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RESEARCH PAPER

Facilitation of central imidazoline I_1-site/extracellular signal-regulated kinase/p38 mitogen-activated protein kinase signalling mediates the hypotensive effect of ethanol in rats with acute renal failure

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Background and purpose: This study investigated the role of central sympathetic activity and related mitogen-activated protein kinase (MAPK) signalling in the cardiovascular effects of ethanol in a model of acute renal failure (ARF).
Experimental approach: The effects of pharmacological interventions that inhibit peripheral or central sympathetic activity or MAPK on the cardiovascular actions of ethanol in rats with ARF induced by glycerol were evaluated.
Key results: Glycerol (50%, 10 mL·kg^{-1}, i.m.) caused progressive increases and decreases in blood pressure (BP) and heart rate (HR) respectively. Subsequent i.v. ethanol (0.25 or 1 g·kg^{-1}) elicited dose-related changes in BP (decreases) and HR (increases). These effects were replicated after intracisternal (i.c.) administration of ethanol. Blockade of nicotinic cholinoceptors (nAChR, hexamethonium, 20 mg·kg^{-1}) or α_1-adrenoceptors (prazosin, 1 mg·kg^{-1}) attenuated cardiovascular effects of ethanol. Ethanol hypotension was also attenuated after the centrally acting sympatholytic drug moxonidine (selective I_1-site agonist, 100 μg·kg^{-1} i.v.), but not guanabenz (selective α_2-receptor agonist, 30 μg·kg^{-1}, i.v.), suggesting involvement of central circuits of I_1 sites in ethanol-evoked hypotension. Selective blockade I_1 sites (efaroxan) but not α_2 (yohimbine) adrenoceptors abolished the hypotensive response to ethanol. Intracisternal administration of PD98059 or SB203580, inhibitors of extracellular signal-regulated kinase (ERK 1/2) and p38 MAPK, respectively, reduced the hypotensive action of moxonidine or ethanol. When used simultaneously, the two MAPK inhibitors produced additive attenuation of ethanol hypotension.
Conclusions and implications: Sympathoinhibitory pathways of central I_1-sites and downstream ERK/p38 MAPK signalling were involved in the hypotensive action of ethanol in ARF.
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Keywords: ethanol; acute renal failure; blood pressure; sympatholytic drugs; mitogen-activated protein kinases

Abbreviations: ARF, acute renal failure; BP, blood pressure; ERK, extracellular signal-regulated kinase; EtOH, ethanol; HR, heart rate; MAP, mean arterial pressure; MAPK, mitogen-activated protein kinase

Introduction

End-stage renal disease is associated with high cardiovascular morbidity and mortality. Also, patients with early renal insufficiency are subject to increased risk of cardiovascular disease such as hypertension, coronary artery disease and nephrosclerosis (Mann *et al.*, 2002; Tuncel and Ram, 2003). Rhabdomyolysis and myoglobinuria play a fundamental role in the pathophysiology of acute renal failure (ARF) in clinical and experimental settings (Vanholder *et al.*, 2000; Rodrigo *et al.*, 2004). Rhabdomyolysis is associated with the release of toxic cellular components into the systemic circulation, which result in intense vasoconstriction and renal tubular necrosis. The intratubular degradation of myoglobin results in the generation of reactive oxygen species and subsequent renal damage (Rodrigo *et al.*, 2004). Reports of ethanol on the progression of renal failure vary. Ethanol intoxication causes renal tubular dysfunction and necrosis (Cecchin and De Marchi, 1996), and predisposes to rhabdomyolysis and ARF (Vamvakas *et al.*, 1998). In contrast, ethanol has no effect on the acute or chronic progressive glomerulonephritis in the anti-thy1 rat model (Peters *et al.*, 2003). Rodrigo *et al.* (2004)

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demonstrated that whereas chronic exposure to ethanol does not alter renal derangements caused by glycerol, it reinforces the renoprotective effect of polyphenolic compounds of alcohol-free red wine. Likewise, ethanol exerts a protective effect against nephrotoxicity induced by ochratoxin (Bertelli *et al*., 2005). The up-regulation of the activity of antioxidant enzymes probably mediates the beneficial effect of ethanol (Rodrigo *et al*., 2004; Bertelli *et al*., 2005).

