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Effects of systemic ethanol on medullary vasomotor neurons and baroreflexes

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The acute effects of systemic ethanol on reticulospinal sympathoexcitatory neurons were examined in vivo in anesthetized, paralyzed rats. Ethanol (0.45 g/kg, i.v.) potentiated the depressant effect of locally applied γ-aminobutyric acid (GABA) but attenuated the excitatory effect of L-glutamate. The baroreflex-mediated inhibition of these neurons and sympathetic nerve activity were partially depressed by the agent while aortic nerve activity and its sensitivity to changes in arterial pressure were not altered. These results suggest that systemic ethanol may markedly influence cardiovascular function by interfering in medullary GABAergic and glutamatergic transmissions involved in central control of the cardiovascular system.

Several lines of evidence indicate that ethanol may have relatively selective interactions with specific cellular mechanisms in neurons. Of particular interest are the actions of ethanol on γ-aminobutyric acid$_A$ ($GABA_A$) [3, 14, 27] and the excitatory amino acid receptor-channel complexes [9, 13]. At pharmacological concentrations ethanol has been reported to augment GABA-stimulated chloride flux [4, 14, 15, 27, 29] and to inhibit the excitatory amino acid-activated Ca^{2+} channels [5, 9]. Electrophysiologically, ethanol has been reported to facilitate neuronal responses to local application of GABA [5, 15] while many others have failed to observe the facilitation (refs. 5, 19, among others).

In the cardiovascular control system, the activity of several groups of neurons in the medulla oblongata determines the basic tone of sympathoexcitatory outflow [2, 7, 8, 18, 20] and mediate the regulation of the arterial pressure by baroreceptors [2, 6, 8, 10, 20, 22]. The principal medullary outflow neurons consist of a small group of tonically active sympathoexcitatory reticulo-spinal neurons within the rostral ventrolateral medulla (RVL) [2, 18, 20]. These neurons are inhibited by arterial baroreceptors over a multisynaptic intramedullary pathway involving: (a) a first-order excitatory input from baroreceptor afferents onto neurons of the nucleus tractus solitarii (NTS) [10, 28] presumably glutaminergic and mediated via the *N*-methyl-D-aspartate (NMDA) subclass; (b) a second order excitatory projection from NTS into a sympathoinhibitory nucleus, the caudal ventrolateral medulla (CVL) [6, 8] which is also presumably glutaminergic and mediated by the NMDA-receptors; and (c) a third order inhibitory GABAergic projection from CVL onto medullary sympathoexcitatory neurons of the RVL [20, 22]. Inhibition, by GABA, of RVL neurons by stimulation of the arterial baroreceptors thereby results in withdrawal of excitation of spinal sympathetic neurons and a fall in mean arterial pressure (MAP). RVL sympathoexcitatory neurons, on the other hand, are excited by glutaminergic inputs from, amongst other areas, the hypothalamus [22].

The facts that both GABA and L-glutamate process the regulation of arterial pressure (AP) by RVL neurons raises the question of whether these neurons would be influenced by acute exposure to ethanol. In this study we demonstrate that ethanol may substantially modify GABAergic and glutamatergic transmission in central cardiovascular neurons.

Adult male Sprague–Dawley rats, weighing 325–350 g, were anesthetized with a mixture of urethane and sodium pentobarbitone (20% and 0.24%, respectively) given via a tail vein at doses adjusted to eliminate the paw-pinch reflex (usually 1.3–1.5 ml). Methods for extracellular single-unit recordings, iontophoresis, isolation of and recording from the lumbar sympathetic chain and aortic depressor nerves have been described elsewhere [20, 21, 23, 24]. Briefly, reticulospinal sympathoexcitatory

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neurons of the RVL were identified by the established criteria [2, 20] of antidromic activation from stimulation of the intermediolateral column of spinal cord, a discharge pattern coupled to the arterial pulse wave, and inhibition by arterial baroreceptor activation [20]. Six-barreled electrodes were used for iontophoresis and single-unit recordings. The recording pipette (3 M NaCl, 4–8 MΩ) protruded 25–35 μm from the other barrels. One barrel was filled with 3 M NaCl for current neutralization and the others were filled with one of the following solutions: 0.8 M GABA (Sigma), pH 4.5; 0.2 M L-glutamate (Sigma), pH 8.5; 0.2 M kynurenate (Sigma), pH 8.5; 0.1 M 2-amino-5-phosphovaleric acid (APV, Sigma), pH 8.5; all in 160 mM NaCl. A retaining current of 10 nA was applied between periods of drug ejection.

