Sigma) solution (Friedemann *et al.*, 1996). A constant potential (+0.9 v vs Ag/AgCl reference) was applied on the electrode for 20 min. The solution was continually stirred during the coating process. Coated electrodes were calibrated before use. The sensitivity was measured by the response to an increasing concentration of NO, by adding increasing volume of standard 2 mM NO solution (Friedemann *et al.*, 1996) while the selectivity was measured against ascorbic acid (AA). Only electrodes whose sensitivity was >50000 (IVEC internal standard), and displayed a NO:AA ratio >500:1, and exhibiting a high degree of linearity ($r > 0.997$) in their response to increasing concentration of NO (2 to 10 μM), were used .

In Vivo Electrochemical studies—Real time NO monitoring was performed by a computer controlled electrochemical instrument (IVEC-10, Medical Systems Corporation) as described (Lin *et al.*, 1996; Wu *et al.*, 2002). An oxidation potential of +0.9 V, with respect to the Ag/AgCl reference electrode, was applied to the carbon fiber electrode for 0.1 sec at a rate of 5 Hz. The electrodes were maintained at a resting potential of 0.0 V between measurements. The sampling rate was set at 5 Hz and data were displayed and stored at 1 Hz. Therefore, each

data point represented the average of five measurements. The system can measure 1-sec NO concentration change, and its detection limit is 35 ± 7 nM NO (Friedemann *et al*., 1996).

Experimental protocols

The method described in our previous study (Li *et al*., 2005a) for simultaneous blood pressure and in vivo electrochemical monitoring was followed. On the day of the experiment, the arterial catheter was connected to a Gould-Statham (Oxnard, CA) pressure transducer and blood pressure was displayed on a Grass polygraph (model 7D, Grass Instrument Co., Quincy, MA); heart rate was computed from blood pressure waveforms by a Grass tachograph, and was displayed in another channel of the polygraph. A pre-calibrated carbon fiber electrode was inserted into the NTS via the guide cannula. The rat was allowed to stabilize for at least 90 min before drug administration. Sixty female rats, which survived the surgery out of 74 rats, were divided into 10 groups used in two experiments.

Experiment 1:Effect of intragastric ethanol on NTS NO and blood pressure— Four groups of sham-operated rats were used in this experiment. The rats in the SO groups were used during the same phase of the estrus cycle (proestrus) for two reasons: (i) to rule out potential impact of the fluctuations in endogenous estrogen (E_2) on the measured variables, and (ii) because the hypotensive effect of ethanol is estrogen-dependent (El-Mas & Abdel-Rahman, 1998). Following stabilization of the NO electrochemical signal and BP, the rats received water, or one of two doses of ethanol (0.5g/kg, or 1.0g/kg) via a nasogastric catheter. Ethanol was diluted with water and the overall volume administered was 1 ml/100 g body weight. The rats in the fourth SO group received LPS (5 mg/kg; i.v.) and served as a positive control because LPS causes increase in NO production in the NTS (Lin *et al*., 1999).

Experiment 2: Effect of intragastric ethanol on NTS NO and blood pressure in the absence or following acute replacement of estrogen—Six groups of OVX rats were used in this experiment. In three OVX groups, saline (0.1ml/100g, i.v., vehicle for E_2), was administered and was followed 30 min later with water, 0.5g/kg, or 1.0g/kg ethanol administered via a nasogastric catheter. The other three OVX groups received intragastric water or one of the two doses of ethanol 30 min after E_2 pretreatment (1μg/kg; i.v.). In all groups, the neurochemical and BP responses were followed for 90 min after ethanol or vehicle (water) administration.

Drugs

Ketamine (Phoenix Pharmaceutical. Inc., CA), alcohol (Midwest Grain Products Co., KS), buprenex (Rickitt & Colman, Richmond, VA), durapen (Vedco, Overland Park, KS), o-PD, nafion, estrogen, LPS (Sigma Chemical Co., MO).

Statistics

Mean arterial pressure was calculated as the diastolic pressure plus one-third the pulse pressure (differences of systolic-diastolic pressures). All data are presented as the mean ± SEM and calculated as the change from the pretreatment base line. Data were analyzed by Student's t-test when comparison was made between two groups, and by repeated measures ANOVA, followed by a Student-Newman-Keuls post-hoc analysis, for time-course comparisons; $p<0.05$ indicates significant difference.

