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The Effect of Acute Ethanol Exposure on the Chronotropic and Inotropic Function of the Rat Right Atrium

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Abstract

Consumption of ethanol (CH_2CH_3OH), both acutely and chronically, is known to affect cardiac function and may alter the autonomic control of the heart. This study investigated the effects of two modes of acute exposure to ethanol on the chronotropy and inotropy of the rat right atrium with emphasis on alterations in the adrenergic responses.

Atria from rats infused with an anesthetizing level of ethanol for 21 h showed a tendency for a greater increase of the unstimulated beating rate with isoproterenol (ISO), while both unstimulated inotropy and the inotropic response to ISO were significantly decreased compared with the control. Right atria in the presence of ethanol in-vitro demonstrated decreased basal active tension development and decreased inotropic responses to ISO. No alteration of the chronotropic response to ISO was evident with any concentration of ethanol.

These results demonstrate both an immediate as well as a persistent effect of ethanol on right atrial chronotropy and inotropy. Alterations in the G-stimulatory subunit of the adenylate cyclase system and alterations in myofilament binding of Ca^{2+} are consistent with these observed ethanol effects.

Consumption of ethanol, both acutely and chronically, is known to affect the function of the cardiovascular system directly and indirectly. Alcohol can directly cause mechanical and electrophysiological changes in the heart (Katz 1982). There is also evidence of a decrease in β-receptor number and alterations of responsiveness to both adrenoceptor and cholinoceptor agonists (Banerjee et al 1978; Pohorecky 1982; Posner et al 1985). The control of the heart by the autonomic nervous system could be compromised by ethanol, resulting in the inability of the heart to efficiently adjust cardiac output. This study was performed to determine if acutely administered ethanol alters the intrinsic beating rate and inotropy of spontaneously beating right atria or alters the effects of adrenergic agents on beating rate and inotropy.

Materials and Methods

Ethanol infusion procedure

Sprague–Dawley rats (300–350 g) were anesthetized with ketamine (10 mg/100 g body weight) and xylazine (0·2 mg/100 g body weight) via an intramuscular injection. A polyethylene catheter (PE-50) was placed in the right jugular vein using aseptic techniques and exteriorized dorsally in the cervical region. Rats were placed in clean cages following surgery.

Two hours after recovery from the anesthesia, rats were given ethanol using a method modified from D'Souza et al (1990). The amount of a 20% ethanol and sterile saline solution that would provide 350 mg/100 g body weight was calculated for each rat and this volume (5–6 mL) was injected intravenously over a 10 min period. This was immediately followed by the constant infusion of ethanol (25–30 mg ethanol/100 g body weight h^{-1}) to maintain a blood level of approximately 400 mg dL^{-1} (88 mM) for 21–22 h. Although blood ethanol levels were not measured, rats were lightly anesthetized (bordering on consciousness) at the blood alcohol levels attained. After the infusion was terminated, rats were fully anesthetized with the inhalant anesthetic enflurane and the right atria were isolated. Concentration–response curves for isoproterenol (ISO) were generated as described below.

Isolated right atria preparation

A modification of the method of Kenakin & Black (1978) was used in the isolation of the rat right

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atria. Rats were anesthetized with the inhalant anesthetic enflurane and were exsanguinated by transection of the carotids. Hearts were removed and placed in cooled Krebs-Ringer bicarbonate buffer. The right atria were cut away from the ventricles, taking care not to damage the sinoatrial node, and cotton thread ties were placed on the apex and base. Each atrium was suspended with its base end anchored to the bottom of a 10-mL organ bath containing Krebs-Ringer bicarbonate buffer and its apex fastened to a force transducer. Passive tension (diastolic tension) was set at 0·5 g. The total time from sacrifice of the rat to placement of the atrium in the organ bath was less than 5 min.

The Krebs-Ringer bicarbonate buffer (pH 7·4) contained (mM): 124 NaCl; 5 KCl; 1·30 $MgCl_2$; 2·24 $CaCl_2$; 25·0 $NaHCO_3$; 0·6 NaH_2PO_4; 10 dextrose; 0·005 17 β-estradiol (to inhibit extraneuronal uptake of ISO (Salt 1972)); 0·03 disodium ethylenediaminetetraacetic acid (Na_2EDTA), and 0·3 ascorbate (both to inhibit oxidation of ISO (Huges & Smith 1978)). The organ baths were maintained at 34°C (to assure viability of the tissue for the duration of the experiment) and gassed with 95% O_2/5% CO_2, bubbled from the bottom of the organ bath.

The atrial beating rate and contractile responses were recorded with Grass force–displacement transducers (model FT.03, Grass Instruments, Inc., Quincy, MA) used in conjunction with an ECG/tachograph equipped Grass Polygraph (model 7D). Force–displacement transducers were calibrated with 1 and 0·5 g weight standards so that the atrial contractile force could be determined from the magnitude of the physiograph pen deflection. Following a 60 min equilibration period, 1 nM ISO was added to prime the tissue. After washout of ISO and on stabilization of the beating rate, cumulative concentration–response curves were constructed for one of the following agents: ISO, a non-selective β-agonist; $CaCl_2$; ethanol.