The effect of ethanol on haemodynamics has been extensively studied. Previous reports including our own showed that acute or chronic administration of ethanol causes increases (Puddey *et al*., 1985; El-Mas and Abdel-Rahman, 1992), decreases (Kawano *et al*., 1992; El-Mas and Abdel-Rahman, 2000) or no changes (Varga and Kunos, 1990) in blood pressure (BP). Clinically, the effect of ethanol on BP follows a J-shaped relationship depending on the duration and amount of ethanol consumed (Klatsky *et al*., 1977). Despite the intimate relationship between cardiovascular and kidney disorders (Mann *et al*., 2002; Tuncel and Ram, 2003), and the high prevalence of alcoholism in patients with renal disease (Hegde *et al*., 2000), current knowledge regarding the cardiovascular effects of ethanol in ARF and possible underlying mechanisms is sparse (Rodrigo *et al*., 2004).

This issue was addressed in the present study by investigating the cardiovascular actions of ethanol in conscious rats with glycerol-induced ARF. This preparation has been used as a model of human ARF (Wolfert and Oken, 1989). Because preliminary findings revealed a dose-related hypotensive effect for ethanol in ARF rats, the study was subsequently extended to assess the involvement of central neural pathways of α_2-adrenoceptors and/or imidazoline I_1 sites, which act tonically to reduce bulbospinal sympathetic discharges from the presympathetic neurons of the rostral ventrolateral medulla (Chalmers and Pilowsky, 1991; Bousquet *et al*., 1992), in the hypotensive action of ethanol. We also assessed the contribution of downstream activation of mitogen-activated protein kinase (MAPK) signalling to the sympathoinhibitory and hypotensive effects of ethanol in ARF. It is important, however, to note that evidence linking I_1 sites to BP control and elicitation of the hypotensive action of clonidine-related imidazolines is still questionable (Szabo, 2002). Our results showed that ethanol dose-dependently lowered BP in ARF rats via inhibition of central sympathetic outflow. The enhancement of central I_1-site/extracellular signal-regulated kinase (ERK)/p38 MAPK signalling constituted the cellular mechanism that underlies the sympathoinhibitory and hypotensive actions of ethanol.

Materials and methods

Animals

All experiments were performed in strict accordance with institutional animal care and use guidelines. Male Wistar rats (200–250 g; High Institute of Public Health, Alexandria, Egypt) were used in the present study.

Intravascular cannulation. The method described in our previous studies (El-Mas and Abdel-Rahman, 1999c; El-Mas *et al*., 2002) for measurement of BP in conscious rats was used. Briefly, rats were anesthetized with thiopental (50 $mg \cdot kg^{-1}$, i.p.); the adequacy of anesthesia was determined by the abolition of the withdrawal and blink reflexes, and by lack of a change in BP in response to tactile stimulation (Lash *et al*., 1992). Catheters (each consisted of a 5 cm polyethylene-10 tubing bonded to a 15 cm polyethylene-50 tubing) were placed in the abdominal aorta and vena cava via the femoral artery and vein for measurement of BP and intravenous administration of drugs respectively. The polyethylene-10 portion was used for the intravascular segment of the catheter. The arterial catheter was connected to a Gould-Statham pressure transducer (Oxnard, CA, USA), and BP was displayed on a Grass polygraph (model 7D, Grass Inst. Co., Quincy, MA, USA). Heart rate (HR) was computed from BP waveforms by a Grass tachograph and was displayed on another channel of the polygraph.

Finally, the catheters were tunneled subcutaneously and exteriorized at the back of the neck between the scapulae. The catheters were flushed with heparin (0.2 mL, 100 $U \cdot mL^{-1}$) and plugged by stainless steel pins. Incisions were closed by surgical clips and swabbed with povidone iodine solution. Each rat received an intramuscular injection of 60 000 U of penicillin G benzathine and penicillin G procaine in an aqueous suspension (Penicid), and was housed in a separate cage. Experiments started 2 days later in conscious rats.

Intracisternal cannulation (i.c.). Five days before starting the experiment (i.e. 3 days before intravascular cannulation), a stainless steel guide cannula was implanted into the cisterna magna under thiopental anesthesia (50 $mg \cdot kg^{-1}$, i.p.) as described in our previous studies (El-Mas and Abdel-Rahman, 1999b,c). A steel cannula (23 G; Small Parts, Miami, FL, USA) was passed between the occipital bone and the cerebellum so that its tip protruded into the cisterna magna. The cannula was secured in place with dental acrylic cement (Glass Ionomer, Shanghai, China). The guide cannula was considered patent when spontaneous outflow of cerebrospinal fluid was observed and by gross post-mortem histological verification following injection of 5 μL of fast green dye (EM Science; Cherry Hill, NJ, USA). After i.c. cannulation, rats were housed individually.

