Impairment of Baroreceptor Reflex Control of Heart Rate But Not Sympathetic Efferent Discharge By Central Neuroadministration of Ethanol

Xin Zhang, Abdel A. Abdel-Rahman, and Wallace R. Wooles

We investigated the acute hemodynamic effects of ethanol microinjection into brain areas known to influence cardiovascular function and reflexes. In chloralose-anesthetized rats, ethanol had no effect on baseline mean arterial pressure, heart rate, or sympathetic efferent discharge when microinjected into the nucleus tractus solitarius, the dorsal motor nucleus of the vagus, the rostral ventrolateral medulla, or the posterior hypothalamus. On the other hand, ethanol microinjection into the anterior hypothalamus caused a site-dependent pressor effect and an increase in sympathetic efferent discharge. Baroreceptor heart rate response but not sympathetic efferent discharge response was impaired by ethanol microinjection into the nucleus tractus solitarius, the dorsal motor nucleus of the vagus, and the rostral ventrolateral medulla, suggesting that ethanol involves one or more of these areas in its inhibitory effect on baroreceptor heart rate response and that ethanol has a selective action on baroreceptor reflex control of heart rate. The findings that 1) the effect was dose dependent and 2) injection of ethanol outside of, or an equal volume of cerebrospinal fluid into, the nucleus tractus solitarius had no effect on the response strongly suggest that the observed effect on baroreceptor heart rate response was ethanol mediated. Ethanol microinjection into the dorsal motor nucleus of the vagus impaired the heart rate response, thus raising the possibility that leakage of ethanol to that area from the nucleus tractus solitarius might have contributed to its effect. These findings show that ethanol has a pressor and sympathoexcitatory site of action within the anterior hypothalamus and that it selectively impairs baroreceptor heart rate response via a central site of action; the mechanisms by which ethanol produces these effects remain to be elucidated. (Hypertension 1989;14:282-292)
nerve stimulation suggested ethanol impairs the
central mediation of baroreceptor reflex responses.\textsuperscript{12}
The sites of action at which ethanol acts within the
central nervous system (CNS) are not known.

In the present study, we investigated potential sites within the CNS at which ethanol may influence cardiovascular variables controlled by baroreceptor reflexes. We initially chose the nucleus tractus solitarius (NTS) because we have already shown that systemic ethanol administration impaired baroreceptor reflex activity\textsuperscript{5-7} and because the NTS has been shown to be the first relay station in the baroreceptor reflex arc.\textsuperscript{13} The amounts of ethanol microinjected were based on calculations of the amount of ethanol expected to reach this area after systemic injection of 0.33, 0.66, and 1 g/kg ethanol, which were administered systemically in our previous studies.\textsuperscript{7,12} We also microinjected ethanol into the anterior and posterior hypothalamus and the rostral ventrolateral medulla (RVL), areas known to be involved in the control of cardiovascular function, to determine if the effects of ethanol were specific to one or more of these areas.

\section*{Materials and Methods}

Male Sprague-Dawley rats (Charles River Breeding
Laboratories, Research Triangle Park, North Carolina) weighing 320–400 g were used in this study. The rats were kept in a room with a 12-hour light/dark cycle, and room temperature was kept between 23° and 24°C. Purina Chow (St. Louis, Missouri) and tap water were supplied ad libitum. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Research Triangle Park, North Carolina) weighing 320–400 g were used in this study. The rats were kept in a room with a 12-hour light/dark cycle, and room temperature was kept between 23° and 24°C. Purina Chow (St. Louis, Missouri) and tap water were supplied ad libitum.

