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Cardiodepressant effects of ethanol on guinea-pig atria: presynaptic and postsynaptic components

LAURA MANTELLI*, VITTORIO CORTI, FABRIZIO LEDDA, *Department of Pharmacology, University of Florence, Viale G.B. Morgagni 65, Florence, Italy*

Ethanol, at concentrations ranging from 0·5 to 1·5%, depressed myocardial contractility of electrically-stimulated guinea-pig atria. This effect was evident in preparations bathed with a low calcium concentration, but was progressively reduced by increasing the extracellular calcium. The same concentrations of ethanol produced a dose-dependent inhibition of the cardiac response to field stimulation of the adrenergic nerve terminals. This effect was again calcium-dependent. These results support the hypothesis that the pre- and postsynaptic components of the cardiodepressant effects of ethanol are due to a reduction in calcium availability both at the nerve endings and in the contractile cells.

Acute exposure to ethanol depresses myocardial contractility in-vivo, both in man (Regan 1971; Ahmed et al 1973; Delgado et al 1975), and in animals (Mierzwiak et al 1972; Nakano & Prancan 1972; Horwitz & Atkins 1974), as well as in isolated cardiac preparations (Gimeno et al 1962; Nakano & Moore 1972; Kobayashi et al 1979).

Although the mechanisms responsible for the myocardial depressant effect of ethanol are still uncertain, it has been proposed that ethanol may interfere with the plasma membrane of the myocardium to disturb excitation-contraction coupling (Nakano & Moore 1972) or may limit the calcium availability to the contractile proteins (Ohnishi et al 1980). However, a cardiac presynaptic inhibitory effect by ethanol has not so far been considered, even though a reduction in the release of several neurotransmitters has been demonstrated both in the central nervous system (Kalant et al 1967; Carmichael & Israel 1975; Clark et al 1977; Sunahara & Kalant 1980) and in peripheral tissues (Mayer et al 1980). However, no report exists on the effects of ethanol on cardiac noradrenaline release. The present study was undertaken to investigate the cardiac effects induced by concentrations of ethanol which are similar to or higher than those present in human blood in the case of acute ethanol intoxication (Hirota et al (1976) on the contractile tension and at the same time on the cardiac adrenergic nerve function, using guinea-pig isolated atrial preparations.

Materials and methods

The experimental methods and apparatus are those described by Ledda & Mantelli (1984). Briefly, isolated guinea-pig atria were mounted vertically in a 15 ml glass chamber containing Tyrode solution of the following composition (mM): NaCl 115; KCl 4·7; $MgSO_4$ 1·2; KH_2PO_4 1·2; $NaHCO_3$ 25; glucose 10; $CaCl_2$ varied from 1·8 to 5·4 mM; the solution was bubbled with a gas mixture of 97% O_2 and 3% CO_2; the pH of the bathing solution was 7·4 and the temperature was maintained at 30 °C. A resting tension of 1 g was applied to the preparations and was maintained throughout the experiment.

The preparations were electrically stimulated at a constant rate, higher than the spontaneous rate (4 Hz), through two platinum electrodes. The isometric contraction was recorded by an isometric transducer and a dc preamplifier on a pen recorder and on a dual beam oscilloscope. After a 60 min equilibration period, field stimulation was started and trains of field pulses (50 mA, 1 ms) were applied at 2 min intervals through two platinum plates parallel to the preparation. Field pulses were delivered one per consecutive contraction; a control circuit allowed timing of the field pulses to begin 10 ms after each consecutive driving pulse, during the absolute refractory period, as described by Angus & Harvey (1981).