The measurements used to assess atrial contractile function in this study were passive tension (diastolic tension) and active tension (systolic minus diastolic tension) development. Basal active tension development where noted refers to active tension development in the absence of ISO or $CaCl_2$.

In-vitro ethanol exposure procedure

The in-vitro function of the rat right atrium during ethanol exposure was investigated using two clinically relevant concentrations (concentrations compatible with life in humans) and one higher concentration of ethanol. The clinically relevant doses were 23 mM (104·5 mg dL^{-1}), which is close to the legally intoxicating blood level in humans (i.e. 0·1%), and 57 mM (259·1 mg dL^{-1}), a blood level that would cause unconsciousness in most humans. A concentration not compatible with life in humans (117 mM or 531·8 mg dL^{-1}) was used to amplify any tendencies seen with the lower doses. The ethanol concentration of the organ baths was determined with a YSI biological analyser for alcohol.

The right atria from naive Sprague–Dawley rats (300–350 g) were removed and placed in 10-mL organ baths using the procedure described below. After priming and stabilization of the tissue, two cumulative concentration–response curves were constructed for either ISO or $CaCl_2$: one before and one during exposure of the atria to one of the three concentrations of ethanol. Between each curve, the organ baths were flushed with fresh Krebs buffer and basal values reattained before the next curve was started. Atria were allowed to stabilize for 5 min after ethanol was added and changes in basal function were monitored. A second curve was then constructed. Atria that did not return to within 0·05 g of the original baseline (the preset 0·5 g passive tension) after the control ISO curve were not included in the inotropic study and were only included in the chronotropic study if no arrhythmias were evident and an increase of more than 100 beats min^{-1} occurred in response to the maximum concentration of ISO.

Cumulative concentration–response curves

The concentrations of studied agents were varied cumulatively. Atria were allowed to stabilize between cumulative concentration–response curves for 30 to 45 min, during which time they were flushed with fresh Krebs buffer every 15 min. Atria with a change in rate of less than 100 beats min^{-1} over the course of the ISO concentration–response curve were eliminated from the study regardless of the inotropic response. All cumulative concentration–rate response curves were evaluated for fit to the following logistics equation:

$$E = E_{max}/\{1 + (EC_{50}/[A])^n\} + \Theta \qquad (1)$$

where E is the increase in rate above basal, E_{max} is the maximum response, EC_{50} is the concentration of agonist, [A], producing half-maximal response, the exponential term, n, is a curve-fitting parameter that defines the slope of the concentration–response curve and Θ is the response in the absence of added agonist. Curve fitting for the rate response was done with the constraint that Θ was

equal to zero using the program Allfit (Delean et al 1978), which was rewritten in Microsoft QuickBasic and compiled for use in an MS-DOS operating system. Curve fitting was not performed on cumulative ISO concentration–inotropic response data because of the decrease in active tension development that followed the peak increase at the higher ISO concentrations.

Preparation of drugs

All drug stock solutions and serial dilutions were prepared immediately before use with Krebs buffer. Solutions of ISO were kept on ice to help retard oxidation. ISO was purchased from Sigma Chemical Co. (St Louis, MO).

Data analysis

An unpaired *t*-test was used to analyse the difference in mean values obtained in the treated group and control group in the ethanol infusion study. A paired *t*-test was used to determine differences before and after treatment in the in-vitro study. A P value of 0·05 or less was accepted as statistically significant.

Results

Rate–tension relationship of the right atria

The inotropic data used in this study were taken from spontaneously beating right atria (more closely approximating the in-vivo situation than an electrically stimulated preparation). Although beating rate can affect the active tension development of atria (Kruta & Stejskalová 1960; Allen 1992), increasing beating rate did not account for the positive active tension development seen with ISO stimulation. This conclusion was reached because right atria that were paced electrically from 250 to 375 beats min^{-1} (the same range of rates produced by ISO stimulation during the steepest part of the concentration–response curve) showed a $36{\cdot}8 \pm 3{\cdot}1\%$ decrease in active tension development with an approximately 7% decrease for every 25 beats min^{-1} increment (Figure 1). The negative rate–tension relationship of the electrically paced adult rat myocardium is well documented (Hollander & Webb 1955; Forester & Mainwood 1974). The negative rate–tension relationship observed in our experimental preparation appeared to be due to an increase in passive tension (note how passive tension mirrors active tension development); possibly a consequence of inadequate Ca^{2+} uptake by the sarcoplasmic reticulum with increasing beating rate (Orchard & Lakatta 1985). Because there was a negative and not a positive rate–tension relationship, any increase in active tension development seen with ISO must have been due to a direct effect by β_1-receptor stimulation on inotropy.

Based solely on the active tension development response of electrically stimulated right atria, the beating rate increase due to ISO may have blunted the positive inotropic response due to ISO. However, passive tension did not show the same pattern of increase in the ISO-stimulated atria as in the paced atria (Figure 2). Passive tension remained unchanged at 333 beats min^{-1} when the rate was increased by ISO. The concentration of ISO that produced this rate of 333 beats min^{-1} is also the approximate concentration that produces the maximum active tension development. The positive inotropic response to ISO was probably not blunted by a rate-induced increase in passive tension because β_1-receptor stimulation enhances Ca^{2+} uptake by the sarcoplasmic reticulum, in addition to its effect on Ca^{2+} influx through the sarcolemma (Shah et al 1994).