Ethanol was diluted in saline and injected intravenously at doses of 45 mg or 0.45 g/kg. All data are expressed as means ± S.E.M. of the mean. The differences are considered to be significant at P values smaller than 0.05 using Student's paired t-test.

Fourteen reticulospinal neurons were identified in 14 rats. Their spontaneous discharge rate was 17.4 spikes/s (±2.0 spikes/s) at a mean arterial pressure (MAP) of 113.6 mmHg (±2.0 mmHg). Their average conduction velocity was 3.1 m/s (±0.4 m/s, n=14, range: 1.3–5.7 m/s). The resting discharge of these neurons was inhibited by a step-wise elevation of MAP (Fig. 1). The 'cut-off MAP', defined as the minimal MAP at which the cell became silent (as determined from the relationship between neuronal discharge frequency/MAP [26]) was 156.9 mmHg (±1.8 mmHg, n=14).

After obtaining baseline values, two doses of ethanol were injected i.v., 45 mg/kg and then 0.45 g/kg in equivalent vols. Injections were over a period of 3 min in each animal separated by 30 min intervals. There were no apparently painful effects of ethanol injection, judging from observations that not much change in AP was evoked during ethanol injection as would have been otherwise [25].

Ethanol at 0.45 g/kg elicited small but statistically insignificant increases in MAP (+4.3±1.4 mmHg, n=14, P>0.05) and in the resting discharge rate of the reticulospinal vasomotor neurons (+0.8±0.4 spikes/s, n=14, P>0.05). However the drug reversibly modified the responses of these neurons to baroreceptor stimulation: by 2 min after injection of ethanol the cut-off MAP increased from approximately 157 mmHg before (see above) to 172.4 mmHg (±2.8 mmHg, n=14), an increase of 15.5 mmHg (±1.7 mmHg, n=14; P<0.05). As a consequence these neurons had significant discharge activity (6.5±0.8 spikes/s, n=14, P<0.05) at the cut-off MAP, i.e. the MAP at which they were silenced by baroreceptor activation before treatment (Fig. $1B_1$). The effects of ethanol on neuronal sensitivity to baroreflex stimulation disappeared by 25–30 min after drug administration (Fig. $1C_1$).

The damping of baroreceptor sensitivity of sympathoexcitatory RVL neurons by ethanol conceivably could result from diminution of the sensitivity of these neurons to GABA and/or enhancement of their responsivity to L-glutamate. These possibilities were directly examined.

GABA, microiontophoretically applied at 15–35 nA for 5–15 s, reproducibly (individually determined to get stable responses for at least 4 continuous trials at regular intervals) depressed the spontaneous discharge of 6 neurons of the group described above. The spontaneous resting activity of these neurons (20.7±2.7 spikes/s) was inhibited by 11.3±1.9 spikes/s (n=6, P<0.05), a reduction of 54.8% (±5.7%, n=6, P<0.05, Fig. $1A_2$ and $1A_3$). Ethanol (0.45 g/kg) did not alter the resting discharge of these neurons. However by 2 min after ethanol administration, GABA, applied at the same current, for the same duration, and at the same intervals was more effective and inhibited the neurons by 16.5 spikes/s (±2.7 spikes/s, n=6), an enhancement of the GABA response by 53.3% (±10.3%, n=6, P<0.05) (Fig. $1B_2$). This profound potentiation of GABA response by ethanol lasted for about 20 min, after which recovery was generally observed (Fig. 1C).

L-Glutamate was applied microiontophoretically to 5 neurons (20–35 nA, 5–10 s). L-Glutamate increased the spontaneous neuronal discharge by 8 spikes/s (±1.4 spikes/s) from 16.6 spikes/s (±3.7 spikes/s; n=5, P<0.05) (Fig. $1A_2$ and $1A_3$). Two minutes after ethanol injection at 0.45 g/kg, i.v., iontophoresis of L-glutamate at the same current, for the same period, at the same intervals elicited a markedly reduced increase in neuronal discharge (3.2±1.1 spikes/s, n=5, P<0.05). This represented a decrease of the response to the amino acid of 4.8 spikes/s (±1.2 spikes/s, n=5). The reduction in reactivity to L-glutamate over the control was 58.8% (±10.4%, n=5, P<0.05). On the other 6 RVL-spinal vasomotor neurons, iontophoretic application of L-glutamate (100 nA, 5–10 s) increased the firing rate of these neurons by 18 spikes/s (±2.2 spikes/s) from 13.2 spikes/s (±3.1 spikes/s, n=6, P<0.05). Iontophoretic application of APV (100 nA, 10–50 s) did not change the spontaneous discharge rate of these neurons but largely abolished the neuronal response to L-glutamate by an average of 74.8% (±8.2%, n=6, P<0.05, not shown). In all cases, the neuronal response to L-glutamate returned to the pre-APV levels within 1 min after termination of APV application.

