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SPECIAL REPORT

Ethanol inhibition of baroreflex bradycardia: role of brainstem GABA receptors

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Ethanol administered i.v. or into the nucleus tractus solitarii (NTS) of rats anaesthetized with urethane inhibits baroreflex bradycardia elicited by phenylephrine. This effect is prevented or reduced by pretreatment of rats with 3-mercaptopropionic acid, bicuculline, or RO 15-4513. Intra-NTS injection of muscimol also inhibits baroreflex bradycardia and causes a pressor response which is potentiated by intra-NTS ethanol. It is proposed that ethanol inhibits baroreflex bradycardia, at least in part, by potentiating the action of endogenous γ-aminobutyric acid (GABA) at $GABA_A$ receptors in the NTS or its vicinity.

Introduction The main inhibitory and excitatory neurotransmitters in the central nervous system are γ-aminobutyric acid (GABA) and glutamate, respectively. Recent evidence indicates that the effects of ethanol on the nervous system may be mediated through its selective potentiation of $GABA_A$ receptor-mediated events (Suzdak *et al.*, 1986) and selective inhibition of effects mediated by the NMDA-type glutamate receptor (Lovinger *et al.*, 1989). In addition to its well known neurotoxic effects, ethanol also affects cardiovascular functions. Clinical and population studies have demonstrated the hypertensive effect of chronic ethanol consumption (Potter & Beevers, 1984). Although the mechanism underlying this effect is not clear, recent studies in the rat indicate that ethanol is a potent inhibitor of the depressor baroreflex response (Zhang *et al.*, 1989), which could contribute to its hypertensive action. The first synapse of the baroreflex arc is in the medullary nucleus of the solitary tract (NTS). While the nature of the baroreflex transmitter in the NTS has not been unequivocally established, hypothalamic GABA-ergic neurones can exert powerful inhibition of the baroreflex by activating $GABA_A$ receptors in the NTS (Barman & Gebber, 1979). Here we provide evidence that the baroreflex inhibitory effect of ethanol in anaesthetized rats is due, at least in part, to its potentiation of $GABA_A$ receptor effects in the area of the NTS.

Methods Male Sprague-Dawley rats weighing 300–350 g were anaesthetized with urethane, $0.3\,g\,kg^{-1}$ i.p. plus $0.8\,g\,kg^{-1}$ i.v. The femoral vein was cannulated for drug injections and a cannula in the femoral artery was connected to a pressure transducer and polygraph for direct measurement of blood pressure and heart rate. For microinjection of drugs into the NTS, the dorsal surface of the medulla was exposed by limited craniotomy and a glass microcannula was inserted into the NTS according to published coordinates (Mastrianni *et al.*, 1989). Drugs or ethanol were microinjected slowly in volumes of 50–100 nl. Baroreflex bradycardia was elicited by the i.v. injection of graded doses of phenylephrine (5, 10, 20, $40\,\mu g\,kg^{-1}$). Peak increases in blood pressure were plotted against the corresponding peak increases in pulse period, and the slope of the regression was taken as an indicator of baroreflex sensitivity.

Results Intravenous injection of $1\,g\,kg^{-1}$ ethanol into rats anaesthetized with urethane produced blood ethanol concentrations of 109 ± 10, 95 ± 1, 74 ± 3, and 61 ± 3 mg% at 5, 15, 40 and 60 min postinjection, respectively, as measured by gas chromatography. Basal blood pressure and heart rate were not significantly changed by ethanol. Intravenous injection of phenylephrine elicited a dose-dependent pressor effect and reflex bradycardia. When phenylephrine was injected following ethanol, the reflex bradycardic response was markedly reduced, resulting in a significant reduction of the slope of the baroreflex function curve (Table 1). In agreement with a recent report (Zhang *et al.*, 1989), microinjection of ethanol into the NTS also inhibited baroreflex bradycardia: the degree of inhibition was the same after the unilateral injection of 200 nmol ethanol or the bilateral injection of 25 nmol/side ethanol (Table 1). The inhibition was reversible with normal baroreflex responses restored within 2 h. No inhibition was observed when ethanol was microinjected as little as 0.5 mm lateral or rostral from the site of injection in the NTS.