The effect of 21-h ethanol infusion on the right atrial chronotropic and inotropic response to β-adrenergic stimulation

Ethanol at a calculated blood level of 400 mg dL^{-1} maintained for 21 h by infusion did not significantly change the chronotropic response of isolated right atria to ISO. There was, however, a tendency for the maximal increase in beating rate above basal rate to be greater in the alcohol group (control = 153 ± 10 beats min^{-1} increase, n = 6; alcohol = 194 ± 19 beats min^{-1} increase, n = 5; $P = 0{\cdot}078$).

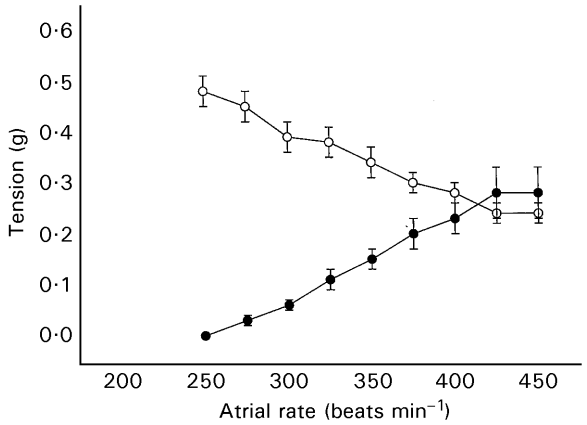

Figure 1. Passive tension (above the unstimulated, baseline passive tension) (●) and active tension (○) development in response to electrical pacing of right atria within a range of beating rates that was obtained with cumulative concentrations of ISO. The data were obtained from one group of right atria. Values represent mean ± s.e.m., n = 4.

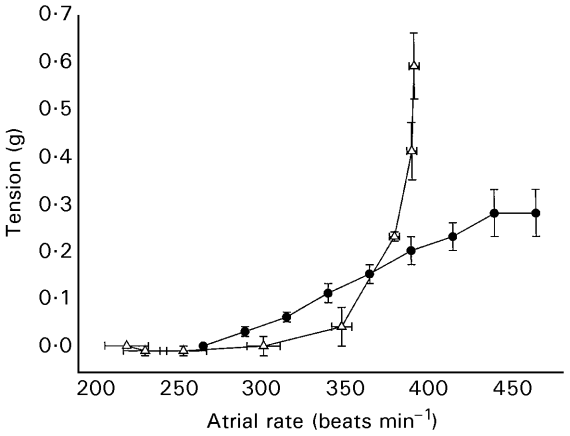

Figure 2. Passive tension (above the unstimulated, baseline passive tension) as a function of increasing beating rate due to either electrical pacing (●) or ISO stimulation (△). The data were obtained from one group of right atria. Values represent mean ± s.e.m., n = 4.

Basal rate was 248 ± 11 beats min^{-1} in the control group (n = 6) and 234 ± 16 beats min^{-1} in the alcohol group (n = 5), while the maximum rates in response to ISO for the control and alcohol groups was 403 ± 9 and 428 ± 16 beats min^{-1}, respectively. The EC_{50} values were $1{\cdot}4 \pm 0{\cdot}2 \times 10^{-9}$ and $2{\cdot}0 \pm 0{\cdot}2 \times 10^{-9}$ M for the control and alcohol-infused groups, respectively.

Spontaneously beating right atria from the ethanol-infused rats had significantly lower basal active tension development than the atria from control rats (Table 1). At 1×10^{-11} M ISO, active tension development (which was the same as under basal conditions) in the alcohol-infused group was 40% of the active tension development in the control atria. A significant decrease was also seen at 1×10^{-10} and 1×10^{-9} M ISO. Although the maximum active tension development in the two groups was not statistically different, the difference between the control and infused groups at all doses of ISO was still 0·21 g, suggesting that ISO may not have overcome the ethanol-induced drop in active tension development.

When the inotropic response of an atrium at each lower dose of ISO was expressed as a percentage of its own maximum response to ISO, the two groups showed a similar responsiveness to ISO (Figure 3). Interestingly, the controls showed a decrease in active tension development at 1×10^{-7} M ISO that was not evident in the alcohol group.

Chronotropic and inotropic response to cumulative ethanol concentrations

Previous studies have shown that ethanol has a positive chronotropic effect on right atria in-vitro (Carpentier & Carpentier 1987). However, in this study cumulative ethanol concentrations up to 200 mM had a minimal effect on the beating rate of the right atria (Table 2). The basal rate was fairly well maintained throughout the range of ethanol concentrations shown. Ethanol did, however, decrease the active tension development of spontaneously beating right atria in a dose-dependent manner. At the highest concentration of ethanol used (200 mM), there was a 41·1 ± 5·1% decrease in active tension development (data not shown).