Stimulus-inotropic response curves were obtained by increasing the number of field pulses from 2 to 12, until the maximum positive inotropic effect was reached. Ethanol was then added; its effect on basal contractile tension was recorded after 10 min of contact, and the stimulus-response curve was determined again. As the response to graded field stimulation in a given preparation remained reproducible for many hours, it was usually possible to test the effect of three increasing concentrations of ethanol in the same experiment. The responses induced by field stimulation were expressed as % changes from the steady-state contraction; the maximum response of the control curve was taken as 100%. Statistical analysis was performed using Student's *t*-test. All the experiments were carried out in the presence of atropine (1 μM) in order to eliminate the parasympathomimetic component of the response to field stimulation.

Results

Inotropic effect of ethanol. The effect of graded concentrations of ethanol (0·5–1–1·5%, corresponding to 86·5, 173 and 259·5 mM respectively) on the contractile force of guinea-pig atria stimulated at 4 Hz and bathed with various Ca^{2+} concentrations is reported in Table 1.

* Correspondence.

Table 1. Effect of increasing ethanol concentrations on the contractile strength of isolated guinea-pig atria, electrically stimulated at 4 Hz and bathed with different calcium concentrations. Number of experiments is indicated in brackets. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$.

Ca^{2+} concn (mM)	Control mg	Ethanol 0·5% mg	Ethanol 1% mg	Ethanol 1·5% mg
1·8 (n = 5)	411·53 ± 23·11	310·93 ± 20·30**	245·81 ± 17·11***	225·58 ± 18·22***
3·6 (n = 6)	521·62 ± 64·93	462·65 ± 55·95*	400·22 ± 45·55*	349·50 ± 40·60***
5·4 (n = 4)	710·82 ± 86·25	675·56 ± 74·48	596·33 ± 71·72	537·02 ± 68·09*

Ethanol significantly depressed the contractile tension of atria in a dose-dependent manner at all the extracellular Ca^{2+} concentrations tested. The depressant effect was greater in preparations bathed with 1·8 mM Ca^{2+}; in fact the contractile tension was reduced to half by the higher ethanol concentration tested. In preparations bathed with 3·6 mM Ca^{2+} the contractile strength was reduced to about 65%; atria bathed with 5·4 mM Ca^{2+} showed a greater control contractile tension (see Table 1); however, also in this case the peak tension was reduced by ethanol to 75% of the control.

Table 1 shows that the cardiodepressant effect of ethanol was partly antagonized by increasing the extracellular Ca^{2+} concentration.

Effect of ethanol on the stimulus-response curve. The stimulation of the sympathetic nerve terminals by trains of field pulses applied during the absolute refractory period induced a graded positive inotropic response. Exposure of the preparations to ethanol (0·5–1–1·5%) for 10 min before field stimulation produced different effects depending on the different calcium concentrations (Fig. 1). In atria bathed with a low calcium concentration (1·8 mM), ethanol significantly reduced the stimulus-response curve in a dose-dependent manner. The concentration of ethanol able to induce a 50% inhibition of the response caused by stimulation of sympathetic nerve terminals (calculated for an intermediate response, i.e. that induced by a train of 6 field pulses) was 1·20 ± 0·02%. The effect of ethanol was reduced by increasing the calcium concentration to 3·6 mM; in this case the ID50 of ethanol was increased to 3·60 ± 0·43%. The inhibitory effect was completely absent in preparations bathed with a higher Ca^{2+} concentration, i.e. 5·4 mM. To ensure that the inhibitory effect of ethanol in preparations bathed with 1·8 mM Ca^{2+} could not be attributed to a blocking effect on postsynaptic β-adrenoceptors, the higher concentration of ethanol (1·5%) was tested on the dose-effect curve for exogenous noradrenaline. It has been observed that 1·5% ethanol did not reduce the positive inotropic effect of exogenously administered noradrenaline (not shown).