Induction of ARF. The glycerol model of ARF (Wolfert and Oken, 1989; Gould *et al*., 1997) was employed. Rats were deprived of drinking water for 24 h. Glycerol 50% (diluted in saline) was injected into the thigh muscle of both hind limbs of rats at a dose of 10 $mL \cdot kg^{-1}$. Control rats were dehydrated and received i.m. injection of equal volume of saline (10 $mL \cdot kg^{-1}$).

Blood analyses. The serum ethanol concentration was measured as in our previous studies (El-Mas and Abdel-Rahman, 1999b,c) by the enzymatic method using the Emit II Plus Ethyl Alcohol Assay (Siemens Healthcare Diagnostics, Deerfield, IL, USA; Viva-E analyzer, Siemens Healthcare Diagnostics). Serum urea was measured by the kinetic urease test (Randox Laboratories Ltd., Crumlin, County Antrim, UK; Hitachi 902 analyzer, Hitachinaka-Shi, Japan), and creatinine by kinetic Jaffe method (Audit Diagnostics, Carrigtwohill Co Cork, Ireland; Hitachi 902 analyzer).

Protocols and experimental groups

Cardiovascular effects of ethanol in rats with ARF. Five groups of rats (n = 6–8) were used in this experiment to investigate the cardiovascular actions of ethanol in conscious freely moving rats with or without ARF. On the day of the experiment, the arterial catheter was connected to a pressure transducer for measurement of BP and HR as mentioned earlier. A period of at least 30 min was allowed at the beginning of each experiment for hemodynamic stabilization. Subsequently, each rat received two consecutive regimens. The first regimen consisted of i.m. glycerol (50%, 10 mL·kg^{-1}) or equal volume of saline as described earlier. The second regimen was given 45 min later and consisted of i.v. ethanol (0.25 or 1 g·kg^{-1}) or equal volume of saline (1.3 mL·kg^{-1}). Ethanol (1 g·kg^{-1}) was administered as 95% in a volume of 1.3 mL·kg^{-1} (El-Mas and Abdel-Rahman, 1992; 1999b). For the lower dose (0.25 g·kg^{-1}) of ethanol, the 95% ethanol was diluted as appropriate in saline. Ethanol or saline was administered slowly over 3 min. The mean arterial pressure (MAP) and HR values before and after drug regimens were measured, and peak changes in both variables were determined. For determination of serum ethanol levels, blood (0.5 mL) was collected through the arterial catheter 15 min after ethanol administration. Another blood sample was collected at the conclusion of the experiment for the measurement of serum ethanol, urea and creatinine levels. Blood samples were centrifuged at 2375× *g* for 5 min, and serum was aspirated and stored at –20°C until analysed.

Effect of sympatholytic drugs on cardiovascular actions of ethanol. Eight groups of glycerol-treated rats (n = 7–8 each) were employed to investigate the effects of peripherally acting (prazosin, α_1-adrenoceptor antagonist; hexamethonium, nicotinic cholinoceptor antagonist) or centrally acting (guanabenz or moxonidine, selective α_2 and I_1 receptor agonists, respectively) sympatholytics on cardiovascular actions of ethanol (1 g·kg^{-1}). Rats were randomly assigned to receive one of the following i.v. regimens: (i) prazosin (1 mg·kg^{-1}) plus saline; (ii) prazosin plus ethanol; (iii) hexamethonium (20 mg·kg^{-1}) plus saline; (iv) hexamethonium plus ethanol; (v) guanabenz (30 μg·kg^{-1}) plus saline; (vi) guanabenz plus ethanol; (vii) moxonidine (100 μg·kg^{-1}) plus saline; or (viii) moxonidine plus ethanol. In each group, the first treatment (prazosin, hexamethonium, guanabenz or moxonidine) was administered 30 min after glycerol, and this was followed by the second treatment (saline or ethanol) 15 min later. BP and HR were monitored for 60 min after the second treatment. Blood samples were collected at the end of the experiment from the guanabenz or moxonidine groups (with or without ethanol) for the measurement of serum urea and creatinine.

To determine whether alterations in α_1-adrenoreceptor responsiveness contributed to ethanol hypotension, we investigated the effect of ethanol (1 g·kg^{-1}) on pressor responses to bolus i.v. doses (1–16 μg·kg^{-1}) of the α_1-adrenoceptor agonist phenylephrine in another group of glycerol-treated rats (n = 7). Another two groups of rats (n = 4–6 each) were used to determine the effect of prazosin (1 mg·kg^{-1}) or its vehicle (5% methanol) on the pressor responses to phenylephrine (1–16 μg·kg^{-1}). This was important to verify the adequacy of α_1-adrenoceptor blockade by prazosin.