Rats were anesthetized by α-chloralose (150 mg/ kg i.p.). The femoral artery and vein were cannulated for blood pressure recording and drug administration, respectively. The arterial catheter was connected to a Gould-Statham pressure transducer (P23 DC) to measure blood pressure using a Grass Intr. Co. polygraph (model 7D, Quincy, Massachusetts). Heart rate was computed from the blood pressure waveforms by a Grass tachograph (Grass Intr. Co.) and was displayed on another channel of the polygraph. Under a dissecting microscope, we exposed the greater splanchnic nerve through a midline abdominal incision. The nerve was cut at its entrance to the celiac ganglion, and a length of approximately 15 mm at the proximal end of the nerve was cleared from surrounding tissues. The abdominal incision was closed and the nerve was then accessed through a retroperitoneal opening, which allowed us to record sympathetic nerve activity from the rat after placing it in the stereotaxic head holder. Splanchnic nerve activity was recorded from the central end of the cut nerve with a bipolar platinum electrode under a mineral oil pool as described in our previous study.\textsuperscript{7} The recorded spikes were amplified by a Grass P511 preamplifier (Grass Intr. Co.), and the amplified nerve activity was visualized on a Tektronix Inc. storage oscilloscope (model D13, Beaverton, Oregon). The output was also fed to a spike processor (Digitimer model D130, Medical Sys. Corp., Great Neck, New York) with a loudspeaker that measured the frequency of the impulses that exceeded a preset lower window level that was set above the background 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 (Grass Intr. Co.) along with blood pressure and heart rate. As a routine at the end of the 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.

Rats were placed in a Kopf Instruments head holder (model 1730, David Kopf Instruments, Tujunga, California) with the bite bar 30 mm below the interaural line for the NTS and dorsal motor nucleus of the vagus (DMV); 12 mm below the interaural line for the RVL and 5 mm above the interaural line for the anterior and posterior hypothalamus. The occipital bone and atlanto-occipital membrane were exposed after the dorsal neck muscles were retracted, and after separation from the dura, the occipital bone was removed. The atlanto-occipital membrane was cut, and the dura overlaying the cerebellum was retracted. The cerebellum was pushed rostrally to expose the floor of the fourth ventricle as far rostrally as the caudal aspect of the inferior cerebellar peduncle. Glass micropipettes were then inserted unilaterally or bilaterally into different brain nuclei according to the coordinates indicated below. The micropipettes were fabricated from glass capillary tubing (2.0 mm o.d., 1.16 mm i.d.; World Precision Instrs. Inc., New Haven, Connecticut) with tips (50 μm) shaped by a standard laboratory micropipette puller. Micropipettes were carried in a stereotaxic micromanipulator and connected by a polyethylene PE10 tubing to a Hamilton microsyringe (Reno, Nevada). The volume of the injectate was 0.5 μl for the NTS, anterior hypothalamus, and posterior hypothalamus and 0.2 μl for the DMV and RVL. The following coordinates were used for the NTS reference point: mediolateral (ML) 0.5, anteroposterior (AP) 0.0, and dorsoventral (DV) 0.9 mm; for the DMV reference point: ML 0.5, AP 0.0, and DV 1.1 mm; for the RVL: ML 2.0, AP 2.0, and DV 3.4 mm; for the anterior hypothalamus: ML 0.75, AP 1.0, and DV 8.0 mm; for the posterior hypothalamus: ML 0.8, AP -1.0, and DV 8.0 mm with the obex as zero for NTS, DMV and calamus scriptorius as zero for RVL, and the bregma as zero for anterior and posterior hypothalamus. Injections were made bilaterally in all areas except the anterior hypothalamus and RVL. Only one dose of ethanol was tested in each rat.

The injection site in all experiments was histologically confirmed by the dye marker technique. Fast
green dye was dissolved in the concentration of ethanol used and was injected in the same volume used in the experiment. The brain was removed, frozen, and cut on a freezing microtome. Verification of the site of injection was by histology. In studies involving the NTS and the RVL, verification of the site of injection was done after insertion of the micropipette by injection of l-glutamate. In the NTS hypotension and bradycardia, comparable to those reported elsewhere occurred after microinjection of 5 nM l-glutamate. In the RVL, injection of 5 nM l-glutamate caused a pressor effect similar to the findings of Ross et al. In some experiments, a second injection of l-glutamate was made at the end of the experiment into both the NTS and the RVL, and the response was similar to that obtained when the injection was made at the beginning of the experiment.

Protocol and Experimental Groups

In all groups of rats studied, the baroreceptor heart rate and sympathetic efferent discharge responses were measured before and after central administration of ethanol or the vehicle cerebrospinal fluid (CSF). This was achieved by injection of graded doses of phenylephrine (0.25–16 μg/kg i.v.), which produced increments in mean arterial pressure of 10–80 mm Hg. The associated reflex decreases in heart rate and sympathetic efferent discharge were plotted against the evoked increments in MAP to construct the baroreceptor reflex curves as detailed in our previous study. The slope of the regression line relating the decreases in heart rate or sympathetic efferent discharge to the increments in MAP was taken as an index of baroreceptor reflex sensitivity.