Chronotropic and inotropic response to β-adrenergic stimulation in the presence of ethanol in-vitro

We next tested the effect of ethanol on the chronotropic effects of β-receptor activation. The

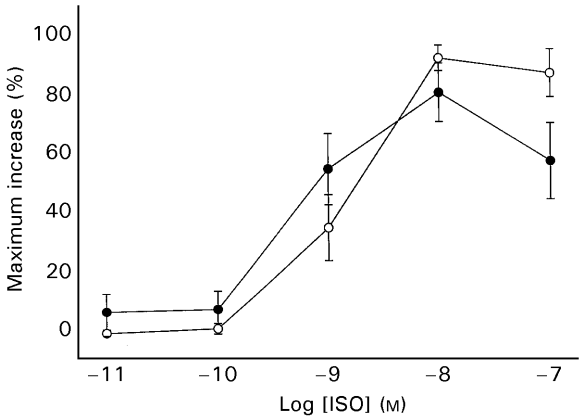

Figure 3. The inotropic response to cumulative concentrations of ISO by right atria from rats infused with ethanol (n = 5) for a period of 21 h (○) compared with the inotropic response of right atria from saline-infused rats (●, n = 6). Values represent mean ± s.e.m.

Table 1. Inotropic response (g) to ISO of spontaneously beating right atria isolated from rats in which blood ethanol levels were maintained at approximately 400 mg dL^{-1} for 21 h compared with that of right atria from saline-infused controls. Also shown is the average maximum active tension development (g).

	log [ISO] (M)					Maximum response to ISO (g)
	−11	−10	−9	−8	−7	
Control (n = 6)	0·35 ± 0·07	0·36 ± 0·06	0·57 ± 0·04	0·73 ± 0·10	0·62 ± 0·12	0·77 ± 0·10
Ethanol infused (n = 5)	0·14 ± 0·04*	0·15 ± 0·05*	0·30 ± 0·09*	0·53 ± 0·12	0·50 ± 0·10	0·56 ± 0·12

ISO concentration is expressed as the logarithm of molar concentration. *$P \leq 0{\cdot}05$, values significantly different to control.

chronotropic response to ISO was the same in the presence or absence of 23 and 57 mM ethanol (Figures 4A and B). The highest concentration of ethanol (117 mM) caused a slight, yet significant, change in basal beating rate ($P = 0{\cdot}037$, paired t-test), but had no effect on the chronotropic response to ISO (Figure 4C).

The inotropic response to ISO in the presence of ethanol was significantly different from the response in the absence of ethanol (Table 3). Basal active tension development (not shown) decreased significantly from $0{\cdot}48 \pm 0{\cdot}07$ to $0{\cdot}45 \pm 0{\cdot}07$ g (n = 4) with 23 mM ethanol, from $0{\cdot}37 \pm 0{\cdot}08$ to $0{\cdot}28 \pm 0{\cdot}05$ g (n = 5) with 57 mM ethanol and from $0{\cdot}76 \pm 0{\cdot}03$ to $0{\cdot}51 \pm 0{\cdot}01$ g (n = 4) with 117 mM ethanol. The inotropic response was significantly lower at 10^{-9} and 10^{-8} M ISO in the presence of 23 mM ethanol. With 57 mM ethanol present, the response was significantly decreased at 10^{-11} to 10^{-9} M. With 117 mM ethanol, the response was decreased at 10^{-10} and 10^{-9} M ISO. The maximum response of each atrium, regardless of the ISO concentration that produced it, was significantly decreased by 57 and 117 mM ethanol. The concentration at which the maximum response was achieved in each individual atrium was not altered by ethanol (not shown). The active tension development of all atria decreased in a dose-dependent manner in both the presence and absence of ethanol with concentrations of 1×10^{-7} M ISO and greater.

Figure 5 shows the increase in atrial active tension development expressed as a percentage of its own maximum ISO-induced increase in active