Effect of blockade of I_1 or α_2 receptors on cardiovascular effects of ethanol. To identify the role of imidazoline I_1 sites in the cardiovascular effects of ethanol or moxonidine, the latter were evaluated in ARF rats after selective blockade of imidazoline I_1 or α_2 adrenoceptors with efaroxan and yohimbine respectively. Four groups of ARF rats were used and received one of the following i.v. regimens: (i) efaroxan (1 mg·kg^{-1}) + moxonidine (100 μg·kg^{-1}); (ii) efaroxan + ethanol (1 g·kg^{-1}); (iii) yohimbine (1 mg·kg^{-1}) + moxonidine; and (iv) yohimbine + ethanol. The 1 mg·kg^{-1} dose of yohimbine or efaroxan has been found in previous studies to produce effective blockade of their respective receptors (Raasch *et al.*, 2003; Jochem, 2004). Efaroxan or yohimbine was administered 30 min after glycerol, and this was followed by moxonidine or ethanol 15 min later.

Role of central MAPKs in the cardiovascular effects of ethanol. This experiment investigated the effect of i.c. infusion of PD98059 or SB203580, selective inhibitors of ERK 1/2 and p38 MAPK, respectively (Lim *et al.*, 2007), or their combination on the cardiovascular actions of moxonidine or ethanol in ARF rats. Six groups of rats (n = 6–8 each) were used in this experiment that received one of the following treatments: (i) PD98059 (10 μg in 10 μL per rat, i.c.) + moxonidine (100 μg·kg^{-1}, i.v.); (ii) PD98059 + ethanol (1 g·kg^{-1}, i.v.); (iii) SB203580 (10 μg in 10 μL per rat, i.c.) + moxonidine; (iv) SB203580 + ethanol; (v) PD98059 + SB203580 + moxonidine; or (vi) PD98059 + SB203580 + ethanol. The i.c. dose of PD98059 or SB203580 was infused slowly over 3–4 min (Lim *et al.*, 2007). In each rat, the first treatment (PD98059, SB203580 or their combination) was administered 20 min after glycerol, and the second treatment (moxonidine or ethanol) was administered 10 min later.

To provide more support for the involvement of central neurons in the hypotensive action of ethanol, we investigated whether the cardiovascular effects of systemic ethanol could be replicated when ethanol was administered i.c. Another two groups of ARF rats (n = 6–7 each) were employed to determine the cardiovascular effects of i.c. administration of ethanol (7.5 mg·rat^{-1}) or its vehicle (saline). Haemodynamic monitoring continued for 60 min.

Data analysis and statistics. Data are expressed as means ± SEM. MAP was calculated as diastolic pressure + one-third pulse pressure (systolic–diastolic pressures). Repeated measures analysis of variance followed by a Newman–Keuls *post hoc* test was used to test for statistical significance. These analyses were performed by GraphPad InStat, software release 3.05. Probability levels less than 0.05 were considered significant.

Materials. Phenylephrine hydrochloride, prazosin hydrochloride, PD98059 [2-(2-amino-3-methoxyphenyl)-4H-1-benzopyran-4-one], SB203580 [4-(4-fluorophenyl)-2-(4-methylsulphinylphenyl)-5-(4-pyridyl)-1H-imidazole], hexamethonium bromide, efaroxan hydrochloride, yohimbine hydrochloride, guanabenz (Sigma Chemical Co., St Louis, MO, USA), thiopental (Thiopental, Sandoz, Germany), glycerol (Chemajet, Alexandria, Egypt), ethanol (Alamia, Cairo, Egypt), povidone iodine solution (Betadine, Nile Pharmaceutical Co., Cairo,

Egypt) and Penicid (Cid Pharmaceutical Co., Cairo, Egypt) were purchased from commercial vendors. Moxonidine was a gift from Solvay Pharmaceuticals GmbH (Hannover, Germany). Moxonidine was dissolved in saline with few drops of 1 M HCl. The pH was then adjusted to 7.4 by 1 M NaOH. Prazosin was dissolved in methanol and then diluted with saline to a final methanol concentration of 5%. PD98059 or SB203580 was dissolved in dimethylsulphoxide (DMSO) and diluted with saline to a final DMSO concentration of 70%. Other drugs were dissolved in saline. The drug/molecular target nomenclature employed in this study follows Alexander *et al.* (2008).