Five groups of rats were used to investigate the acute hemodynamic and electrophysiological effects of ethanol and its effect on baroreceptor reflexes when microinjected into the NTS. Three groups received three doses of ethanol, 5.5 μg (n=8), 11 μg (n=7), or 16 μg (n=11); the fourth group received an equal volume of CSF and served as control (n=10). The fifth group received the highest dose of ethanol outside the NTS (n=6) to determine whether leakage to nearby areas had contributed to the observed effects. Injection of the dye in the same site outside the NTS caused a partial coverage of the NTS by the dye (Figure 5). It must be noted that the physico-chemical properties of ethanol (ETOH) and fast green dye are different, and thus, the volume of distribution after the injection of equal volumes of both agents into brain areas is expected to be different. We acknowledge this limitation, especially when the high lipid solubility of ethanol is considered.

Four more groups of rats were used to investigate the acute effects of a single dose of ethanol in the other brain areas: DMV (6.4 μg ethanol, n=7), anterior hypothalamus (16 μg ethanol, n=20), posterior hypothalamus (16 μg ethanol, n=9), and RVL (6.4 μg ethanol, n=16). The difference in the doses used was related to the size of the area under study.

Drugs

The following drugs were used: ethanol was diluted in CSF to make 1.4, 2.7, and 4.1% solutions for the three doses of 5.5, 11, and 16 μg, respectively. α-Chloralose (1% solution) and phenylephrine (Sigma Chemical Company, St. Louis, Missouri) were dissolved in 0.9% sodium chloride solution. Artificial CSF of the following composition (mM) was used: NaCl 123, CaCl2 0.86, KCl 3.0, MgCl2 0.89, NaHCO3 25, NaH2PO4 0.50, Na2HPO4 0.25, aerated with 95% O2-5% CO2, pH 7.4 as described by Tseng et al.

Statistical Analysis

Values presented are mean±SEM. Student’s t test was used in the analysis of paired and unpaired means and the stimulus–response curves were analyzed by one-way analysis of variance. The relation between increases in MAP and the associated decreases in heart rate and sympathetic efferent discharge was assessed by regression analysis for each rat. The regression coefficient (slope of regression line) was taken as an index of reflex sensitivity, and the difference in slopes in response to treatment was determined by the paired or unpaired t test. Probability levels of less than 0.05 were considered statistically significant.

Results

Microinjection of either artificial CSF or ethanol into the NTS, DMV, posterior hypothalamus, or RVL did not cause any change in baseline MAP or heart rate (Table 1). The only change in baseline values of any cardiovascular parameter studied was a 30% and 40% increase in sympathetic efferent discharge that occurred at 12 and 20 minutes, respectively, after microinjection of ethanol into the RVL (Table 1) and a 15 mm Hg increase in MAP associated with a corresponding increase in sympathetic efferent discharge when ethanol was microinjected into the anterior hypothalamus (Figure 1). This effect of ethanol was not time related but was related to the distance from the bregma where the ethanol was injected (Figure 1). Equivolume injections of artificial CSF had no effect on baseline values of any cardiovascular parameter tested (Figure 1).

Microinjection of 5.5, 11, or 16 μg ethanol into the NTS produced a dose-related impairment of the baroreceptor reflex heart rate response but had no effect on the reflex response of the sympathetic efferent discharge to baroreceptor activation (Figures 2 and 3, Table 2). Thus, for a comparable rise in MAP evoked by phenylephrine, the reflex decrease in heart rate was significantly smaller after ethanol (Figure 3). Microinjection of an equivolume of artificial CSF into the NTS had no effect on the reflex responses of heart rate and sympathetic
TABLE 1. Baseline Mean Arterial Pressure, Heart Rate, and Sympathetic Efferent Discharge Before and After Ethanol or Microinjection