Results

Effect of ethanol on NTS NO and BP in proestrus SO rats

The pretreatment baseline values for blood pressure and heart rate were similar in all groups of rats used in the study (Table1). The NTS NO, BP, and heart rate (HR) responses elicited by intragastric ethanol in SO rats are shown in Fig. 1 (representative tracings) and Fig 2. In a group of rats that served as positive control, LPS (5mg/kg; i.v.) increased NTS NO and lowered BP (data not shown). These findings, which agree with reported findings (Lin *et al.*, 1999), verified the identity of the measured NO in the present study. Compared with corresponding control (water), intragastric ethanol elicited dose-related increases in NTS NO and hypotensive responses; the increase in NTS NO level started within 10 min after ethanol administration and continued throughout the 90 min observation period (Fig. 2). The data presented as "area under the curve" also demonstrate the dose-dependent responses (Fig. 2, right panels). The neurochemical response was associated with a reduction in BP that started 10 min after ethanol administration, and continued throughout the observation period. The maximal hypotensive response occurred approximately 20 min after ethanol and BP tended to recover to pretreatment level by the end of the experiment (Fig. 2). The changes in HR in the ethanol groups were not statistically different from the corresponding control values (Fig. 2).

Effect of ethanol on NTS NO and BP in OVX rats

Baseline BP and HR of the 3 OVX groups that received water (control) or either dose of ethanol were similar (Table 1). As shown in Fig. 3, neither dose (0.5 or 1 g/kg) of intragastric ethanol caused any significant change in NTS NO or BP. On the other hand, comparison of the findings with the 1 g/kg dose of intragastric ethanol in OVX and age-matched SO rats clearly demonstrate that the neurochemical (increased NTS NO) and BP reduction caused by intragastric ethanol in SO rats were virtually abolished by prior ovariectomy (Fig. 2 and Fig 3). Baseline BP and HR values were similar in OVX and SO rats (Table 1).

Restoration of the neurochemical and BP effects of ethanol following acute pretreatment with estrogen in OVX rats

Three groups of OVX rats received i.v. E_2 (1 μg/kg) 30 min before intragastric ethanol (0.5 or 1 g/kg) or the vehicle (water). Acute E_2 (1 μg/kg; i.v.) administration had no effect on baseline BP or HR of OVX rats as compared to the corresponding values obtained in vehicle (saline)-treated OVX or SO rats (Table 1). However, in OVX E_2 pretreated rats, intragastric ethanol elicited dose-related increases in NTS NO and reductions in BP (Fig. 4). The neurochemical and BP responses elicited by the 1 g/kg dose of ethanol in E_2 pretreated OVX rats (Fig. 4) were significantly ($p<0.05$) greater than the corresponding responses observed in OVX rats (Fig. 3). Further, the neurochemical and BP responses elicited by intragastric ethanol in E_2 pretreated OVX rats were very similar ($p>0.05$) to the corresponding responses obtained in age matched proestrus SO rats (Fig. 2 and Fig 4). These findings were corroborated when the data were presented as "area under the curve" in Fig. 2 –Fig 4(right panels). The sites of NO measurement in the NTS are shown in Fig. 5.

Discussion

The objective of this study was to test the hypothesis that increased NO production in the NTS contributes to ethanol-evoked hypotension and that this neurochemical effect of ethanol is dependent, at least partly, on *rapid* estrogen signaling. The most important findings of the present study are: (i) ethanol elicited dose-related increases in NTS NO and reductions in BP in proestrus SO rats; (ii) the neurochemical and BP effects of ethanol were virtually abolished in OVX rats; (iii) acute (30 min) pretreatment with E_2 (1μg/kg) in OVX rats restored the neurochemical and BP responses elicited by ethanol to levels comparable to those seen in SO

rats. It is important to note that surgical (ovariectomy) or pharmacological (E_2) interventions employed in the study had no effect on baseline BP or HR. Together, the present findings yield insight into the role of *rapid* estrogen signaling in mediating a neurochemical effect of ethanol, which underlies, at least partly, the estrogen dependent hypotensive action of ethanol.

The present finding that systemic (intragastric) ethanol increased NTS NO is the first direct evidence of ethanol ability to enhance NO production in a brainstem area (NTS) that is implicated in blood pressure regulation. Earlier studies including ours utilized pharmacological NOS inhibitors to confirm the involvement of NOS-derived NO in BP effects of ethanol and focused on the role of peripheral (cardiac and/or vascular) NO in the observed responses (El-Mas *et al.*, 2006). We utilized in vivo electrochemistry to monitor instantaneous changes in NO in NTS neurons so that we can establish a temporal relationship between NTS NO and BP responses elicited by ethanol. This methodology has been used in a number of neuroscience and behavioral studies (Kobayashi *et al.*, 2000; Griveau *et al.*, 2007) but very few studies utilized this powerful tool in cardiovascular research. The present study showed that the hypotension elicited by ethanol in female rats, which is consistent with our previous findings (El-Mas & Abdel-Rahman, 1999a), was associated with significant increase in NTS NO level. The involvement of NO in the ethanol-evoked hypotension has been suggested by our previous finding that response was abolished by a nonselective NOS inhibitor (El-Mas *et al.*, 2006). Notably, nonselective NOS inhibition elicited hypertensive response, which might have confounded the data interpretation. The use of selective NOS inhibitors that caused little or no change in baseline blood pressure in our more recent studies circumvented such a limitation (El-Mas et al., 2008). Nonetheless, it was not possible to discern a role for central NO in ethanol-evoked hypotension in these previous studies.

