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A Differential Action for Ethanol on Baroreceptor Reflex Control of Heart Rate and Sympathetic Efferent Discharge in Rats (42630)

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Abstract. The acute effects of ethanol (0.33, 0.66, or 1 g/kg) on baroreflex control of heart rate (HR) and sympathetic efferent discharge (SED) were investigated in chloralose-anesthetized rats. The two higher doses of ethanol caused a progressive and significant increase in baseline SED and a slight increase in HR. That these effects were ethanol mediated is suggested by the absence of any change in blood pressure following ethanol injection in any amount used and the finding that equivolume saline had no effect on any of the tested parameters. On the other hand, the baroreflex slope of the MAP-SED relationship after ethanol was similar to the control (preethanol) value in contrast to a significant decrease in the baroreflex slope of MAP-HR under the same conditions. These findings suggest that the sensitivity of the reflex control of SED was preserved whereas that of HR was impaired after acute ethanol administration. Since these findings were obtained in the same animals, our data suggest that acute ethanol has a differential action on reflex control of SED and HR. Further, the significant increase in SED after moderate and high doses of ethanol suggests an increased central sympathetic tone as recordings were made from preganglionic nerve fibers (splanchnic nerve). The absence of an increase in baseline MAP, in spite of a significant increase in baseline SED following acute ethanol injection, could be explained, at least in part, by an ethanol-evoked reduction in pressor responsiveness to phenylephrine, an α-adrenergic agonist.

The responses of the cardiovascular variables blood pressure (BP) and heart rate (HR) following the acute administration of ethanol have been reported to vary from no change (1–3) to a slight increase (4, 5). These differences are due, in part, to differences in the dose of ethanol employed and the different protocols of the experiments but are most likely due to the complex central and peripheral effects of ethanol. For example, acute ethanol administration produces a direct cardiac depressant effect (6, 7) which may be masked by ethanol-induced sympathetic overactivity (7). Increased sympathetic activity after either acute (4) or chronic (8, 9) ethanol administration has been shown by our experiments involving cardiac autonomic blockade (8) and by others as an increase in circulating plasma catecholamines (9). It is not known if these changes involve the peripheral nervous system (e.g., modifying feedback or ganglionic transmission) or central sympathetic tone since information about the effect of ethanol on the direct changes in neural activity is lacking.

In a series of studies designed to evaluate the role of arterial baroreceptors in the development of ethanol-induced hypertension we have recently shown that acute ethanol administration impaired the baroreceptor reflex control of heart rate in normotensive humans and that this effect was related to blood ethanol levels (10). Furthermore, we have also shown that ethanol feeding in rats substantially impaired the baroreflex control of heart rate even in the absence of any change in blood pressure (11), an effect which preceded the development of ethanol-induced hypertension in this model (8). These findings lead us to believe that an ethanol-induced abnormality in arterial baroreceptors may be an important factor in ethanol-induced hypertension and that the rat could be used as an appropriate animal model for studies which could not be performed in humans since our findings in both species were similar. However, we recognize that although the baroreflex control of HR is widely used as a measure of baroreflex function (12–15) it may (16) or may not (17, 18) be indicative of changes in peripheral vascular resistance or of changes in sympathetic efferent discharge (SED). Therefore, we felt it important to determine, in the same animal,

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the effects of acute ethanol administration on the simultaneous baroreflex control of HR and SED. In this study we report the effects of acute administration of ethanol on central sympathetic activity as measured by recording splanchnic nerve activity, baroreflex control of HR and SED, and pressor responsiveness to phenylephrine with doses of ethanol which have no effect on baseline blood pressure.

Materials and Methods. *General methods and preparation.* Male Sprague–Dawley rats weighing 330–400 g were anesthetized with ip α chloralose (100 mg/kg). A PE50 catheter was inserted into a carotid artery and a jugular vein was cannulated for iv injections of phenylephrine and ethanol or saline. The arterial cannula was connected to a Statham P23db pressure transducer and blood pressure was displayed on a Grass Model 7D polygraph. Heart rate was recorded with a Grass tachograph and simultaneously recorded on another channel of the polygraph. The blood gases and pH were monitored periodically and none of the doses of ethanol used had any significant effect on these parameters during the course of the experiment. Rectal temperature was measured and maintained between 36 and 38°C by external warming.