<table>
<thead>
<tr>
<th>Area</th>
<th>n</th>
<th>Parameter</th>
<th>-4</th>
<th>4</th>
<th>12</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ethanol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTS</td>
<td>11</td>
<td>MAP</td>
<td>100±0.58</td>
<td>99±0.60</td>
<td>105±0.74</td>
<td>108±0.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HR</td>
<td>384±19.02</td>
<td>371±22.43</td>
<td>366±20.35</td>
<td>360±20.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SED*</td>
<td>100±0.00</td>
<td>102±0.55</td>
<td>108±0.09</td>
<td>122±12.70</td>
</tr>
<tr>
<td>DMV</td>
<td>7</td>
<td>MAP</td>
<td>123±0.08</td>
<td>121±0.07</td>
<td>131±0.51</td>
<td>135±0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HR</td>
<td>343±22.21</td>
<td>339±22.96</td>
<td>340±24.96</td>
<td>330±19.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SED</td>
<td>100±0.00</td>
<td>102±0.75</td>
<td>110±0.83</td>
<td>105±11.97</td>
</tr>
<tr>
<td>PHT</td>
<td>9</td>
<td>MAP</td>
<td>116±0.98</td>
<td>117±0.59</td>
<td>119±0.75</td>
<td>109±0.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HR</td>
<td>460±15.80</td>
<td>458±15.76</td>
<td>463±15.85</td>
<td>473±17.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SED</td>
<td>100±0.00</td>
<td>111±13.67</td>
<td>126±00.33</td>
<td>107±13.00</td>
</tr>
<tr>
<td>RVL</td>
<td>16</td>
<td>MAP</td>
<td>94±0.88</td>
<td>98±0.86</td>
<td>98±0.46</td>
<td>98±0.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HR</td>
<td>347±20.64</td>
<td>352±21.06</td>
<td>342±29.50</td>
<td>358±20.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SED</td>
<td>100±0.00</td>
<td>106±0.25</td>
<td>130±14.39</td>
<td>139±17.85</td>
</tr>
<tr>
<td><strong>CSF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTS</td>
<td>7</td>
<td>MAP</td>
<td>115±11.04</td>
<td>115±09.97</td>
<td>121±09.03</td>
<td>123±03.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HR</td>
<td>402±27.24</td>
<td>396±26.68</td>
<td>398±30.80</td>
<td>395±32.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SED</td>
<td>100±0.00</td>
<td>92±09.15</td>
<td>112±24.37</td>
<td>121±13.25</td>
</tr>
<tr>
<td>DMV</td>
<td>6</td>
<td>MAP</td>
<td>113±0.70</td>
<td>114±08.20</td>
<td>115±08.40</td>
<td>117±07.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HR</td>
<td>381±19.60</td>
<td>381±16.40</td>
<td>369±18.81</td>
<td>374±25.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SED</td>
<td>100±0.00</td>
<td>122±19.30</td>
<td>98±10.72</td>
<td>87±11.26</td>
</tr>
<tr>
<td>PHT</td>
<td>5</td>
<td>MAP</td>
<td>130±0.43</td>
<td>128±04.08</td>
<td>128±03.42</td>
<td>130±05.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HR</td>
<td>427±22.76</td>
<td>428±25.19</td>
<td>434±25.78</td>
<td>444±22.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SED</td>
<td>100±0.00</td>
<td>96±13.04</td>
<td>112±24.38</td>
<td>92±23.51</td>
</tr>
<tr>
<td>RVL</td>
<td>6</td>
<td>MAP</td>
<td>90±07.91</td>
<td>90±07.41</td>
<td>91±07.66</td>
<td>89±08.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HR</td>
<td>372±16.68</td>
<td>373±16.80</td>
<td>367±16.59</td>
<td>370±17.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SED</td>
<td>100±0.00</td>
<td>97±02.35</td>
<td>99±02.18</td>
<td>113±02.47</td>
</tr>
</tbody>
</table>

NTS, nucleus tractus solitarii; MAP, mean arterial pressure (mm Hg); HR, heart rate (beats/min); SED, sympathetic efferent discharge (%); DMV, dorsal motor nucleus of vagus; PHT, posterior hypothalamus; RVL, rostral ventrolateral medulla.