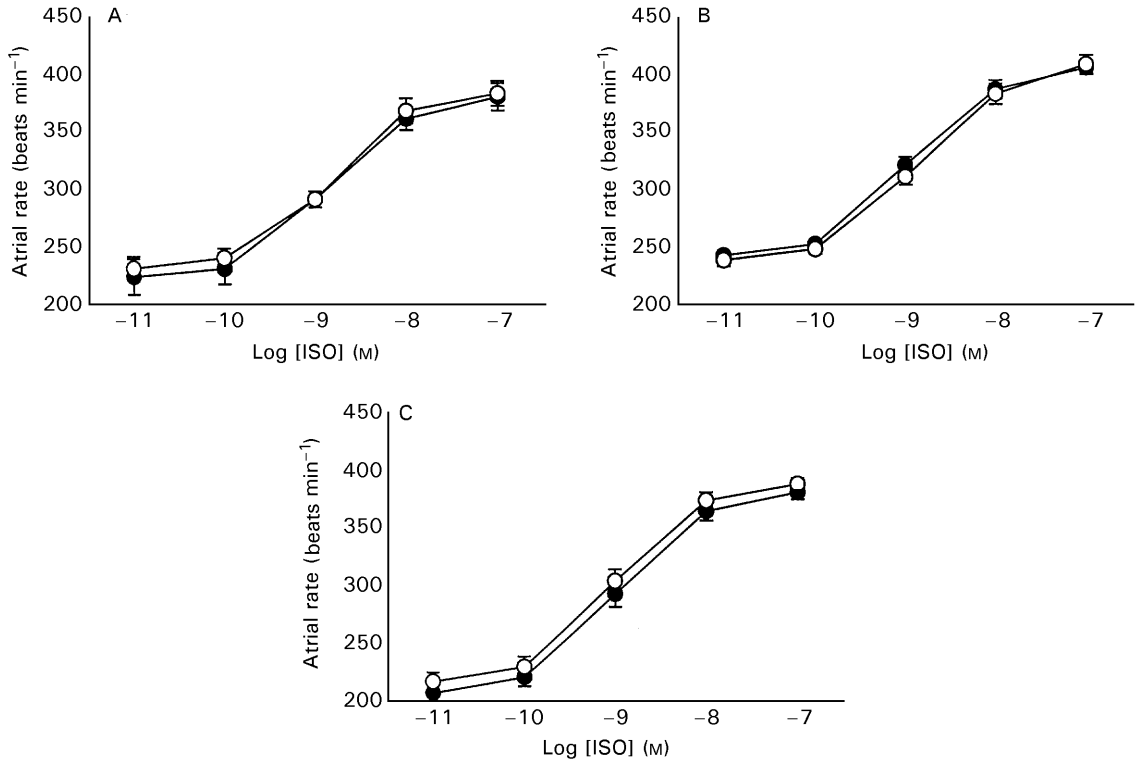

Figure 4. Chronotropic response to ISO of right atria in the absence of ethanol (A, n = 4; B, n = 5; C, n = 7) (●) and then in the presence of 23 (A, n = 4), 57 (B, n = 5) and 117 (C, n = 7) mM ethanol (○, n = 4). Values represent mean ± s.e.m.

Table 2. Chronotropic response in beats min^{-1} and as a percentage increase in beating rate of spontaneously beating right atria (n = 4) to cumulative concentrations of ethanol.

	Ethanol Concn (mM)										
	0	20	40	60	80	100	120	140	160	180	200
Beats min^{-1}	230 ± 12	231 ± 11	232 ± 11	234 ± 12	237 ± 12	241 ± 12	243 ± 12	246 ± 12	254 ± 13	257 ± 13	262 ± 13
Increase (%)	0	0·6 ± 0·8	1·0 ± 0·6	1·9 ± 1·2	3·2 ± 1·0	4·9 ± 1·4	5·8 ± 1·8	7·1 ± 2·0	10·6 ± 2·3	11·9 ± 2·8	14·2 ± 2·9

Table 3. Paired data showing the inotropic response (g) to cumulative concentrations of ISO in the absence of ethanol or in the presence of 23, 57 or 117 mM of ethanol. Also shown is the average maximum active tension development.

	log [ISO] (M)					Maximum response to [ISO] (g)
	−11	−10	−9	−8	−7	
Ethanol concn (mM)						
0 (n = 4)	0·50 ± 0·08	0·57 ± 0·11	0·81 ± 0·15	0·85 ± 0·11	0·66 ± 0·09	0·87 ± 0·13
23 mM	0·51 ± 0·11	0·51 ± 0·10	0·65 ± 0·11*	0·76 ± 0·09*	0·58 ± 0·06	0·76 ± 0·09
0 (n = 5)	0·42 ± 0·06	0·47 ± 0·08	0·70 ± 0·10	0·76 ± 0·09	0·58 ± 0·09	0·80 ± 0·10
57 mM	0·30 ± 0·06*	0·34 ± 0·07*	0·52 ± 0·11*	0·69 ± 0·11	0·61 ± 0·10	0·69 ± 0·10*
0 (n = 4)	0·68 ± 0·07	0·92 ± 0·06	1·39 ± 0·11	1·06 ± 0·10	0·78 ± 0·06	1·40 ± 0·11
117 mM	0·53 ± 0·02	0·68 ± 0·03*	0·98 ± 0·05*	0·89 ± 0·09	0·71 ± 0·07	0·99 ± 0·05*

ISO concentration is expressed as the logarithm of the molar concentration. Values represent means ± s.e.m. *$P \leq 0{\cdot}05$, values significantly different to control.

tension development in the absence of ethanol. The greatest effect of ethanol occurred at 1×10^{-9} M ISO for all three concentrations of ethanol tested. Figures 5A, B and C, respectively, show that 91·4 ± 5·1%, 87·2 ± 5·4% and 99·5 ± 0·5% of the control maximum was attained with 10^{-9} M ISO in the absence of ethanol as opposed to 74·9 ± 2·4, 63·8 ± 8·0 and 70·7 ± 2·7% in the presence of the three concentrations of ethanol tested. The sensitivity of the inotropic response to ISO was the same in the presence and absence of all concentrations of ethanol.

After the ISO concentration–response curves were constructed in the presence of 117 mM ethanol and the tissues were allowed to recover in the absence of ethanol, addition of 1×10^{-8} M ISO increased active tension development to a similar extent as before addition of ethanol (control = 1·02 ± 0·07 g, n = 7; post-ethanol = 1·15 ± 0·41 g, n = 7). This suggested a reversal of the ethanol effect.