Under a dissecting microscope, the greater splanchnic nerve was exposed through a midline abdominal incision. Nerve activity was recorded from the central end of the cut splanchnic nerve using a bipolar platinum electrode under a mineral oil pool according to the technique described by Guo and Abboud (18). The recorded spikes were amplified by a Grass P511 preamplifier, and the amplified nerve activity was visualized on a Tektronix storage oscilloscope. The output was also fed to a Spike Digitimer D130 processor with a loud speaker that measured the frequency of the impulses that exceeded a preset lower window level which is set above the noise level. Digital output (spikes/5 sec) was read off the counter and the output in analog form was displayed as a time–frequency histogram on a third channel of the Grass polygraph along with blood pressure and heart rate. As a routine, at the end of each experiment the nerve was crushed proximal to the recording point and the experiment was counted successful if the Spike processor output fell to zero or to not more than 10% of the recorded activity; the noise remaining after crushing the nerve was subtracted from the values recorded during the experiment.

Protocol. A period of at least 30 min was allowed for stabilization of BP, HR, and SED after completion of the surgical procedures. Each rat served as his control and we examined the baroreflex responses of HR and SED following evoked graded elevations in blood pressure by iv phenylephrine. Arterial pressure was raised by bolus iv injections of phenylephrine (0.25–16 μg/kg). At least five sequential doses of phenylephrine were injected in the pretreatment control period which raised blood pressure over the range of 20 to 75 mm Hg. These rises in blood pressure were associated with reflex decreases in HR and SED and a highly significant correlation ($r = 0.8$–0.99) existed between MAP as the independent variable and SED or HR as the dependent variable. The decreases in HR and SED induced by rises in blood pressure within this range were abolished by sinoaortic baroreceptor denervation, indicating the reflex nature of the response and its mediation predominantly through the arterial baroreceptors. Ethanol (0.33, 0.66, or 1.0 g/kg) or saline was infused iv over a 3-min period and the same protocol was repeated with increasing the doses of phenylephrine as needed to attain rises in blood pressure similar to those obtained before treatment. Blood ethanol concentration was determined at 15, 30, and 45 min after iv infusion of 0.5 g/kg of ethanol by the method of Bonnichsen and Lundgren (19). Each rat received only one treatment (ethanol or saline) and 6–11 rats per group were used in the four groups. Phenylephrine injections started 5 min after completion of ethanol or saline infusion and lasted for at least 30 min with 5 min allowed between injections.

Data analysis. Mean values ± SE are presented. The relationship between increases in MAP and associated decreases in HR or percent decrease in SED was assessed by regression analysis for each animal. The regression coefficient (slope of the regression line) was taken as an index of reflex sensitivity and the difference in slopes in response to treatment

was determined by the paired t test, unpaired t test, or analysis of variance. Probability levels of less than 0.05 were considered statistically significant.

Results. None of the doses of ethanol used nor an equivalent volume of saline caused any change in baseline blood pressure or heart rate. Although baseline heart rate and blood pressure values before and after all doses of ethanol are not presented, Fig. 1 shows that baseline heart rate and blood pressure were not affected by administration of 1 g/kg of ethanol. However, doses of 0.66 and 1 g/kg of ethanol caused a progressive increase in baseline SED which began within 5 min after ethanol administration and was still elevated 30 min later which was the usual time the experiment was terminated. The increase after 0.66 g/kg was 18% at 5 min and 20% at 30 min whereas after 1 g/kg of ethanol the increases were 55 and 80% at 5 and 30 min, respectively. Neither the 0.33