In order to test whether endogenous GABA is involved in the baroreflex inhibitory effect of ethanol, rats were treated with the GABA depleting agent 3-mercaptopropionic acid at a

Table 1 The effect on ethanol on baroreflex sensitivity

			Ethanol	
		$1\,mg\,kg^{-1}$	NTS, unilat.	NTS, bilat.
Pretreatment	*Control*	i.v.	200 nmol	25 nmol/side
—	0.84 ± 0.10	0.32 ± 0.04*		
—	0.69 ± 0.15			0.37 ± 0.08*
—	0.68 ± 0.10		0.37 ± 0.08*	
3-MP	0.83 ± 0.27	0.97 ± 0.27		
3-MP	0.54 ± 0.12		0.75 ± 0.21	
RO 15-4513	0.81 ± 0.26	0.72 ± 0.15		
Bicuculline	0.71 ± 0.13	0.53 ± 0.12*		

In each animal, baroreflex sensitivity was determined before (control) and after the administration of ethanol, as described in Methods. Numbers represent the slope of the regression in ms $mmHg^{-1}$ (means ± s.e., $n = 4$–6). Asterisks indicate significant difference from the corresponding control value, * $P < 0.005$. 3-MP (3-mercaptopropionic acid, $100\,mg\,kg^{-1}$ i.p.), bicuculline ($2\,mg\,kg^{-1}$ i.p.) or RO 15-4513 ($5\,mg\,kg^{-1}$ i.p.) were given 5 min before the first test dose of phenylephrine in the control period.

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dose of 100 mg kg^{-1} i.p., which causes a substantial decrease in brain GABA content within 15 min which lasts for more than 60 min (Roberts *et al.*, 1978). 3-Mercaptopropionic acid treatment caused a small decrease in basal blood pressure. When ethanol was administered to these rats either i.v. or intra-NTS, it did not inhibit baroreflex bradycardia (Table 1). These results suggest that endogenous GABA localized in the NTS is involved in the baroreflex inhibitory action of ethanol. In other experiments, treatment of rats with the $GABA_A$ receptor antagonist bicuculline, 2 mg kg^{-1} i.p., also attenuated, although did not abolish, the baroreflex inhibitory action of ethanol (Table 1). These findings suggest that activation of $GABA_A$ receptors contributes to the baroreflex inhibitory action of ethanol. Further evidence for this was obtained in experiments with the benzodiazepine inverse agonist, RO 15-4513 (ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-[1,5α],[1,4]benzodiazepine-3-carboxylate), a compound that inhibits certain actions of ethanol presumably by interfering with the interaction between ethanol and the $GABA_A$ receptor complex (Ticku & Kulkarni, 1988). In rats pretreated with RO 15-4513, 5 mg kg^{-1} i.p., ethanol failed to inhibit baroreflex bradycardia (Table 1).

To test whether ethanol interacts with $GABA_A$ receptors in the NTS, we examined its effect on the response to the $GABA_A$ receptor agonist, muscimol. Muscimol microinjected into the NTS elicited a dose-dependent pressor response with no change in heart rate (Figure 1), and inhibited baroreflex bradycardia (baroreflex slope: 0.89 ± 0.12 vs 0.27 ± 0.08 ms mmHg^{-1} before and after 40 pmol muscimol, respectively, $P < 0.005$). When muscimol was tested after the intra-NTS injection of 200 nmol ethanol, it caused significant tachycardia and its pressor effect was markedly potentiated (Figure 1). The effect of ethanol on the muscimol response was selective: intra-NTS injection of 200 nmol ethanol did not influence the hypotensive and bradycardic response to 1 nmol glutamate microinjected into the same site (not shown).