We reasoned that ethanol evoked enhancement of NO production in NTS neurons might contribute to ethanol-evoked hypotension in female rats for the following reasons. (i) NTS NO plays an important role in cardiovascular regulation (Dias *et al.*, 2005) and increased production of NO in the NTS leads to hypotension (Tseng *et al.*, 1996). (ii) Ethanol enhances NOS activity in many brain areas (Davda *et al.*, 1993; Xia *et al.*, 1999). Our results are consistent with the notion that enhanced NO production in the NTS contributes to ethanol evoked hypotension. However, because ethanol-evoked hypotension is estrogen-dependent and estrogen was injected intravenously in the present study, it is important to comment on the ability of *acutely* administered estrogen to modulate neuronal signaling within the NTS. This is particularly important because we did not measure estrogen level in CSF in the present study. Notably, the findings of the following reported studies including ours support the ability of systemically administered estrogen to elicit rapid neuronal signaling within brain nuclei. First, systemically administered estradiol enhances BRS in OVX rats, at least partly, via a central mechanism of action (Mohamed et al., 1999). Second, more direct evidence demonstrated the ability of microinjected estrogen into the NTS to enhance BRS in OVX rats (Saleh et al., 2000). Importantly, intravenously administered estradiol reaches peak levels in plasma and CSF within 15 min (van den Berg et al., 2004). Together, these findings suggest that systemically administered estrogen can influence neuronal signaling in the NTS. Whether estrogen is locally produced in the NTS has not been investigated in the present study. Notably, the estrogen synthetizing enzyme aromatase exists in many brain areas (Lephart, 1996) but there are no reports on whether the enzyme is present in the NTS. This issue needs to be investigated in future studies.

The possibility must be considered that enhanced NO production in other brain areas or peripheral tissues might contribute to the hypotensive response because ethanol was administered systematically. For example, our recent studies showed that increased vascular iNOS content/activity (El-Mas *et al.*, 2006) or cardiac NOS activity (El-Mas *et al.*, 2008) contributes to ethanol-evoked hypotension in female rats. Interestingly, these effects of ethanol

on vascular and cardiac NOS are mediated, at least partly, by ethanol-evoked endotoxemia, which is reported in a number of studies (Fukui, 2005) and confirmed in our recent study (El-Mas *et al.*, 2008). It is imperative to note that systemic endotoxins (e.g. LPS) cause increase in NTS NO along with the hypotensive response (Lin *et al.*, 1999). We have replicated these latter findings in our model system by demonstrating the ability of i.v. LPS to increase NTS NO along with the hypotensive response (data not shown). It is possible, therefore, that ethanol enhancement of NTS NO production could be a result of direct action of ethanol on brainstem neurons and/or indirectly mediated through the systemic iNOS. However, regardless of the mechanism implicated, it might be argued that the increase in NTS NO was a consequence, rather than a cause, of the hypotensive response elicited by ethanol. This possibility is unlikely for two reasons. First, the rise and peak of NTS NO preceded the reduction in BP (see representative tracing in Fig.1). Second, hypotension elicits neuronal signaling that leads to sympathoexcitation (Julien *et al.*, 1995) while NTS NO is implicated in sympathoinhibition (Wu *et al.*, 2002). The latter notion is further supported by the findings that nonselective inhibition of central NOS virtually abolished clonidine-evoked hypotension, which is mediated by central sympathoinhibition (Nassar & Abdel-Rahman, 2008). Together, these findings support the contribution of NTS NO to ethanol-evoked hypotension in female rats.