Figure 6 compares the passive tension response to ISO in the absence and presence of 117 mM ethanol. The smaller decrease in active tension development at high concentrations of ISO in the presence of ethanol (Table 3) may be due to the smaller increase in passive tension at these concentrations of ISO in the presence of ethanol.

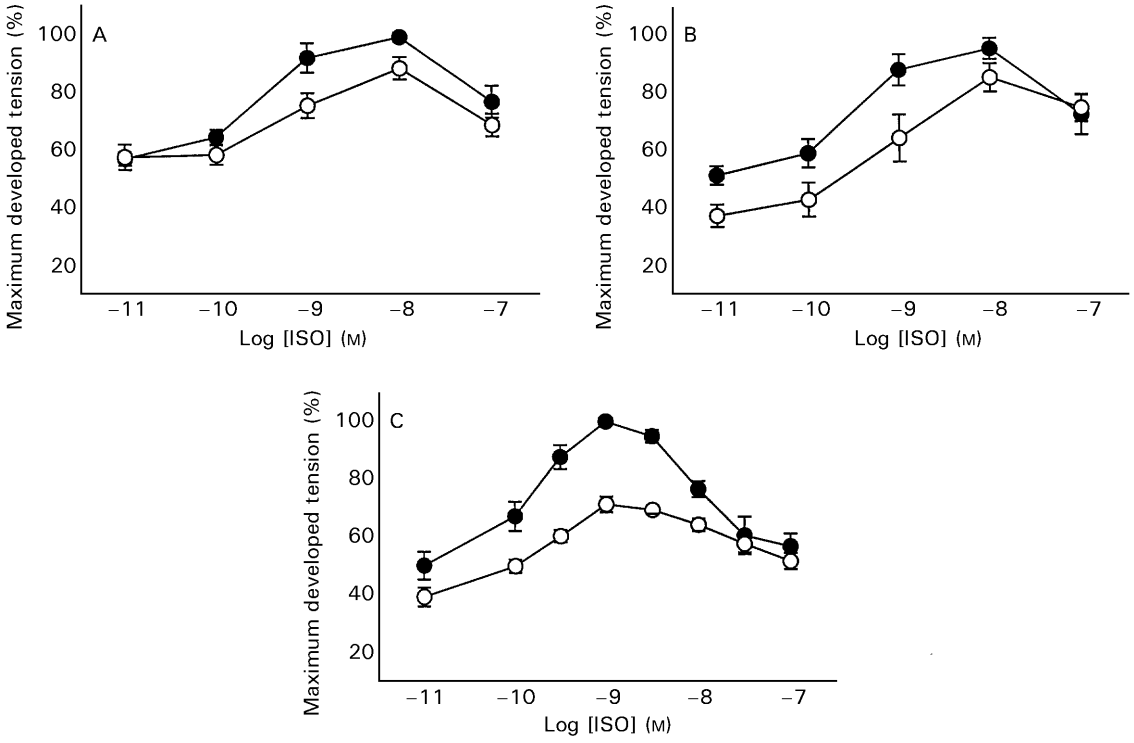

Figure 5. Inotropic response to ISO of right atria in the absence of ethanol (A, n = 4; B, n = 5; C, n = 4) (●) and then in the presence of 23 (A, n = 4), 57 (B, n = 5) and 117 (C, n = 4) mM ethanol (○). Values represent mean ± s.e.m.

The effect of ethanol on the positive inotropic response of spontaneously beating right atria to Ca^{2+}

The positive inotropic response to Ca^{2+}, like that of ISO, was significantly decreased by ethanol (Figure 7). We used Ca^{2+} as another positive inotrope to further elucidate which part of the excitation–contraction coupling event was affected in this in-vitro exposure to ethanol. Increasing the $CaCl_2$ concentration from 1 to 6 mM increased active tension development in both the presence and absence of 57 mM ethanol (Figure 7). Active tension development attained a plateau at 6 mM $CaCl_2$, which continued through 10 mM $CaCl_2$ with and without ethanol present (not graphed). Basal active tension development was $0{\cdot}31 \pm 0{\cdot}06$ g with 1 mM $CaCl_2$ for the first curve and returned to $0{\cdot}28 \pm 0{\cdot}04$ g when 10 mM $CaCl_2$ was replaced with 1 mM $CaCl_2$. In the presence of 57 mM ethanol, however, there was a significant $32{\cdot}5 \pm 6{\cdot}2\%$ decrease in active tension development at the lowest $CaCl_2$ concentration (1 mM), as well as at the highest concentration graphed (6 mM). Thus the increase in Ca^{2+} concentration could not overcome the negative inotropic effect of ethanol.

When the inotropic response to increasing Ca^{2+} was expressed as a percentage of the maximum increase, the response in the presence or absence of 57 mM ethanol was not different. Approximately 50% of the maximum increase occurred at 3 mM $CaCl_2$ in both groups; thus the sensitivity to the inotropic effects of Ca^{2+} was not altered by alcohol. The beating rate showed little increase as $CaCl_2$ concentration was increased from 1 to 6 mM. Ethanol did not change this lack of a chronotropic response to Ca^{2+}.