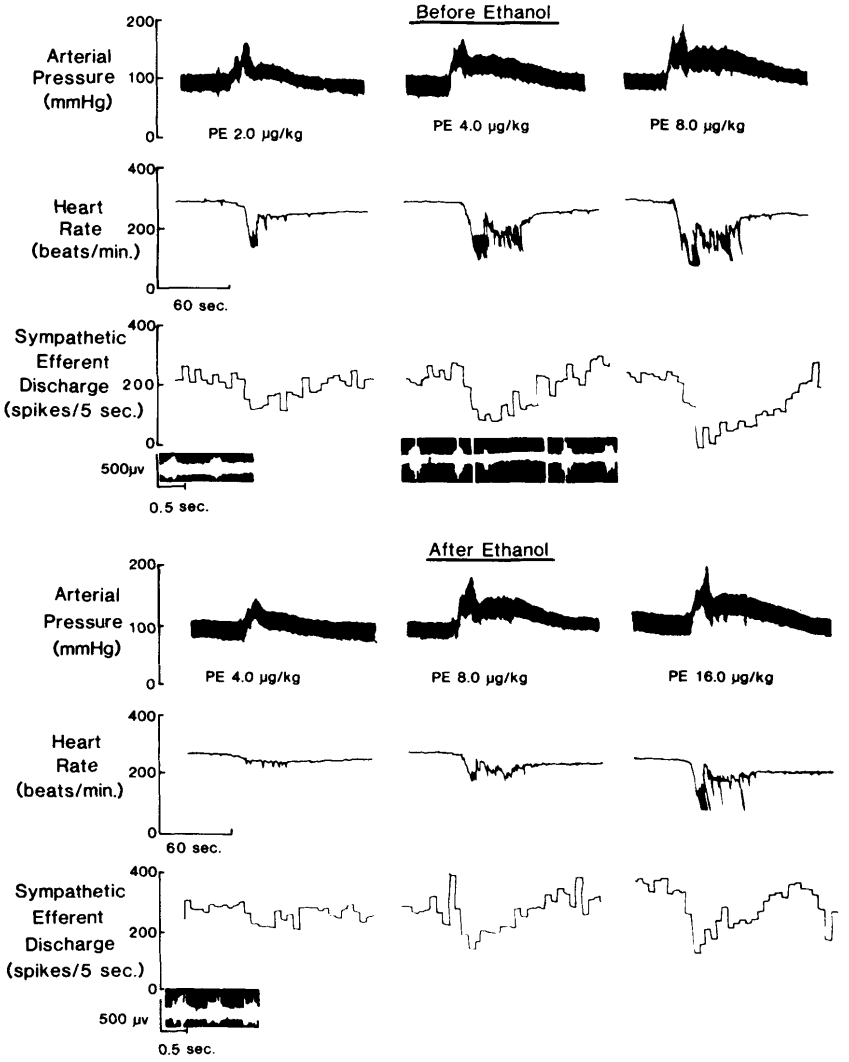

FIG. 1. Original records showing arterial baroreflex responses to increases in arterial pressure induced by phenylephrine (PE) in a chloralose-anesthetized rat before (top) and after (lower) iv infusion of 1 g/kg ethanol. Note that reflex bradycardia, but not reflex sympathoinhibition, is less after ethanol and that a twofold increase in PE dosage is required after ethanol to evoke similar rises in arterial pressure.

g/kg dose of ethanol nor the saline had any effect on baseline SED.

A representative record of the increase in baseline SED and of the reflex response of HR and SED during evoked increases in BP by phenylephrine before and after the infusion of 1 g/kg of ethanol is shown in Fig. 1. The reflex bradycardia associated with comparable increases in evoked BP was much less after ethanol even though baseline BP and HR after ethanol were similar to the control values. However, as also shown in Fig. 1, the reflex inhibition in SED was still preserved after acute ethanol administration. Baroreflex curves, relating percent decreases in SED to PE-evoked increases in BP are shown in Fig. 2 and baroreflex sensitivity values, expressed as the slopes of regression lines of these relationships, are reported in Table I. These data show that in spite of a small upward shift in the baroreflex curves after ethanol but not saline administration (Fig. 2), the baroreflex sensitivity values were similar after the three doses of ethanol as compared to control values (Table I). Thus, ethanol does not seem to alter the baroreflex-mediated sympathoinhibition.