Discussion The findings presented strongly suggest that potentiation of the effects of endogenous GABA at $GABA_A$ receptors in the NTS contributes to the inhibition of baroreflex bradycardia by ethanol. When ethanol was microinjected into the NTS, it inhibited baroreflex bradycardia, potentiated the effects of similarly administered muscimol and did not affect the response to glutamate. This selectivity and the reversibility of the inhibition of baroreflex bradycardia make it unlikely that the effects of ethanol in the NTS would be caused by non-specific tissue damage. The lack of effect of ethanol on the glutamate response is not in conflict with the reported inhibition by ethanol of NMDA-receptor-mediated responses (Lovinger *et al.*, 1989), as the effects of glutamate in the NTS are not affected by either NMDA or non-NMDA antagonists (Leone & Gordon, 1989).

Because of the close proximity of the NTS to the dorsal vagal nucleus it is possible that intra-NTS ethanol may have reached and interacted with $GABA_A$ receptors at the latter site (Zhang *et al.*, 1989; Mastrianni *et al.*, 1989). Indeed, there is evidence that the baroreflex inhibitory action of both ethanol (Zhang *et al.*, 1989) and GABA (Barman & Gebber, 1979) is primarily due to inhibition of the vagal outflow to the heart. Another possible site where GABA may be involved in the baroreflex inhibitory action of systemically administered ethanol is the caudal ventrolateral medulla where GABA inhibits depressor baroreflex responses through stimulation of $GABA_A$ receptors (Willette *et al.*, 1983). In any case, the present observations provide an example of the selective interaction of ethanol with a specific type of neurotransmitter receptor as the basis for one of ethanol's biological effects.

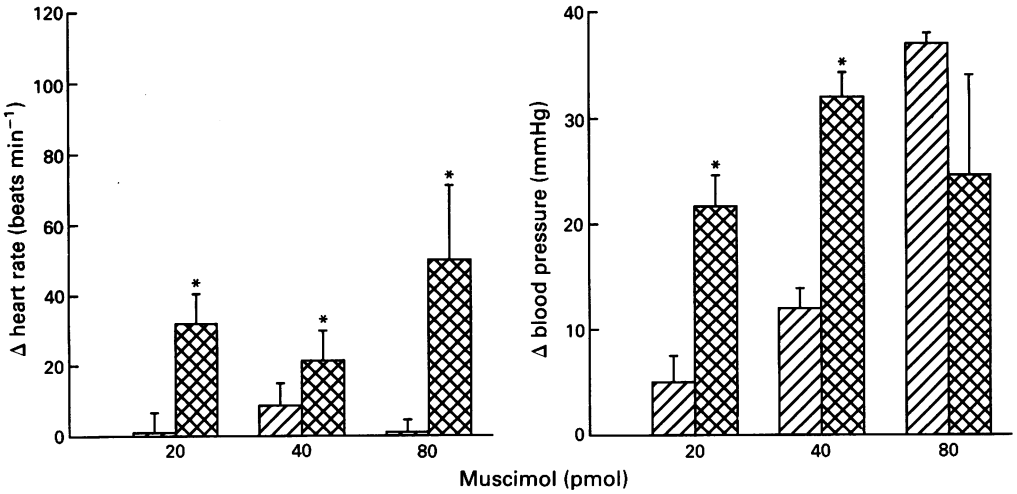

Figure 1 Ethanol potentiates the pressor and tachycardic effects of muscimol. Muscimol was injected bilaterally into the nucleus tractus solitarii (NTS) before (hatched columns) and after the bilateral intra-NTS injection of 200 nmol ethanol (cross-hatched columns). Numbers indicate the dose of muscimol in pmol/side. Vertical bars represent s.e., $n = 3$. Each rat was tested with only one dose of muscimol. * indicates significant difference between corresponding paired control and post-ethanol effects ($P < 0.05$).

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