In addition to confirming our previous findings that ethanol-evoked hypotension is absent in OVX rats (El-Mas & Abdel-Rahman, 1999a), we present evidence that ethanol enhancement of NO production in the NTS is dependent on ovarian hormones. These findings are consistent with the conclusion that the neurochemical effect (increased NTS NO) underlies, at least partly, the hypotensive response elicited by intragastric ethanol. However, to confirm the dependence of the neurochemical effect of ethanol on ovarian hormones, particularly estrogen, we investigated the effects of ethanol on NTS NO and BP following estrogen replacement. In our previous studies, we demonstrated restoration of ethanol-evoked hypotension in OVX rats that received s.c. E_2 in osmotic mini-pumps or silastic tubing at least 2 days before ethanol administration. Nonetheless, we felt it important in the present study to determine whether ethanol-evoked hypotension could be restored along with the increase in NTS NO following acute administration of E_2 in OVX rats. In addition to the long-term regulation of gene expression (hours to days), *rapid* (nongenomic) , estrogen signaling occurs within minutes and involves activation of cellular kinases, synthases and ion channels (Edwards, 2005), that are dependent on NOS-NO signaling. Recent findings showed that GPR30, a member of the G-protein-coupled receptor (GPCR) super-family, expressed predominantly at the cell surface, might take part in mediating the *rapid* effect of estrogen (Prossnitz *et al.*, 2008). More pertinent to the present study the findings that estrogen-dependent neurobiological responses occur within the NTS within minutes of estrogen administration (Saleh *et al.*, 2000). The present findings are the first that demonstrated the restoration of ethanol-evoked hypotension within minutes of estrogen replacement. Therefore, the estrogen-dependent hypotensive response elicited by ethanol in our previous studies (El-Mas & Abdel-Rahman, 1999a) seems to be mediated, at least partly, via rapid estrogen receptor signaling. In support of this notion, we demonstrate the ability of intragastric ethanol to enhance NTS NO production within minutes after estrogen administration in OVX rats. Together, these findings highlight a pivotal role for rapid estrogen signaling in ethanol-evoked hypotension. It must be remembered that since ethanol and E_2 were administered systemically, the possibility cannot be overlooked that NO-dependent signaling in peripheral tissues or brain nuclei other than the NTS or E_2-signaling unrelated to NO might contribute to the hypotensive response.

In summery, we present evidence that systemic (intragastric) ethanol enhances the production of NO in NTS neurons. This neurochemical effect of ethanol mediates, at least partly, the hypotensive effect of ethanol in female rats. The neurochemical and blood pressure responses elicited by ethanol are estrogen dependent because they are abolished in OVX rats and restored following estrogen replacement. Equally important, these effects of ethanol are dependent on

rapid estrogen signaling. Although the molecular mechanisms that underlie the dependence of the neurobiological effects of ethanol on estrogen signaling remain to be elucidated, the present findings yield insight into the dependence of a neurochemical (NTS NO) that underlies, at least partly, the hypotensive action of ethanol on *rapid* estrogen signaling.

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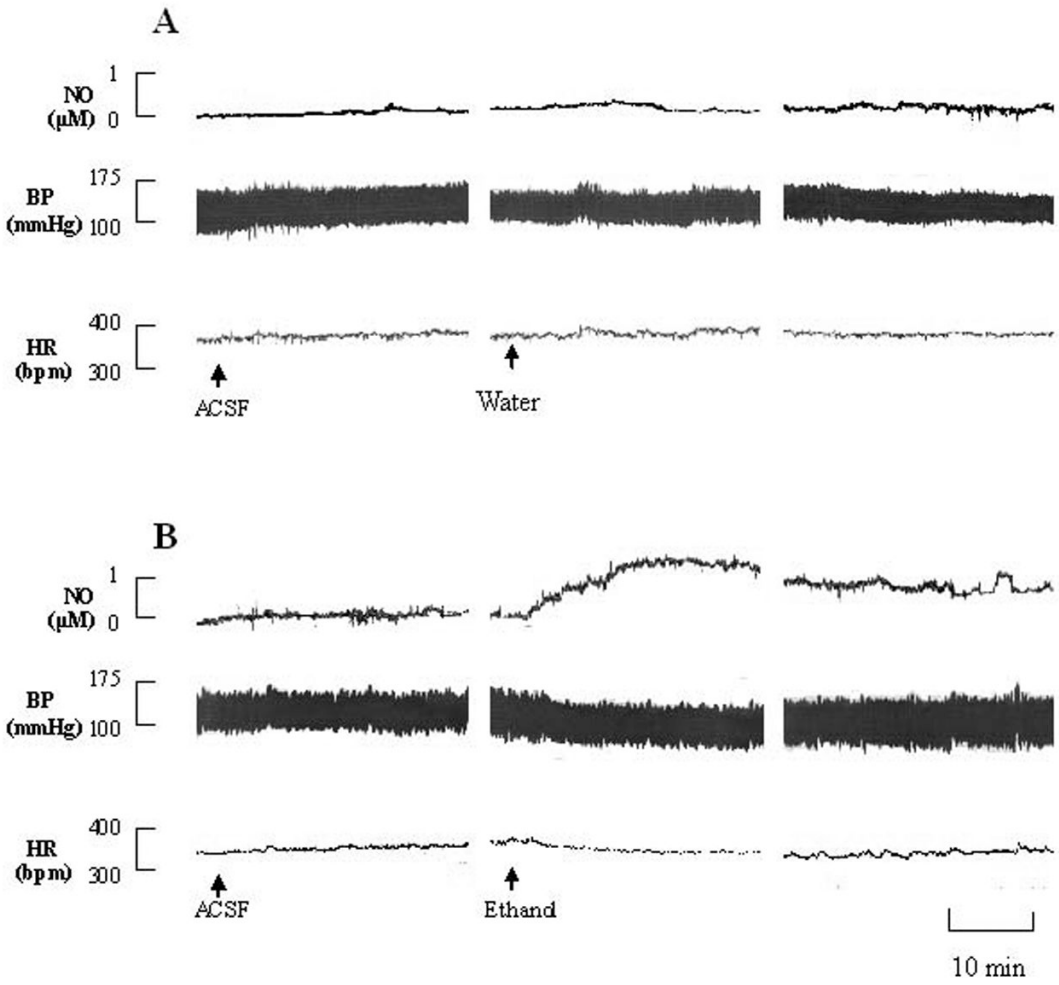

Fig. 1.