On the other hand, when baroreflex curves, relating the baroreflex mediated bradycardia to evoked rises in blood pres-

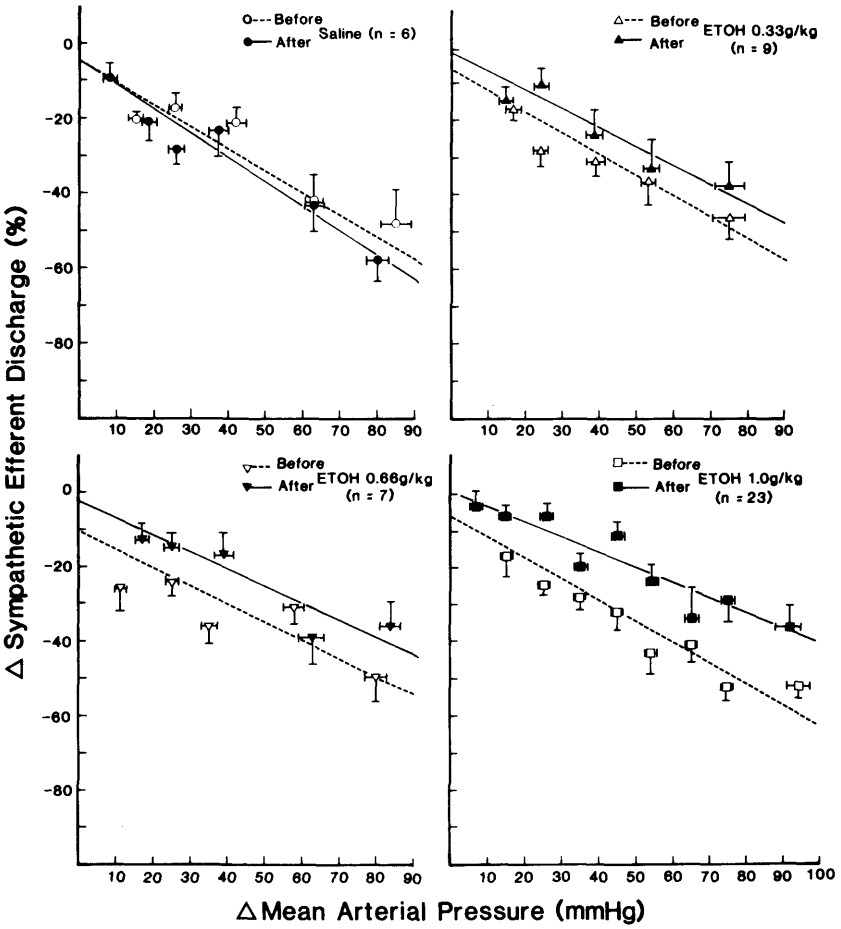

FIG. 2. Mean changes in sympathetic efferent discharge in response to increments in arterial pressure evoked by phenylephrine before and after saline or ethanol administration; the doses of ethanol and the number of rats in each group are shown. The relationship between the changes in both variables is highly significant in the four groups both before and after treatment and ethanol has no effect on the slope of the linear regression lines (baroreflex sensitivity) (see Table I). Values are means ± SE.

TABLE I. REGRESSION COEFFICIENTS OF REFLEX CHANGES IN SYMPATHETIC EFFERENT DISCHARGE AND HEART RATE FOLLOWING PHENYLEPHRINE INJECTIONS