Ethanol at the smaller dose (45 mg/kg, i.v.) did not significantly change the responses of RVL neurons to GABA or L-glutamate (not shown).

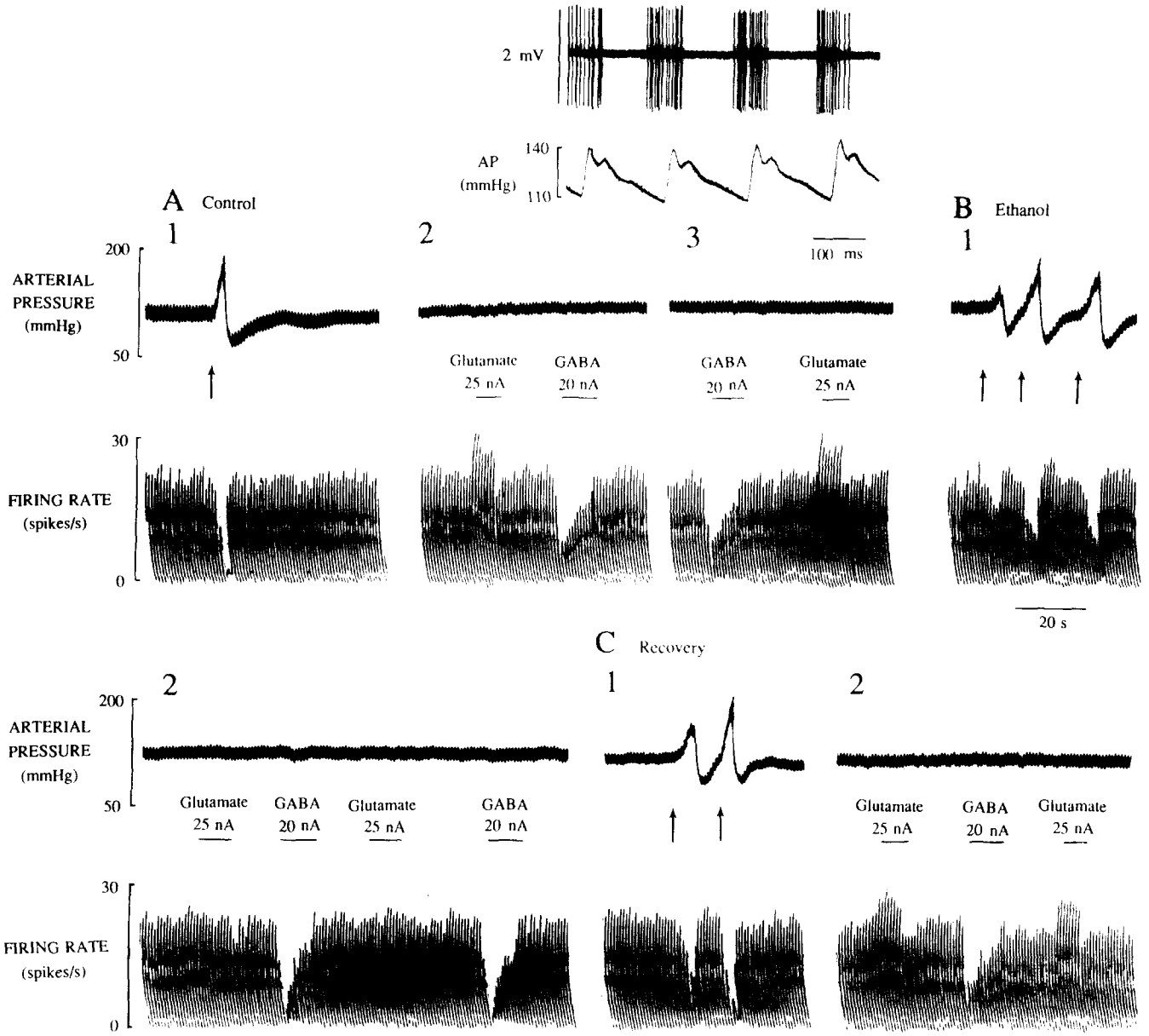

Fig. 1. Effects of ethanol on GABA-induced and glutamate-induced responses of reticulospinal vasomotor neurons. Neuronal integrated activity (bottom) of an identified reticulospinal vasomotor neuron with axon conduction velocity of 3.2 m/s in response to iontophoretic application of GABA or L-glutamate (at bars), aortic constriction (starting at arrows). A: before ethanol administration. Note that segments A_2 and A_3 were 5 min apart showing the stable response to GABA and glutamate during continuous application. B: 2 min after ethanol injection (0.45 g/kg, i.v.). Note the depressed responses to glutamate (B_2) and baroreflex inhibition (B_1) and enhanced GABA effect (B_2). C: 25 min after ethanol injection. Note recovery of the ethanol effects. Inset: pulse-synchronous discharge of a rostral ventrolateral medulla (RVL)-spinal vasomotor neuron showing 10 consecutive sweeps of ECG-triggered trace of neuronal discharge (top) and a single sweep of arterial pressure (bottom).

In 5 rats the effects of systemic administration of ethanol, at either high (0.45 g/kg) or low (45 mg/kg i.v.) doses had no significant effects upon MAP, HR, the resting discharge of lumbar sympathetic neurons or the integrated discharge of baroreceptor afferent fibers recorded from the aortic depressor nerve (ADN, Fig. 2A,B). However, at 0.45 g/kg, ethanol reversibly depressed the integrated discharge of lumbar sympathetic nerves to stimulation of arterial baroreceptors (Fig. 2A) without modifying the discharge of baroreceptor afferents in the ADN (Fig. 2B).

In the present study, we have observed that ethanol significantly alters the electrophysiological effects of GABA and L-glutamate on reticulospinal vasomotor neurons and their sensitivity to baroreflex inhibition. These effects could not be attributed to so-called 'warm-up' or 'cool-down' effects of iontophoresis barrels since stable responses were observed before ethanol ad-