Representative tracings depicting the changes in nucleus tractus solitarius (NTS) nitric oxide (NO), blood pressure (BP) and heart rate (HR) elicited by intragastric ethanol (1g/kg), compared with equal volume of vehicle (water), in conscious freely moving sham-operated SD female rats.

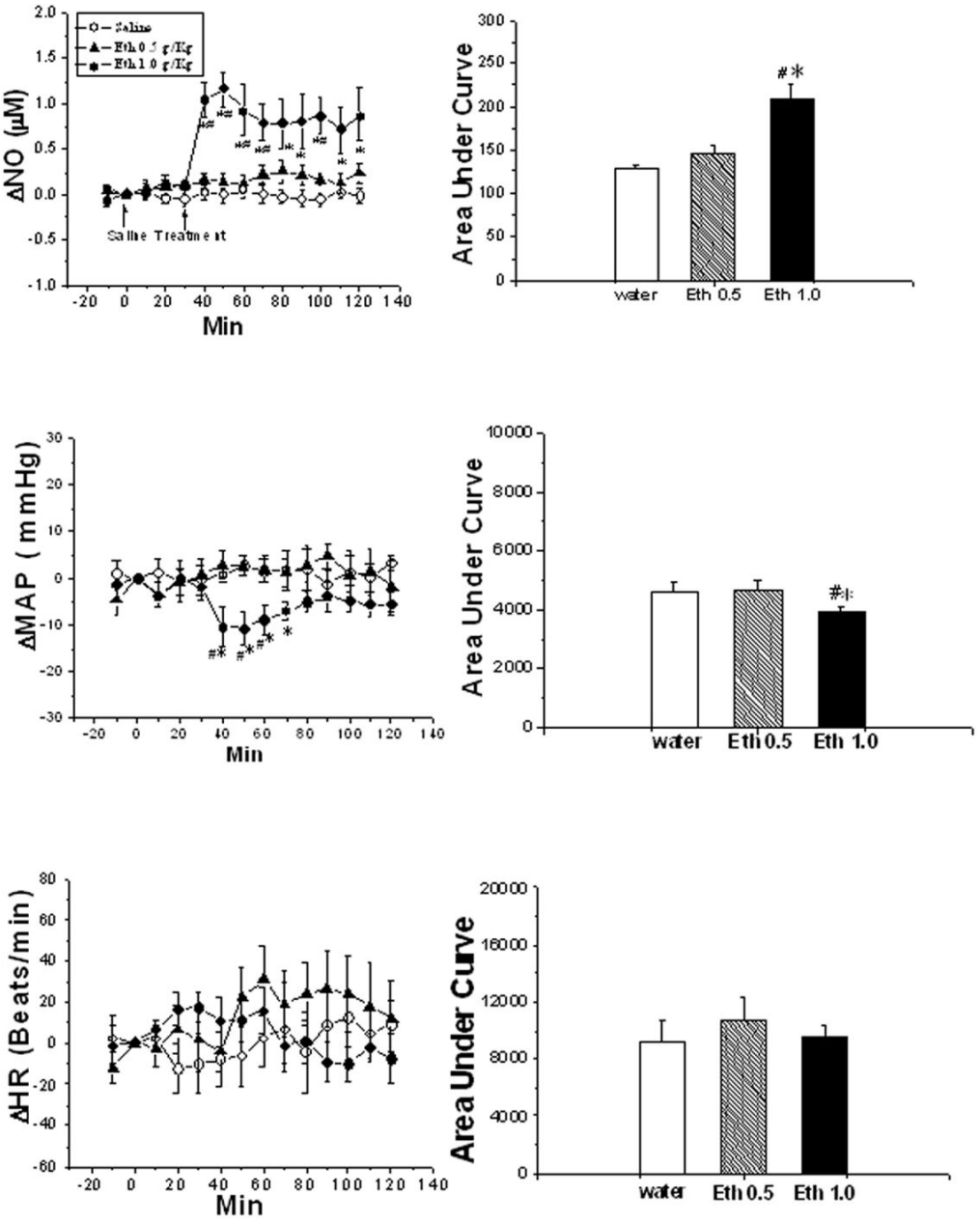

Fig. 2.