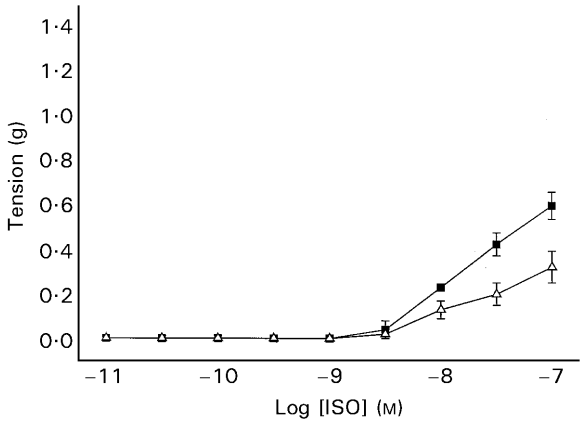

Figure 6. Passive tension (above the unstimulated, baseline passive tension) in ISO stimulated right atria in the absence of ethanol (■) and then in the presence of 117 mM ethanol (△). Values represent mean ± s.e.m., n = 4.

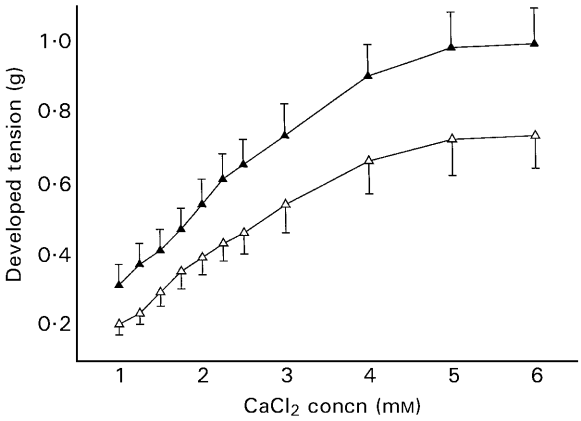

Figure 7. The inotropic response of right atria to an increasing concentration of calcium in the absence (▲) and presence (△) of 57 mM ethanol. Values represent mean ± s.e.m., n = 7.

Discussion

This study investigated the effect of 21 h of ethanol infusion in-vivo and ethanol exposure in-vitro on the spontaneous beating rate and contractile performance of rat right atria in-vitro. In addition, ethanol-induced changes in β-adrenergic responsiveness were investigated. In both modes of ethanol exposure, inotropy was primarily affected, with only small changes in chronotropy.

The physiological effects of ethanol vary depending on whether blood ethanol levels are rising or falling (Ekman et al 1964). For this reason an infusion protocol was used in order to amplify the changes that might occur while ethanol is at its highest concentration in the blood. An ethanol blood level calculated to be approximately 400 mg dL^{-1} (88 mM) and maintained for 21 h by infusion did not significantly change the in-vitro right atrial basal beating rate or the chronotropic response to β_1-receptor stimulation. There was, however, a tendency for the maximum increase from the basal beating rate to be greater in the ethanol infused group. In view of the fact that ethanol-induced catecholamine release has been documented (Pohorecky 1977; Adams & Hirst 1983) we had predicted a diminished responsiveness to adrenoceptor agonists due to the down-regulation of β-receptors (Kenakin & Ferris 1983). Furthermore, a significantly increased catecholamine excretion (indicative of an increased catecholamine release) was demonstrated in a study by King & Hirst (1990) using rats in which the blood ethanol levels (74·7–92·7 mM) and the maintenance period of these levels (24 h) were very similar to that of our study. The absence of a decrease in the chronotropic response to ISO in our study could be due to the development of tolerance to the constant

blood ethanol level, resulting in catecholamine release returning to normal. Conversely, continual increasing and decreasing of blood ethanol levels would tend to inhibit development of tolerance. Just such a situation would have existed in the King & Hirst (1990) study owing to their method of ethanol administration. It is also possible that stress-induced catecholamine release was minimized with our infusion method of ethanol administration. For whatever reason, the predicted decrease in chronotropic response to ISO did not occur.

As with the constant infusion model, the right atrial basal beating rate did not change in response to 23 or 57 mM ethanol in-vitro, but there was a small, yet significant, increase in beating rate with 117 mM ethanol. An increase in beating rate due to in-vitro ethanol exposure has been observed with lower concentrations of ethanol by other investigators (Carpentier & Carpentier 1987). Carpentier & Carpentier (1987) attributed this response to the direct effect of ethanol on the sinoatrial node. In contrast, this increase was only evident at the highest ethanol concentration in our study. No observable change was noted in the chronotropic response to β-receptor stimulation by ISO at any in-vitro ethanol concentration in our study.

Other investigators have observed that in intact cells and in cell-free striatal and cerebral cortical tissue preparations, ethanol appears to alter the function of the G-protein component of the adenylate cyclase system. Luthin & Tabakoff (1984) observed that 75 mM ethanol in-vitro increased guanine nucleotide and agonist-stimulated adenylate cyclase activity in the striatal membrane. In addition, they demonstrated the possibility that there is a specific effect on the G_s protein. Saito et al (1987) demonstrated the same for cerebral cortical membrane preparations with 50 mM ethanol. This documented effect of ethanol on the G_s protein suggests a possible explanation for the observed tendency for a greater maximum rate response to ISO after ethanol infusion. It also may explain the higher basal beating rate observed with 117 mM ethanol in-vitro.