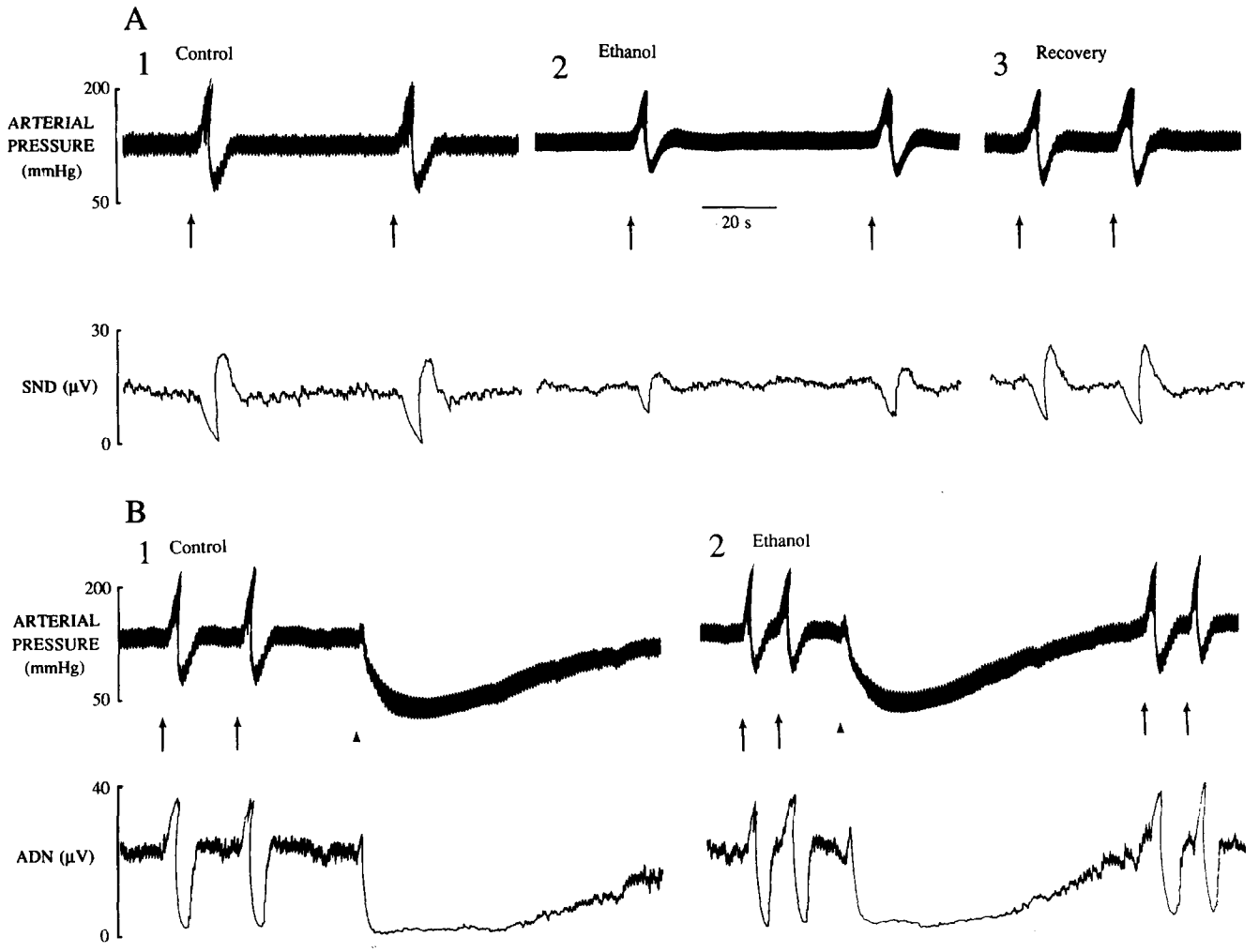

Fig. 2. Effects of ethanol on activity of sympathetic nerve (SND, A) and aortic depressor nerve (ADN, B). A_1, B_1: before ethanol administration; A_2, B_2: 2 min after ethanol injection (0.45 g/kg, i.v.); A_3: 25 min after ethanol administration. Arrows indicate the time when aortic constrictions were applied, while arrowheads, the application of sodium nitroprusside (0.01 mg, i.v.).

ministration and recovery was observed in all cases. Such effects may play a role in the cardiovascular component of acute ethanol intoxication.

Ethanol has been reported to have a variety of actions on synaptic transmission, both pre- and post-synaptically. A direct effect on the reticulospinal vasomotor neurons is strongly suggested by our results since the neuronal responses to locally applied GABA and L-glutamate were affected. The potentiation by ethanol of the GABA-mediated inhibition of the reticulospinal neurons could be produced via enhancement of GABA-induced Cl^- influx [14, 27, 29]. It is known that the chloride channel complex contains receptors for at least 4 types of drugs: GABA, benzodiazepines, some convulsants (e.g. picrotoxin) and barbiturates. GABA agonists activate the chloride channel, and this action is allosterically enhanced by benzodiazepine agonists or barbiturates and allosterically inhibited by benzodiazepine inverse agonists and convulsants [1]. There is evidence that the inverse benzodiazepine antagonists, R0154513 and FG7142, can antagonize certain effects of ethanol [12, 16, 26], suggesting its mechanism of action, though possible involvement of an ethanol-GABA-barbiturate interaction could not be ruled out.