	Saline (n = 6)		Ethanol (0.33 g/kg) (n = 9)		Ethanol (0.66 g/kg) (n = 7)		Ethanol (1 g/kg) (n = 23)	
	Before	After	Before	After	Before	After	Before	After
SED responses								
% SED/mm Hg	0.53 ± 0.09	0.65 ± 0.03	0.56 ± 0.03	0.50 ± 0.03	0.47 ± 0.07	0.46 ± 0.02	0.55 ± 0.02	0.42 ± 0.07
r	0.95 ± 0.02	0.97 ± 0.06	0.95 ± 0.01	0.96 ± 0.02	0.87 ± 0.05	0.95 ± 0.06	0.96 ± 0.05	0.96 ± 0.02
HR responses								
beats · min^{-1} · mm Hg^{-1}	1.19 ± 0.15	0.94 ± 0.19	0.56 ± 0.13	0.39 ± 0.03	0.65 ± 0.11	0.35 ± 0.07*	1.20 ± 0.15[a]	0.65 ± 0.07*
r	0.98 ± 0.02	0.93 ± 0.06	0.96 ± 0.14	0.87 ± 0.02	0.99 ± 0.03	0.94 ± 0.04	0.93 ± 0.04	0.90 ± 0.03

Note. Values represent mean regression coefficient (slopes; baroreflex sensitivity) ± SE; r values under each regression coefficient indicate correlation coefficient. SED, sympathetic efferent discharge; HR, heart rate.

[a] HR responses were obtained from 12 out of the 23 rats used in this group.

* Indicates significantly different ($P < 0.05$) from pretreatment values.

sure, were compared in the same animals before and after ethanol or saline the data clearly showed that ethanol caused a significant upward shift in baroreflex curves with a reduction in the slope (Fig. 3). This effect was ethanol mediated as equivalent volume of saline was without an effect on the baroreceptor heart rate response curve (Fig. 3). The inhibitory effect of ethanol on baroreflex HR sensitivity was dose related since it caused an inhibition of baroreflex sensitivity of 30, 46, and 48% following administration of 0.33, 0.66, and 1 g/kg, respectively (Table I).

The effect of acute ethanol administration on the pressor responsiveness to phenylephrine is shown in Fig. 4. Neither saline nor the 0.33 g/kg of ethanol had any significant effect on pressor responsiveness to phenylephrine. However, doses of 0.66 and 1 g/kg caused a significant downward shift in the phenylephrine dose–response curve, indicating a reduced pressor responsiveness. Blood ethanol levels at 15, 30, and 45 min after administration of 0.5 g/kg of ethanol were 0.5 ± 0.15, 0.35 ± 0.03, and 0.14 ± 0.05 mg/ml, respectively.

Discussion. The major finding of the present study was that acute ethanol administration had a differential effect on baroreflex control of heart rate and SED: it impaired baroreflex control of heart rate but not that of SED. This differential effect of ethanol supports the observations (18) that changes in baroreflex heart rate response cannot accurately reflect other changes in baroreceptor-controlled variables. Because of this and because of our previous findings that ethanol impaired the heart rate response in rats (11) and humans (10) we felt it was important to simultaneously evaluate the effects of ethanol on two baroreflex-controlled variables, HR and SED, in the same animal. We recorded sympathetic neural activity from a preganglionic nerve (splanchnic nerve) and the data suggested that ethanol induced a state of augmented central sympathetic tone. This finding agrees well with studies which measured plasma norepinephrine levels as an index of sympathetic neural activity and which found that acute ethanol administration, in doses comparable to those used in the present study, increased the level of this catecholamine (4). However, in spite of the substantial increase in SED after all doses of ethanol there was no change in baseline MAP or HR.

The lack of ethanol-induced heart rate changes in the presence of enhanced SED is somewhat surprising since previous studies from our laboratory have shown that similar doses of ethanol increased baseline heart rate of normotensive humans (10) and rats (1). It is known, however, that the final chronotropic change after ethanol administration is