Results

Cardiovascular effects of ethanol in rats with ARF

The baseline values of MAP (112 ± 4 vs. 121 ± 3 mm Hg) and HR (354 ± 19 vs. 346 ± 13 beats·min^{-1}) in rats subsequently receiving i.m. saline or glycerol were not statistically different. The haemodynamic responses elicited by glycerol and subsequent treatment with ethanol in conscious rats are illustrated in Figure 1. Compared with control (saline, i.m.) values, glycerol caused abrupt and progressive increases in MAP that reached peak levels after approximately 85 min (Figure 1A). The pressor effect of glycerol was associated with significant and sustained decreases in HR (Figure 1B). The intravenous administration of ethanol (0.25 or 1 g·kg^{-1}), 45 min after glycerol, attenuated the progressive changes in MAP and HR induced by glycerol during the following 60 min (Figure 1). In rats treated with i.m. saline, the subsequent treatment with ethanol caused no changes in MAP or HR (Figure 1). Representative tracings of the cardiovascular effects of glycerol and subsequent ethanol or saline administration are shown in Figure 2. Serum ethanol concentrations measured 15 min post ethanol (1360 ± 50 vs. 1420 ± 140 mg·L^{-1}) or at the conclusion of the experiment (650 ± 20 vs. 760 ± 50 mg·L^{-1}) in rats pretreated with i.m. saline or glycerol were not

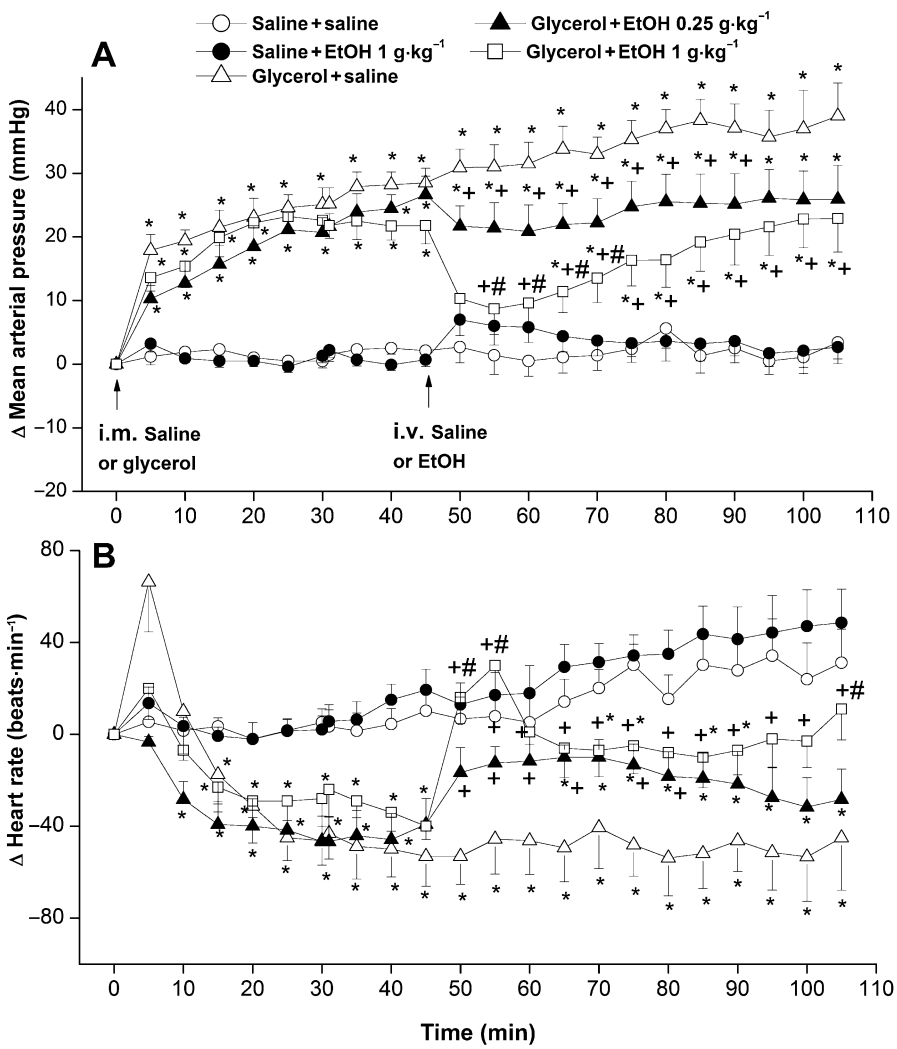

Figure 1 Changes in mean arterial pressure (A) and heart rate (B) evoked by consecutive administration of glycerol (50%, 10 mL·kg^{-1}, i.m.) and ethanol (EtOH, 0.25 or 1 g·kg^{-1}, i.v.) in conscious Wistar rats. Values are means ± SEM of six to eight observations. *P < 0.05 versus respective 'saline + saline' values, ^{+}P < 0.05 versus respective 'glycerol + saline' values, $^{\#}P$ < 0.05 versus respective 'glycerol + ethanol 0.25 g·kg^{-1}' values.