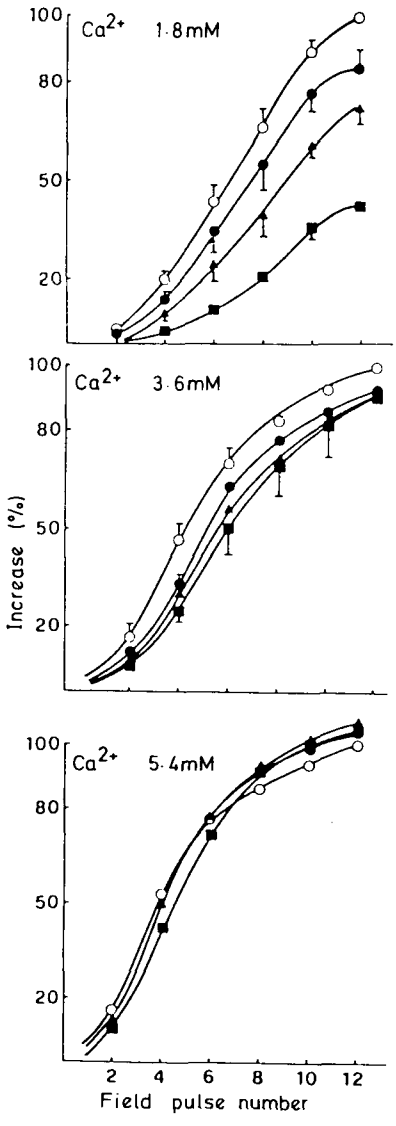

FIG. 1. Effect of different ethanol doses on the cardiac response to sympathetic nerve stimulation in preparations bathed with different calcium concentrations. (○), control; (●), ethanol 0·5%; (▲), ethanol 1%; (■), ethanol 1·5%. Points represent mean values of 4–6 experiments. Vertical bars indicate standard errors; where not indicated they are obscured by the symbols.

Discussion

The results of the present study have demonstrated that ethanol (0·5–1·5%) produces a dose- and calcium-dependent negative inotropic effect in guinea-pig iso-

lated atria, and a reduction of the positive inotropic reponse induced by sympathetic nerve stimulation. Both these phenomena, in different ways, are dependent on the cellular calcium availability. Calcium ions are necessary for excitation-contraction coupling as well as for neurotransmitter release.

The cardiodepressant effect of ethanol has been attributed to an interaction with the plasma membranes of the myocardium resulting in an enhanced calcium binding to membrane proteins and a reduction of the calcium supply to the contractile proteins (Nakano & Moore 1972; Rudolph et al 1979; Ohnishi et al 1980). Moreover, electrophysiological studies have shown that ethanol shortens the plateau of the cardiac action potential, a phase which is known to be sustained by a calcium inward current (Snoy et al 1980; Williams et al 1980).

The hypothesis that ethanol interferes with the contractile process by reducing the calcium availability at the contractile proteins agrees with our finding that the negative inotropic effect of ethanol is reduced by increasing the extracellular calcium concentration. With regard to the observation that ethanol reduces the positive inotropic effect of sympathetic nerve stimulation, the finding that this effect is antagonized by increasing calcium concentration suggests that ethanol may also reduce cellular calcium availability in nerve cells. In fact, calcium fluxes caused by depolarization are blocked by ethanol in isolated brain synaptosomes (Harris & Hood 1980; Harris 1981; Stokes & Harris 1982) and in smooth muscle (Sanders & Bauer 1982). Moreover, our data on the calcium dependence of the effect of ethanol are in agreement with those of Mayer et al (1980), who found that the inhibition of electrically-evoked contractions of guinea-pig ileum by ethanol was partially reversed by raising extracellular calcium concentration. In conclusion, the results of the present study indicate that ethanol reduces electrically-stimulated noradrenaline release whilst it impairs cardiac contractility. The calcium-dependence of both these effects suggests an inhibitory action of ethanol on calcium fluxes both at the nerve and at the muscle cells.

From a toxicological point of view the sum of the two distinct, but both negative, effects (the pre- and post-synaptic ones) demonstrated in our in-vitro preparations with high doses of ethanol, may justify the cardiodepressant effects reported in-vivo both in animals and in man with even lower doses of ethanol than the ones we have tested.

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