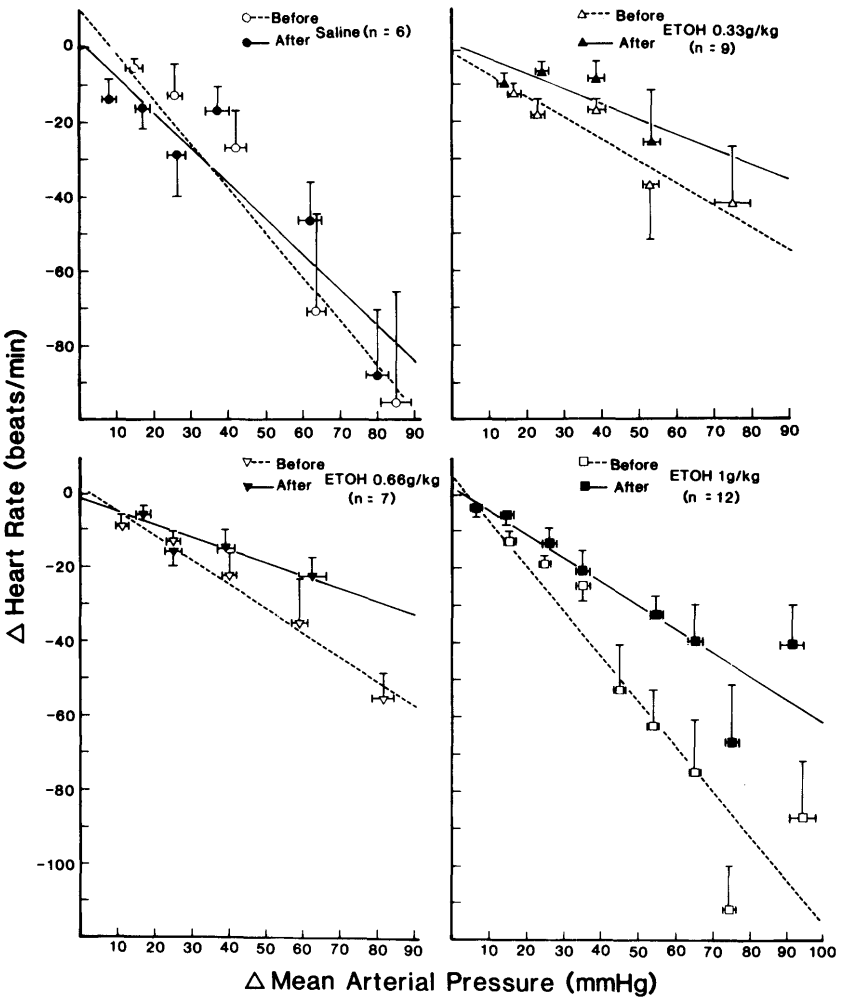

FIG. 3. Baroreflex curves depicting the inverse relationship between evoked rises in mean arterial pressure by graded doses of phenylephrine and the associated decreases in heart rate before and after ethanol or saline treatment. The data presented in this figure and in Fig. 2 were obtained from the same rats (see Fig. 2 for details). The slopes of linear regression lines (baroreflex sensitivity) are significantly lower after the three doses of ethanol (see Table I).

the net effect of a direct cardiac depressant action vs an indirect positive chronotropic action which involves the sympathetic nervous system (7). The apparent discrepancy between our present and previous findings may relate, at least in part, to the route of administration; in this study ethanol was administered iv versus ip in our previous rat experiments (1, 11) and po in the human study (10). The blood ethanol concentration 15 min after the iv injection of 0.5 g/kg of ethanol was 0.53 ± 0.15 mg/ml which is slightly below the levels obtained in humans when the same dose was administered orally (10). Even though we did not use this dose of ethanol in this study it was chosen because it fell within the range of doses used and allowed us to compare the results to those found in humans (10).