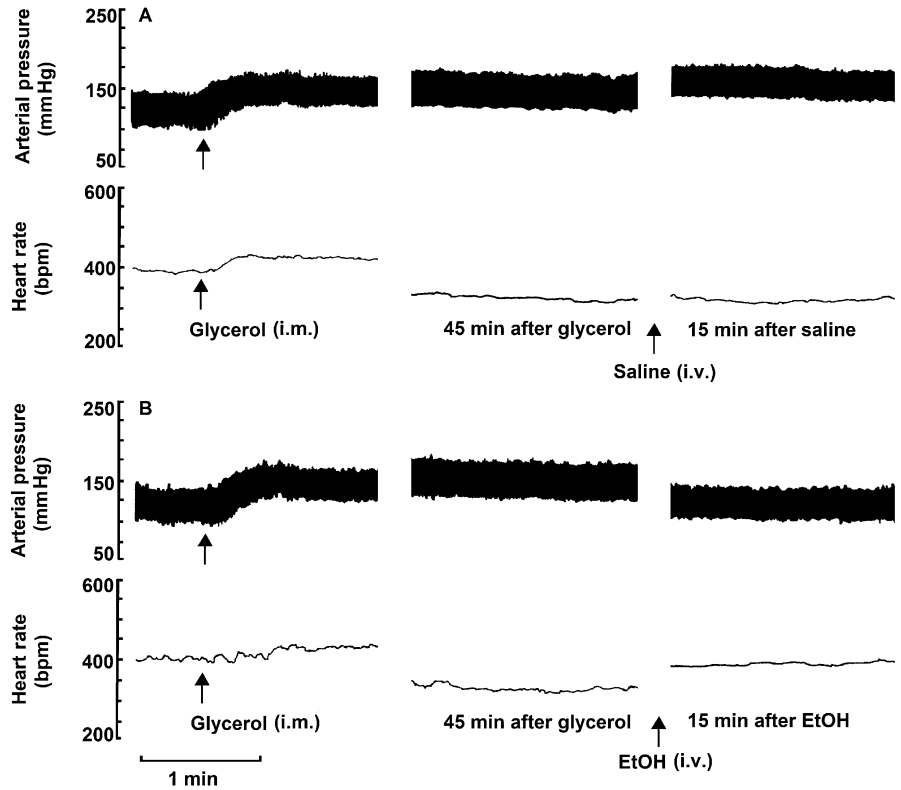

Figure 2 Representative tracings showing the effect of i.m. glycerol (50%, 10 mL·kg^{-1}) and subsequent i.v. ethanol (EtOH, 1 g·kg^{-1}, B) or equal volume of saline (A) on arterial pressure and heart rate in conscious freely moving rats.

Table 1 Increases and decreases in mean arterial pressure (MAP) and heart rate (HR), respectively, caused by phenylephrine (PE, 1–16 μg·kg^{-1}, i.v.) before and after treatment with ethanol (1 g·kg^{-1}, i.v.) or prazosin (1 mg·kg^{-1}, i.v.) in glycerol (50%, 10 mL·kg^{-1}, i.m.)-treated rats

PE	*Before ethanol*		*After ethanol*		*Before prazosin*		*After prazosin*	
	ΔMAP	*ΔHR*	*ΔMAP*	*ΔHR*	*ΔMAP*	*ΔHR*	*ΔMAP*	*ΔHR*
1	12 ± 3	–10 ± 2	8 ± 3	–9 ± 2	14 ± 2	–11 ± 1	3 ± 1*	–4 ± 3*
2	17 ± 3	–17 ± 4	14 ± 2	–14 ± 2	18 ± 3	–16 ± 1	3 ± 1*	–2 ± 4*
4	20 ± 3	–29 ± 4	19 ± 3	–17 ± 3	25 ± 4	–20 ± 3	1 ± 1*	–7 ± 3*
8	28 ± 4	–30 ± 4	27 ± 5	–21 ± 4	30 ± 5	–35 ± 10	1 ± 2*	–12 ± 3*
16	34 ± 5	–42 ± 7	30 ± 6	–43 ± 11	33 ± 5	–48 ± 13	2 ± 2*	–1 ± 4*

Values are means ± SEM of six to seven observations. *$P < 0.05$ vs. respective 'before prazosin' values.

statistically different. Also, glycerol significantly increased serum urea (Figure 3A) and creatinine (Figure 3B). Treatment of glycerol-treated rats with ethanol (1 g·kg^{-1}) restored serum urea and creatinine to control values (Figure 3).