*Expressed as percent of control values.
†p<0.05 compared with cerebrospinal data.

efferent discharge (Figure 4, Table 2) eliminating the possibility that the changes observed were volume related. To eliminate the possibility that the observed changes were due to leakage of ethanol from the NTS or DMV, 16 /μg ethanol was microinjected 0.5 mm lateral to the NTS/DMV complex. As shown in Figure 4, ethanol microinjection outside of the NTS had no effect on the baroreceptor reflex variables.

Routine histological verification of the site of injection of ethanol or artificial CSF showed that the DMV was stained by the dye microinjected into the NTS (Figure 5). Because of the close proximity of the NTS to the DMV and because the DMV contributes to the control of central vagal tone, we decided to investigate the effect of low doses of ethanol into the DMV. A dose of 6.4 μg microinjected into the DMV produced a greater inhibition of the baroreceptor heart rate response than did the dose of 16 μg into the NTS (Figure 6, Table 2). This effect was solely due to ethanol since an equivalent volume of artificial CSF microinjected into the same area had no effect on the baroreceptor reflex control of heart rate. Ethanol microinjected into the DMV had no effect on the baroreceptor reflex control of sympathetic efferent discharge similar to the results obtained when ethanol was administered into the NTS (Table 2).
Changes in mean arterial pressure, heart rate, and sympathetic efferent discharge elicited by microinjection of ethanol (ETOH) (16 µg) or cerebrospinal fluid (CSF) into anterior hypothalamus (AHT). Medial-lateral coordinate is given in lower right corner of the brain section drawing. Open circles in sagittal section of brain show spread of dye microinjected at end of experiment. SC, suprachiasmatic nucleus; OT, optic tract.

A dose of 6.4 µg ethanol microinjected into the RVL caused an attenuation of the baroreceptor reflex-mediated bradycardia but did not have any effect on baroreceptor reflex control of sympathetic efferent discharge (Figure 7, Table 2). Equivolume artificial CSF was without effect. However, the inhibition of baroreceptor reflex-mediated bradycardia was much less than that obtained after microinjection of the same amount into the DMV (90% vs. 62%, respectively). Finally, microinjection of 16 µg ethanol into the anterior (Table 2) or the posterior hypothalamus (Figure 8, Table 2) had no effect on baroreceptor reflex control of heart rate or sympathetic efferent discharge.

**Discussion**

We have previously shown that systemically administered ethanol impaired the bradycardic and depressor responses to stimulation of the aortic nerve in animals with sino-aortic denervation. This data provided indirect evidence of a possible impairment of central mediation in the baroreceptor reflex control of heart rate. In the present study, we examined the effects of microinjection of ethanol in some CNS sites that are possibly involved in the baroreceptor reflex arc. Since our previous findings also showed that systemic ethanol administration augmented preganglionic sympathetic discharge, we expanded our study to include the effects of centrally administered ethanol on the baroreceptor reflex control of heart rate and sympathetic efferent discharge.

The role of the NTS in modulating the activity of baroreceptor reflexes, as the first relay station, is well established. The ability of a number of chemical transmitters injected into the NTS to increase or to decrease systemic blood pressure suggests that the NTS has a functional role in the regulation of blood pressure. The fact that ethanol microinjected into the NTS was without activity on baseline sympathetic efferent discharge (Table 1) seems to rule it out as the site of action of systemically administered ethanol on sympathetic efferent discharge observed in our earlier study.

The use of the dye marker (fast green) technique, which we used to verify the site of injection and the extent of leakage, deserves some comment because of its potential importance to the study. Fast green is a water soluble dye, does not have the same degree of lipid solubility as ethanol, and has a much higher molecular weight. Therefore, it is difficult to definitively claim that the spread of the dye from the site of injection represents the extent of diffusion of ethanol, even though we dissolved the dye in the concentration of ethanol used in the study. The greater lipid solubility of ethanol alone would make one suspect the spread of ethanol would be greater than the dye. However, Myers has shown that the spread of a dye in the CNS is more related to the volume of fluid injected. In our study, we found that the degree of spread of the dye marker was also positively related to the volume (0.2 and 0.5 µl) used.