Effect of intragastric ethanol (Eth 0.5 or 1.0 g/kg), compared with vehicle (water), on nucleus tractus solitarius (NTS) nitric oxide (NO) level, blood pressure (MAP), and heart rate (HR) in conscious freely moving sham-operated female SD rats. The bar graphs show the Area Under the Curve for the measured variables. Values are mean ± SEM. P<0.05 compared with corresponding control, #P<0.05 compared with ethanol 0.5g/kg group.

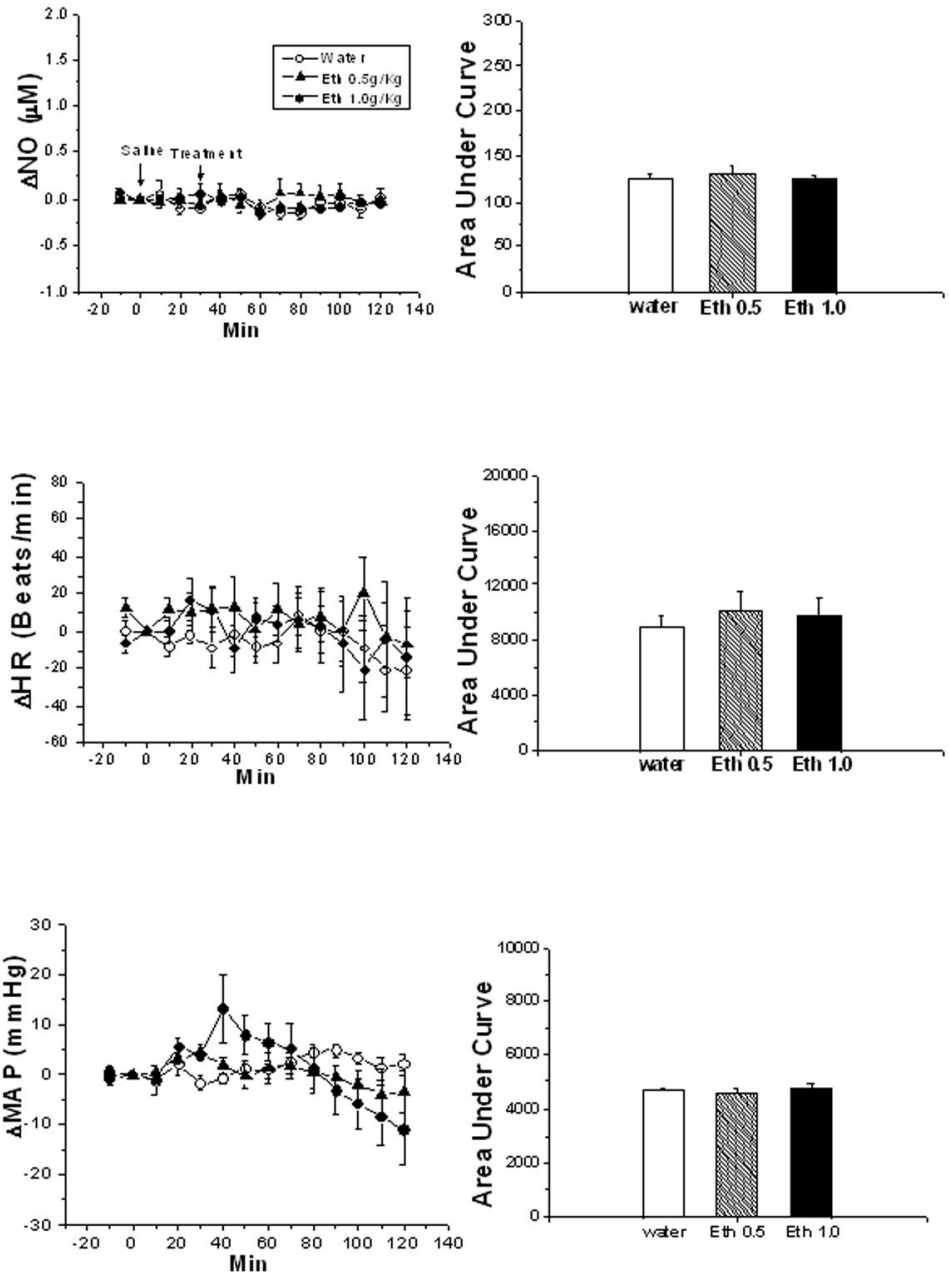

Fig. 3.

Effect of intragastric ethanol (Eth 0.5 or 1.0 g/kg), compared with vehicle (water), on nucleus tractus solitarius (NTS) nitric oxide (NO) level, blood pressure (MAP), and heart rate (HR) in conscious freely moving ovariectomized SD rats. The bar graphs show the Area Under the Curve for the measured variables. Values are mean ± SEM.