Effect of sympatholytic drugs on cardiovascular effects of ethanol in ARF rats

Figures 4–6 depict the effects of peripherally or centrally acting sympatholytic drugs on the cardiovascular effects of ethanol in ARF rats. Rats in different experimental groups exhibited similar baseline values of MAP and HR (data not shown). Intravenous administration of prazosin (α_1-adrenoceptor antagonist, 1 mg·kg^{-1}, Figure 4A,B) or hexamethonium (nAChR antagonist, 20 mg·kg^{-1}, Figure 4C,D) caused significant decreases and increases in BP and HR respectively. In prazosin- or hexamethonium-pretreated ARF rats, the hypotension and tachycardia induced by ethanol (1 g·kg^{-1}, i.v.) were not evident. On the other hand, as shown in Table 1, the dose-related pressor and associated reflex bradycardic responses elicited by bolus i.v. doses (1–16 μg·kg^{-1}) of the α_1-adrenocceptor agonist phenylephrine were not affected by ethanol in ARF rats, but were virtually abolished in the presence of prazosin (1 mg·kg^{-1}). The vehicle of prazosin (5% methanol) had no effect on phenylephrine responses (data not shown).

Intravenous administration of moxonidine (100 μg·kg^{-1}) or guanabenz (30 μg·kg^{-1}) produced short-lived (approximately 2 min) and similar increases in BP that were followed by more sustained decreases in MAP (Figure 5A). HR was also reduced after moxonidine or guanabenz (Figure 5B). The cardiovascular effects of ethanol (1 g·kg^{-1} i.v.), the

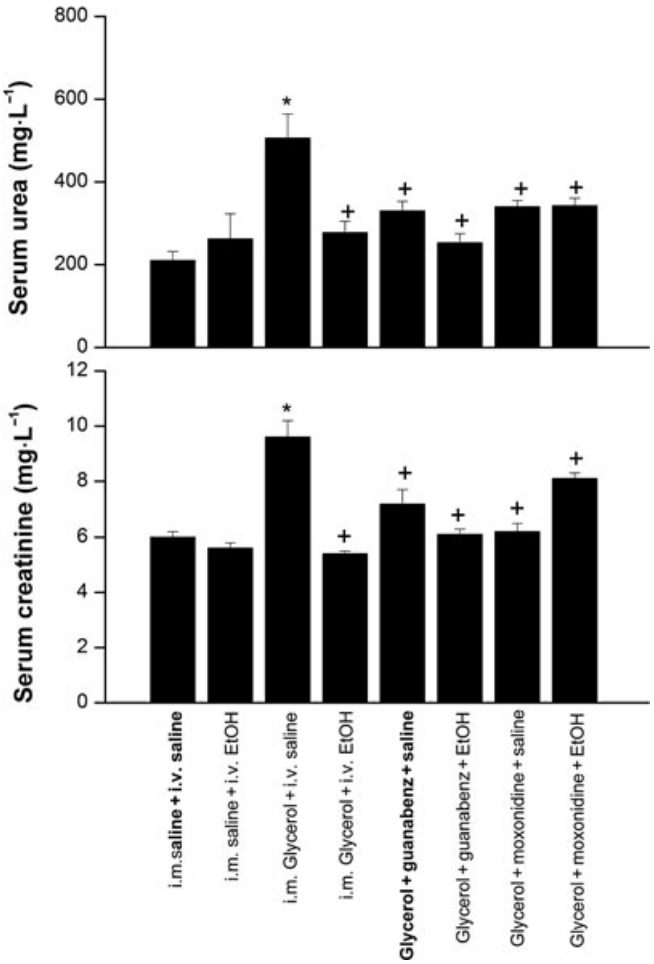

Figure 3 Effect of ethanol (EtOH, 1 g·kg^{-1}, i.v.) on serum urea and creatinine in Wistar rats pretreated with i.m. glycerol (50%, 10 mL·kg^{-1}) or equal volume of saline in the absence or presence of i.v. moxonidine (I_1-site agonist, 100 μg·kg^{-1}) or guanabenz (α_2-adrenoceptor agonist, 30 μg·kg^{-1}). Values are means ± SEM of six to eight observations. *$P < 0.05$ versus respective 'i.m. saline + i.v. saline' values, $^{+}P < 0.05$ versus respective 'i.m. glycerol + i.v. saline' values.