Although ethanol injected in the NTS had no effect on baseline heart rate, sympathetic efferent discharge, and MAP, it did produce a dose-related decrease in baroreceptor reflex activity secondary to loading of baroreceptors by phenylephrine. This effect was selective, however, to the heart rate response (Figure 3, Table 2). Since equivolume
artificial CSF was without effect, the effect of ethanol is not a volume effect and is probably a direct effect of ethanol or a metabolite. Similarly, injection of the largest amount of ethanol used lateral to the NTS was without activity. Since histological verification of the site of injection raised the possibility of leakage of ethanol from the NTS to the DMV, we investigated the effect of direct injection of ethanol into the DMV. We used the lowest amount of ethanol and in a smaller volume and the response obtained was greater than that obtained with the largest dose of ethanol injected into the NTS. This suggested that the DMV may be more involved than the NTS. However, the findings do not rule out the possibility that ethanol exerted its effect via an alteration in the activity of interneurons connecting the two areas. The greater effect of a smaller dose of ethanol in the DMV may also be related to the fact that the dose/unit weight ratio may be greater in the DMV.

Ethanol microinjected into the RVL medulla also produced an inhibitory effect on the baroreceptor heart rate response but did not affect baroreceptor reflex control of sympathetic efferent discharge. These data suggest this area is involved in the baroreceptor reflex–mediated bradycardia observed after either parenteral16,7,12 or oral5 administration of ethanol. Since the RVL medulla is relatively distant anatomically from the NTS23 or the DMV, it is unlikely that ethanol leakage to either or both areas could account for the impairment of baroreceptor reflex–mediated bradycardia. However, given the limitations of the dye marker technique discussed above, the possibility must be considered that leakage of ethanol to the nucleus ambiguus after its microinjection into the RVL may be involved in that effect. This possibility is strengthened by: 1) the nucleus ambiguus is a major control area of central vagal tone24 and 2) it is anatomically close to the RVL.24 If we can assume a similar spread of ethanol from the RVL to the nucleus ambiguus as occurred from the NTS to the DMV, there should have been a greater impairment of baroreceptor reflex control of heart rate when ethanol was injected into the RVL because of the greater role of the nucleus ambiguus than the DMV in controlling central vagal tone in the rat.25 However, the magnitude of the impairment of the baroreceptor heart rate response was much less after injection of ethanol into the RVL than the NTS. Thus, it is unlikely that the nucleus ambiguus was involved, as a primary site, in the inhibitory action of ethanol after its microinjection into the RVL.

Injection of ethanol into the forebrain regions of the anterior and posterior hypothalamus had no effect on baroreceptor reflex–mediated bradycardia. However, ethanol injection into the anterior, but not the posterior hypothalamus, was associated with a modest but significant increase in baseline MAP, which was associated with an excitatory effect on sympathetic efferent discharge (Figure 1). These data suggest the anterior hypothalamus as
the possible site of action of enhanced sympathetic efferent activity observed after systemic ethanol administration. This finding is consistent with a pressor effect of ethanol of central origin. However, many studies including our own have shown that acute ethanol administered systemically did not increase blood pressure, although it did increase sympathetic efferent discharge. This may be because of the systemic effects of ethanol such as an activity similar to alpha blocking, which can counteract the pressor effect.

It is interesting that the effect of ethanol on baroreceptor reflex-mediated bradycardia was specific to the medullary areas (NTS, DMV, and the RVL). However, since the baroreceptor reflex control of sympathetic efferent discharge was not altered by ethanol microinjection into any of these areas (Figures 3, 6, and 7, Table 2), the data suggest that the baroreceptor reflex-mediated bradycardia did not involve significant changes in the sympathetic component of the reflex. Therefore, this effect of ethanol is mediated at either the NTS, the DMV, or the RVL, or on interneurons that connect the areas and seems to involve the efferent vagal component.

Ethanol did not have any effect on the baroreceptor reflex control of sympathetic efferent discharge when administered in any of the medullary or forebrain areas we studied. This finding confirms our previous observation that systemic administration of ethanol had no effect on baroreceptor reflex control of sympathetic efferent discharge. Both findings clearly establish that ethanol has a selective effect on baroreceptor reflex control of heart rate. Systemically administered ethanol would have equal access to all areas of the brain and it is reasonable to expect it has a comparable effect on all neural membranes. The impairment of baroreceptor reflex control of heart rate but not sympathetic efferent discharge suggests a selective effect of ethanol.