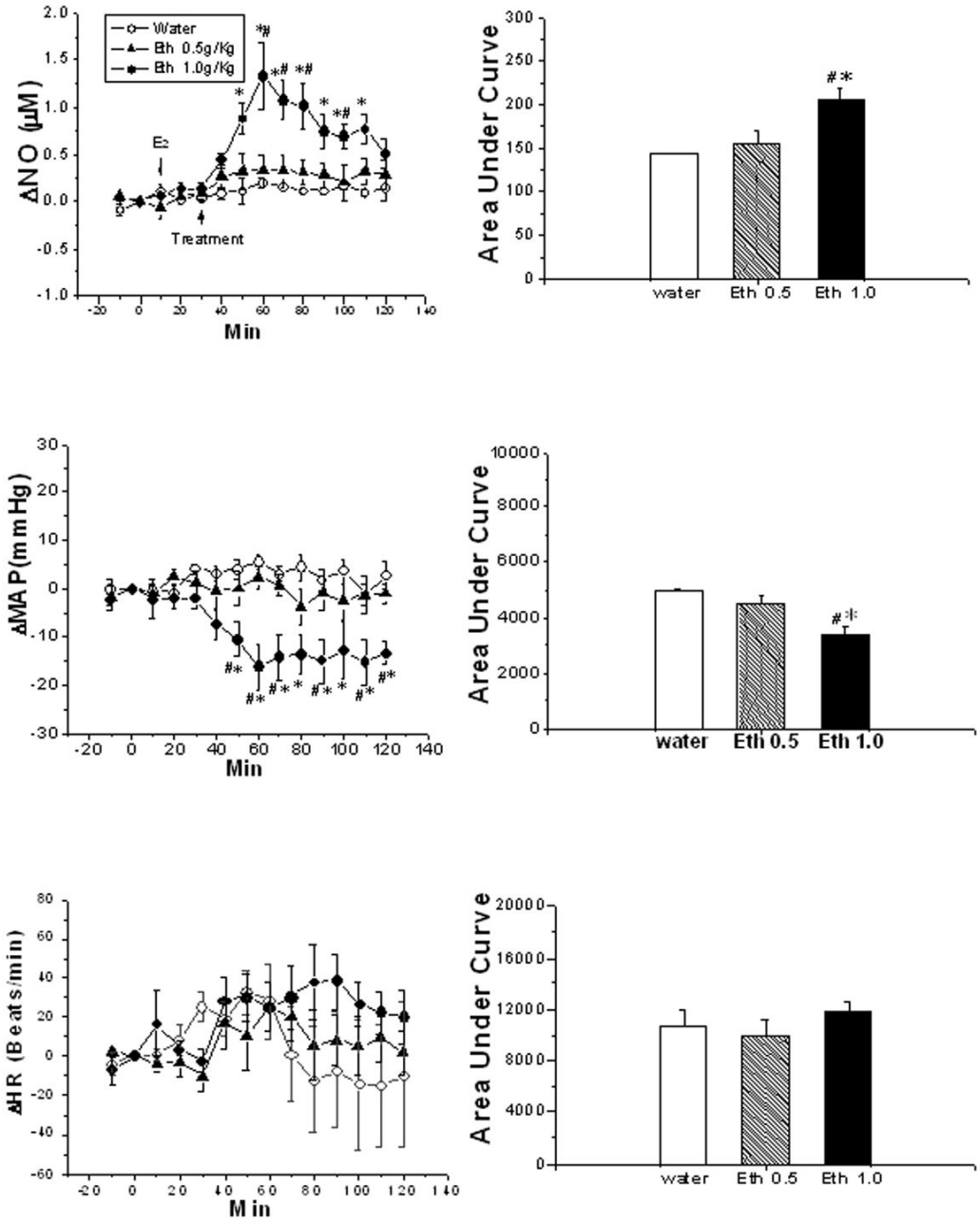

Fig. 4.

Effect of acute (30 min) estrogen (E_2, 1μg/kg, i.v.) pretreatment in conscious ovariectomized rats on the neurochemical (NTS NO), blood pressure (MAP), and heart rate (HR) responses elicited by intragastric ethanol (Eth 0.5 or 1.0 g/kg) or the vehicle (water). The bar graphs show the Area Under the Curve for the measured variables. Values are mean ± SEM. *P<0.05 compared with corresponding control, #P<0.05 compared with ethanol 0.5g/kg group.