The effects of the two different modes of exposure to ethanol on right atrial inotropy were also investigated in this study. Significant effects on inotropy were observed in both the infusion and the in-vitro models of ethanol presentation.

The 21-h maintenance of an ethanol blood level of 400 mg dL^{-1} significantly decreased the basal inotropy of isolated right atria and this depression was not overcome with β-receptor stimulation by ISO. Similar levels of ethanol were attained and maintained in the anesthetizing range for a period of 24 h by gastric tube feeding in the study by King & Hirst (1990). They suggested that a recorded increase in heart weight was due to β-receptor activation-dependent hypertrophy. Ethanol-induced cardiac hypertrophy is associated with compromised inotropic function (Regan 1984; Urbano-Marquez et al 1989). This may be one explanation for the compromised inotropy of the right atria in the present study. Danzinger et al (1991) suggested, from in-vitro experiments, that clinically relevant levels of ethanol (100–150 mg dL^{-1}) reversibly decrease Ca^{2+} binding to the myofilaments. Thus, another possible reason for the decreased basal active tension development is a prolonged consequence of this ethanol effect proposed by Danzinger et al (1991).

As was the case with the right atria from the ethanol infused rats, right atrial basal inotropy and inotropic response to β-receptor stimulation were decreased at all the levels of ethanol tested in-vitro. Because we showed that rate changes little with increasing ethanol concentrations in-vitro, these decreases could not have been due to a negative rate–tension relationship but instead had to be due to a direct effect of ethanol. Although the negative inotropic response of rat isolated left atria to clinically relevant levels of ethanol was seen as early as 1962 (Gimeno et al 1962), the effect of ethanol on the response to β_1-receptor stimulation of spontaneously beating right atria by ISO has not been extensively studied. As mentioned above, Danzinger et al (1991) proposed that an observed contractile depression of rat cardiac myocytes in the presence of clinically relevant ethanol concentrations may be due to a decreased ability of myofilaments to bind Ca^{2+}. Norepinephrine in the continued presence of ethanol reversed this negative inotropic response. However, our findings showed that the decrease in basal active tension development in the presence of ethanol was not reversed by β-receptor stimulation, and the maximum response to β-receptor stimulation was always lower in the presence of ethanol. Thus the normal reserve for increase in inotropy is reduced in the presence of ethanol. If the same is true for the ventricles, a heart in the presence of ethanol may not be able to meet the cardiac output demands of the body in situations in which sympathetic nervous system stimulation is required to generate high cardiac outputs.

As was the case with β-receptor stimulation by ISO, an increase of $CaCl_2$ from 1 to 6 mM did not overcome the negative inotropic effect of 57 mM ethanol on unpaced right atria, i.e. the inotropic response to 6 mM $CaCl_2$ was significantly lower in the presence of 57 mM ethanol than in the absence of ethanol. The occurrence of an ethanol-induced alteration of the Ca^{2+} transient and or Ca^{2+} binding

to myofiliments that was independent of receptor or adenylate cyclase therefore seems likely. A decrease in the Ca^{2+} transient caused by ethanol might be overridden with higher concentrations of $CaCl_2$; however, we did not observe an override.

Finally, yet another demonstration that ethanol inhibits the Ca^{2+} transient or binding to the myofilaments occurred fortuitously. A concentration-dependent decrease in atrial active tension development occurred with concentrations of ISO higher than that which resulted in peak active tension development (approximately 3×10^{-9} M ISO). This decrease was due to a concentration-dependent increase in passive tension. Interestingly, there was a tendency for this increase in passive tension to be less in the presence of 117 mM ethanol. If this increase in diastolic tension is due to a build up of intracellular Ca^{2+}, as we suspect, then the ethanol must have decreased the amount of Ca^{2+} entering or decreased the ability of myofilaments to bind Ca^{2+}, as proposed by Danzinger et al (1991).

In conclusion, the most significant effects of ethanol in this study were on the inotropy of the right atrium. The decrease in right atrial basal active tension development with clinically relevant concentrations of ethanol in-vitro were consistent with other studies. β-Adrenergic stimulation did not override the compromised active tension development that occurred in the presence of ethanol. In other words, the maximum inotropic response in the presence of ethanol was less than the maximum response in the absence of ethanol. In addition, the compromised inotropy seen in the presence of ethanol in-vitro also occurred in the absence of ethanol in-vitro in right atria from rats that were exposed to anesthetizing levels of ethanol for a period of 21 h. These findings are most consistent with the proposal that myofilament binding of Ca^{2+} is inhibited by ethanol.

In concurrence with other studies investigating the effect of ethanol on the heart, this study shows that ethanol is not a benign drug. More investigation is required to determine the extent to which ethanol affects inotropy of the human heart. The clinical implications of our results are that the heart of an individual with acute alcohol intoxication may lose the capacity to increase inotropy and therefore show a compromised response to stresses placed on the cardiovascular system such as might occur with trauma, infection or injury.

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