The selective action of ethanol on baroreceptor reflex control of heart rate deserves some comment. First, leakage of ethanol to the DMV and nucleus ambiguus, which control central vagal tone may explain the effect of ethanol on heart rate responses. However, the finding that ethanol increased sympathetic efferent discharge after its administration into the anterior hypothalamus indicates its ability to interact with central sympathetic structures as well. Second, it is possible that ethanol impairs baroreceptor reflex control of sympathetic activity in some sympathetic nerves and not in others as reported by other investigators who showed that baroreceptor reflex control of splanchnic nerve activity was preserved while that of renal nerve activity was impaired under the same conditions. This may be related to the concept of nonuniformity in
TABLE 2. Effect of Ethanol on Baroreceptor Reflex Slopes

<table>
<thead>
<tr>
<th>Area</th>
<th>n</th>
<th>HR responses (beats/min/mm Hg) Before</th>
<th>After</th>
<th>SED responses (% SED/mm Hg) Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTS</td>
<td></td>
<td>-1.82±0.15</td>
<td>-2.22±0.11</td>
<td>-0.62±0.09</td>
<td>-0.85±0.10</td>
</tr>
<tr>
<td>CSF</td>
<td>10</td>
<td>-1.23±0.09</td>
<td>-0.84±0.06*</td>
<td>-0.66±0.02</td>
<td>-0.52±0.04</td>
</tr>
<tr>
<td>ET 5.5 µg</td>
<td>8</td>
<td>-1.67±0.20</td>
<td>-0.80±0.09*</td>
<td>-0.64±0.09</td>
<td>-0.44±0.13</td>
</tr>
<tr>
<td>ET 11 µg</td>
<td>7</td>
<td>-1.41±0.14</td>
<td>-0.76±0.08*</td>
<td>-0.67±0.07</td>
<td>-0.51±0.08</td>
</tr>
<tr>
<td>ET 16 µg</td>
<td>6</td>
<td>-1.30±0.11</td>
<td>-1.55±0.12</td>
<td>-0.80±0.05</td>
<td>-0.73±0.11</td>
</tr>
<tr>
<td>DMV</td>
<td></td>
<td>-1.26±0.13</td>
<td>-1.35±0.11</td>
<td>-0.76±0.05</td>
<td>-0.82±0.05</td>
</tr>
<tr>
<td>CSF</td>
<td>6</td>
<td>-1.43±0.08</td>
<td>-0.71±0.04*</td>
<td>-0.91±0.06</td>
<td>-0.89±0.05</td>
</tr>
<tr>
<td>ET 6.4 µg</td>
<td>7</td>
<td>-2.22±0.38</td>
<td>-2.23±0.26</td>
<td>-0.87±0.08</td>
<td>-0.84±0.08</td>
</tr>
<tr>
<td>PHT</td>
<td></td>
<td>-2.55±0.14</td>
<td>-1.71±0.16</td>
<td>-0.86±0.06</td>
<td>-0.76±0.07</td>
</tr>
<tr>
<td>AHT</td>
<td></td>
<td>-0.98±0.06</td>
<td>-1.20±0.06</td>
<td>-0.74±0.07</td>
<td>-0.81±0.06</td>
</tr>
<tr>
<td>ET 16 µg</td>
<td>7</td>
<td>-1.62±0.13</td>
<td>-1.59±0.11</td>
<td>-0.87±0.06</td>
<td>-0.74±0.07</td>
</tr>
<tr>
<td>RVL</td>
<td></td>
<td>-2.38±0.19</td>
<td>-2.13±0.37</td>
<td>-0.92±0.06</td>
<td>-0.77±0.14</td>
</tr>
<tr>
<td>CSF</td>
<td>6</td>
<td>-1.69±0.11</td>
<td>-1.31±0.08*</td>
<td>-0.91±0.06</td>
<td>-0.82±0.05</td>
</tr>
</tbody>
</table>

HR, heart rate; SED, sympathetic efferent discharge; NTS, nucleus tractus solitarii; CSF, cerebrospinal fluid; ET, ethanol; DMV, dorsal motor nucleus of vagus; PHT, posterior hypothalamus; AHT, anterior hypothalamus; RVL, rostral ventrolateral medulla.