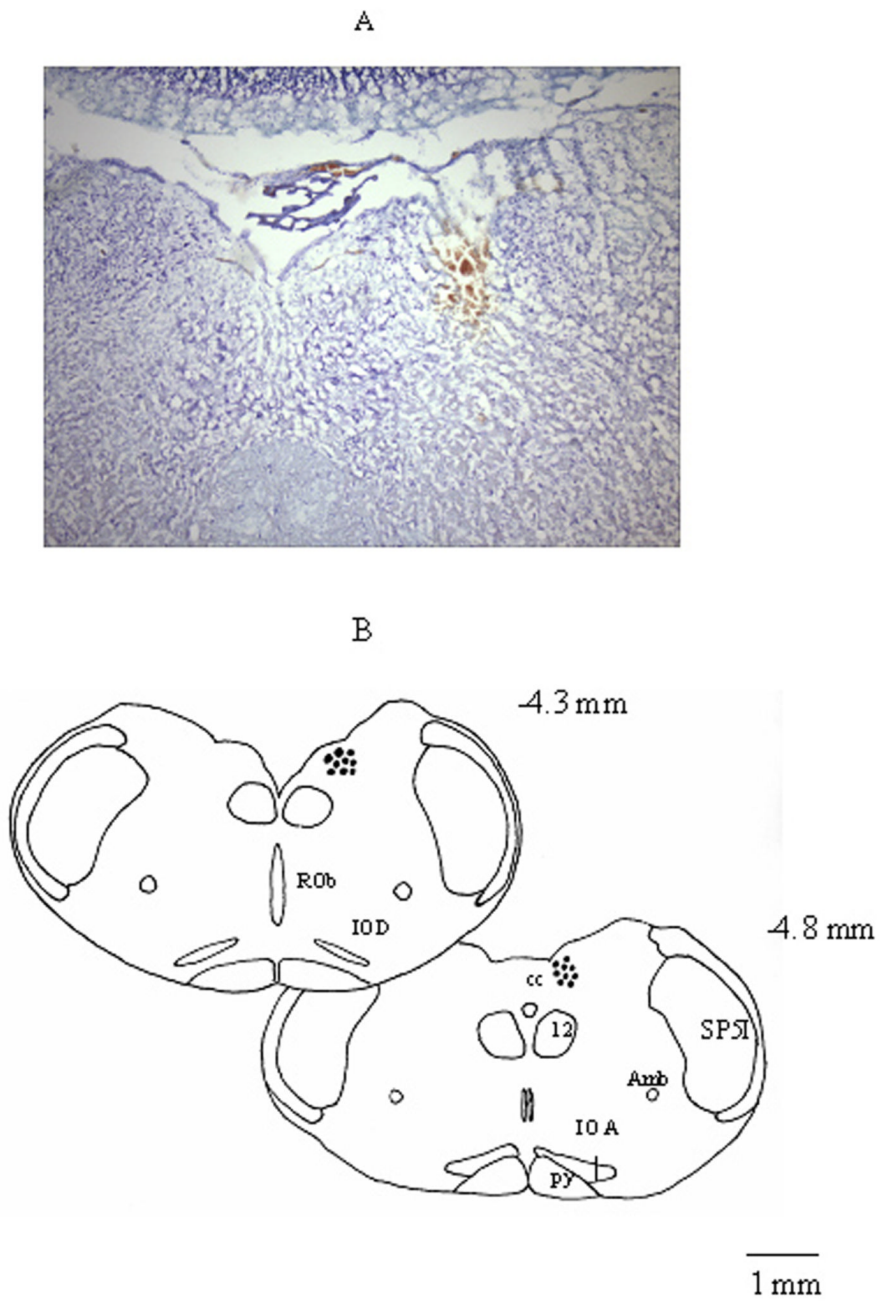

Fig. 5.

Picture (A) and schematic diagrams (B) of coronal sections of rat brainstem showing the electrochemical recording sites in the NTS. The rostro-caudal coordinates related to interaural line are in the upper right corner of each diagram (B). Py, pyramidal tract; ROb, raphe obscurus nu; IOD, inferior olive, dorsal nu; cc, central canal; SP5I, spinal 5 nu, interpolar part; Amb, ambiguus nu; 12, hypoglossal nu; IOA, IO, subnu Aof medial nu. According to Paxinos and Watson (Paxinos & Watson, 1998).

Table 1

Baseline Blood Pressure and Heart Rate

Baseline (pretreatment) values of mean arterial pressure (MAP, mmHg) and heart rate (HR, beat/min) of conscious sham operated (SO), ovariectomized (OVX) or OVX-estrogen (E_2)- pretreated female SD rats

Treatment	ACSF		Ethanol (0.5g/kg)		Ethanol (1g/kg)	
	MAP	HR	MAP	HR	MAP	HR
SO	122 ± 4	355 ± 22	123 ± 6	338± 17	129 ± 7	352 ± 23
OVX	121 ± 5	331 ± 24	128 ± 7	367 ± 20	118 ± 7	363 ± 22
OVX+E_2	119 ± 4	325 ± 20	118 ± 4	356 ± 21	127 ± 6	337 ± 30

Values are mean ± SEM; n = 6 in each group.