Ethanol also suppressed the excitation of reticulospinal vasomotor neurons by L-glutamate. The data are consistent with the hypothesis that ethanol acts as an antagonist at glutamate receptors particularly of the NMDA subclass [9, 13], since the neuronal response to L-glutamate was largely abolished by APV, a selective NMDA subreceptor antagonist [4]. Whether the effect also involves an interaction with the glycine (strychnine-insensitive) co-agonist site of NMDA receptor-channel complex [17] remains to be studied.

The effects of ethanol to facilitate GABAergic transmission on the reticulospinal vasomotor neurons would be expected to enhance baroreflex-mediated inhibition of sympathetic nerve activity. However, the inhibition of

sympathetic nerve activity by baroreceptor stimulation was depressed by ethanol. Since ethanol did not affect the discharge of baroreceptors in the ADN the effect must be central. The most likely explanation for the discrepancy is that ethanol acts on more than one site within the central baroreceptor reflex arc, including NTS and CVL. In both of these regions facilitation of GABAergic and inhibition of glutamatergic transmission would have opposite effects from those produced by the same transmitters in the RVL upon sympathetic nerve activity, AP and baroreflex-sensitivity: in NTS or CVL GABA would enhance sympathetic nerve discharge and depress baroreflex activity while L-glutamate would have opposite effects [6, 8, 10, 28]. Thus while the actions of ethanol on specific transmitters may be uniform within the baroreflex arc the effect on the integrated reflex will depend upon the net effect of the drug in a network. Such considerations may also explain why ethanol did not markedly affect the resting activity of the reticulospinal vasomotor neurons, sympathetic nerve activity and arterial pressure but did influence the baroreflex.

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