We have previously shown that the baroreflex control of heart rate is impaired by acute ethanol administration in humans (10) and in rats (1, 11). The findings of this study also show that acute administration of moderate doses of ethanol caused impairment in the baroreflex control of HR (Fig. 3) and

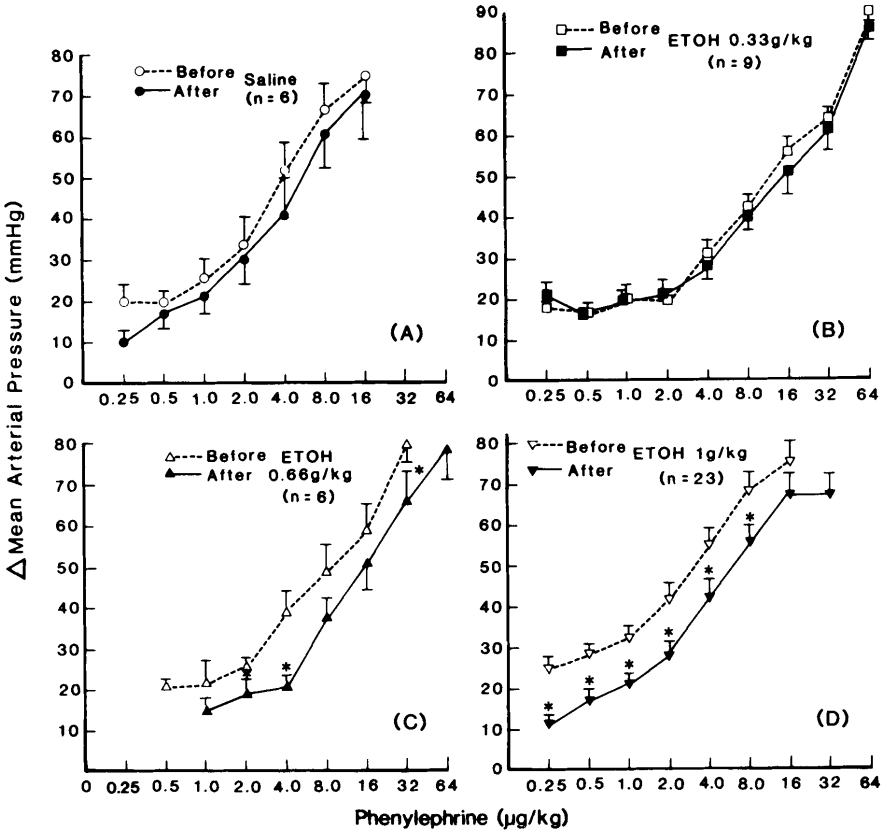

FIG. 4. Phenylephrine dose–pressor response curves obtained before and after saline or ethanol treatment (doses of ethanol and number of rats in each group are shown). The downward shift of the dose–response curves after the two higher doses of ethanol (C and D) is statistically significant ($P < 0.05$). Data are presented as means ± SE.

thus support our previous findings. The reason(s) for this selective action of ethanol on baroreflex control of heart rate is not readily available from our data. Whether this differential action involves selective impairment of central vagal tone remains to be elucidated.

The mechanism(s) by which ethanol impairs the baroreflex control of heart rate is not known. However, it is helpful to suggest three possibilities for which there is partial evidence. First, ethanol could evoke a change in the afferent limb of the baroreflex arc which may involve receptors, the distensibility of blood vessel walls, or physical coupling between the receptors and the vessel wall. In support of this possibility are the observations that alcohol *in vitro* inhibits neuronal action potentials and decreases the duration and magnitude of the action potential (20, 21). However, the concentration of ethanol used in these studies was much higher than that used in our study and the relationship to *in vivo* studies is unclear. Second, ethanol could impair the central component of the baroreflex control of heart rate. In support of this possibility we have shown that heart rate responses to electrical stimulation of the central end of the left aortic depressor nerve were reduced after similar doses of ethanol (22). Third, altered chronotropic response to baroreceptor activation at the effector organ level and/or the parasympathetic ganglion may explain the reduced baroreflex control of heart rate after ethanol. However, since we have previously shown that the negative chronotropic response to efferent vagal nerve stimulation was not altered by similar doses

of ethanol (22) it is unlikely that these peripheral mechanisms are involved in the inhibitory action of ethanol on baroreflex control of heart rate. Finally, it must be emphasized that the differential action of ethanol on baroreflex control of HR and SED was observed after an acute dose of ethanol. The effect of chronic ethanol administration on these two baroreceptor-controlled variables remains to be elucidated.

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