*p<0.05 compared with pretreatment.
†Ethanol microinjection was made 0.5 mm lateral to NTS.

central control of sympathetic outflow. Third, the selective effect of ethanol may suggest that potentially different physiological processes are involved in the baroreceptor reflex control of heart rate and sympathetic efferent discharge. These processes include transmitters or cotransmitters (e.g., glutamate and norepinephrine), different receptors or subreceptor mechanisms, or a combination of both. Finally, this finding rules out the possibility that ethanol-evoked nonspecific cell damage contributed to the
FIGURE 5. Diagramatic representation of coronal section of rat brain. Dark areas show spread of dye injected at completion of experiments in which ethanol or cerebrospinal fluid (CSF) was injected into the nucleus tractus solitarii (NTS), rostral ventrolateral medulla (RVL), posterior hypothalamus (PHT), or anterior hypothalamus (AHT). Hatched area shows spread of dye when tip of micropipettes were positioned 0.5 mm lateral to NTS. XII, nucleus originser nervi hypoglossic; TS, tractus solitarii; DMV, dorsal motor nucleus of vagus; IVN, inferior vestibular nucleus; MVN, medial vestibular nucleus; IO, inferior olivary nucleus; CST, corticospinal tract; PMD, dorsal preimillary nucleus; ARH, arcuate nucleus of the hypothalamus; SM, stria medullaris thalami; CO, chiasma opticum.

FIGURE 6. Ethanol (6.4 μg) or cerebrospinal fluid (CSF) microinjection into dorsal motor nucleus of the vagus (DMV) caused impairment of baroreceptor reflex heart rate response (top panel) but had no effect on baroreceptor reflex control of sympathetic efferent discharge (lower panel) in same rats (see Table 2).
observed effect. Further studies are needed to investigate the mechanism by which ethanol selectively influences baroreceptor reflex control of heart rate.

Our data do not suggest whether the action of ethanol is on the cell bodies located in the various areas studied on or fibers of passage or both. The excitatory amino acid, L-glutamate, has been shown to have a selective effect on cell bodies and not fibers of passage.29 Since an ethanol effect was observed in those areas in which the excitatory amino acid L-glutamate produced the expected cardiovascular responses, we believe that the effect of ethanol is on cell bodies in the areas studied. This is also supported by the finding that ethanol had no effect on the tested parameters when injected lateral to the NTS.

Figure 7. An inhibitory effect of ethanol (6.4 µg) when microinjected into rostral ventrolateral medulla (RVL) on baroreceptor reflex control of heart rate (top panel); baroreceptor reflex control of sympathetic efferent discharge (lower panel) was not influenced by ethanol (see Table 2 for regression coefficient values). CSF, cerebrospinal fluid.

Figure 8. Failure of ethanol (16 µg) microinjection into anterior hypothalamus (AHT) to alter the baroreceptor reflex control of heart rate and sympathetic efferent discharge in response to phenylephrine evoked increments in blood pressure. Similar data were obtained after ethanol microinjection into posterior hypothalamus (PHT). CSF, cerebrospinal fluid.
The results of our study only pertain to the acute effects of ethanol on baroreceptor reflex control of cardiovascular variables and no information concerning the chronic effects of ethanol on these parameters is yet available. Further studies are needed to establish if chronic ethanol administration, which impairs baroreceptor reflex control of heart rate, before and after the development of ethanol-induced hypertension would have a positive or negative effect on sympathetic efferent discharge.

References
8. Altura BM, Altura BT: Peripheral and cerebrovascular actions of ethanol, acetaldehyde and acetate: Relationship to divalent cations. Alcoholism (NY) 1987;11:99-111
ergic receptors in the brainstem mediate hypertension and bradycardia. Hypertension 1988;11:191-197

KEY WORDS • ethanol • baroreceptor reflex • nucleus tractus solitarii • hypothalamus • heart rate • vagus nerve • blood pressure
Impairment of baroreceptor reflex control of heart rate but not sympathetic efferent discharge by central neuroadministration of ethanol.
X Zhang, A A Abdel-Rahman and W R Wooles

Hypertension. 1989;14:282-292
doi: 10.1161/01.HYP.14.3.282

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/14/3/282

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/