decrease and increase in MAP (Figure 6A) and HR (Figure 6B), respectively, were not altered in rats pretreated with guanabenz. On the other hand, the hypotensive effect of ethanol was abolished in moxonidine-pretreated rats (Figure 6A), whereas the associated tachycardia was preserved (Figure 6B). Treatment with guanabenz or moxonidine prevented the glycerol-induced increases in serum urea and creatinine (Figure 3). Similar effects were observed upon combined treatment with ethanol and either sympatholytic drug (Figure 3).

Effect of yohimbine or efaroxan on cardiovascular effects of ethanol or moxonidine

The effect of selective blockade of imidazoline I_1 sites or α_2-adrenoceptors with efaroxan and yohimbine, respectively, on the cardiovascular effects of moxonidine or ethanol is shown in Figure 7. The intravenous administration of efaroxan or yohimbine (1 mg·kg^{-1} each) produced no haemodynamic changes (data not shown). The hypotensive response elicited by moxonidine (Figure 7A) or ethanol (Figure 7C) and associated HR responses (Figure 7B,D) were abolished in efaroxan-pretreated ARF rats. Yohimbine caused partial inhibition of the hypotensive effect of moxonidine (Figure 7A), but failed to affect the ethanol-evoked hypotension (Figure 7C). Yohimbine also had no effect on HR responses to moxonidine (bradycardia; Figure 7B) or ethanol (tachycardia; Figure 7D).

Effect of inhibiting central MAPKs on the cardiovascular actions of ethanol or moxonidine

Figure 8 shows the individual or combined inhibition of ERK 1/2 (PD98059, 10 μg per rat, i.c.) and p38 MAPK (SB203580, 10 μg per rat, i.c.) on the cardiovascular actions of i.v. moxonidine (100 μg·kg^{-1}) or ethanol (1 g·kg^{-1}) in ARF rats. When used alone, PD98059 or SB203580 significantly reduced the hypotensive effect of moxonidine (Figure 8A) or ethanol (Figure 8B). The simultaneous i.c. administration of the two MAPK inhibitors produced additive inhibitory effects on the hypotension caused by ethanol (Figure 8B). As when given intravenously, the i.c. administration of ethanol (7.5 mg per rat) caused significant decreases in MAP (Figure 9). The baseline values of MAP of rats subsequently treated with glycerol plus i.c. saline or glycerol plus i.c. ethanol were similar (110 ± 4 vs. 105 ± 3 mm Hg).

Discussion

Despite the wealth of information on the effect of ethanol on haemodynamics (Puddey *et al.*, 1985; El-Mas and Abdel-Rahman, 1992; 2000; Kawano *et al.*, 1992), there has been no study, clinical or experimental, that systematically assessed the cardiovascular effects of ethanol under conditions of compromised renal function. The present study evaluated the cardiovascular effects of ethanol in rats with ARF and the causal relationship between ethanol effects and central sympathetic activity. Ethanol, administered systemically or intracisternally, caused significant falls in BP in conscious ARF rats. The following observations favour a critical role for central sympathoinhibitory pathways of imidazoline I_1-sites in ethanol hypotension. First, the inhibition of the hypotensive action of ethanol by hexamethonium suggests a role for sympathetic neural activity in response to ethanol. Second, perturbations (activation or inhibition by moxonidine and efaroxan, respectively) of central I_1-sites abolished the hypotensive effect of ethanol, whereas similar manipulations of α_2-adrenoceptors failed to do so. Third, central inhibition of ERK 1/2 and p38 MAPK abolished the hypotension caused by ethanol or moxonidine. It is concluded that facilitation of central I_1-site/ERK/p38 MAPK signalling underlies, at least partly, the hypotensive action of ethanol in ARF rats.

The glycerol rat model simulates acute tubular necrosis caused by rhabdomyolysis in humans and is characterized by rapid myoglobinuria, reduced glomerular filtration rate and oliguria (Wolfert and Oken, 1989; Vanholder *et al.*, 2000; Rodrigo *et al.*, 2004). Although the mechanism is not fully understood, the hypertensive response elicited by glycerol might be accounted for by the dramatic increase in plasma

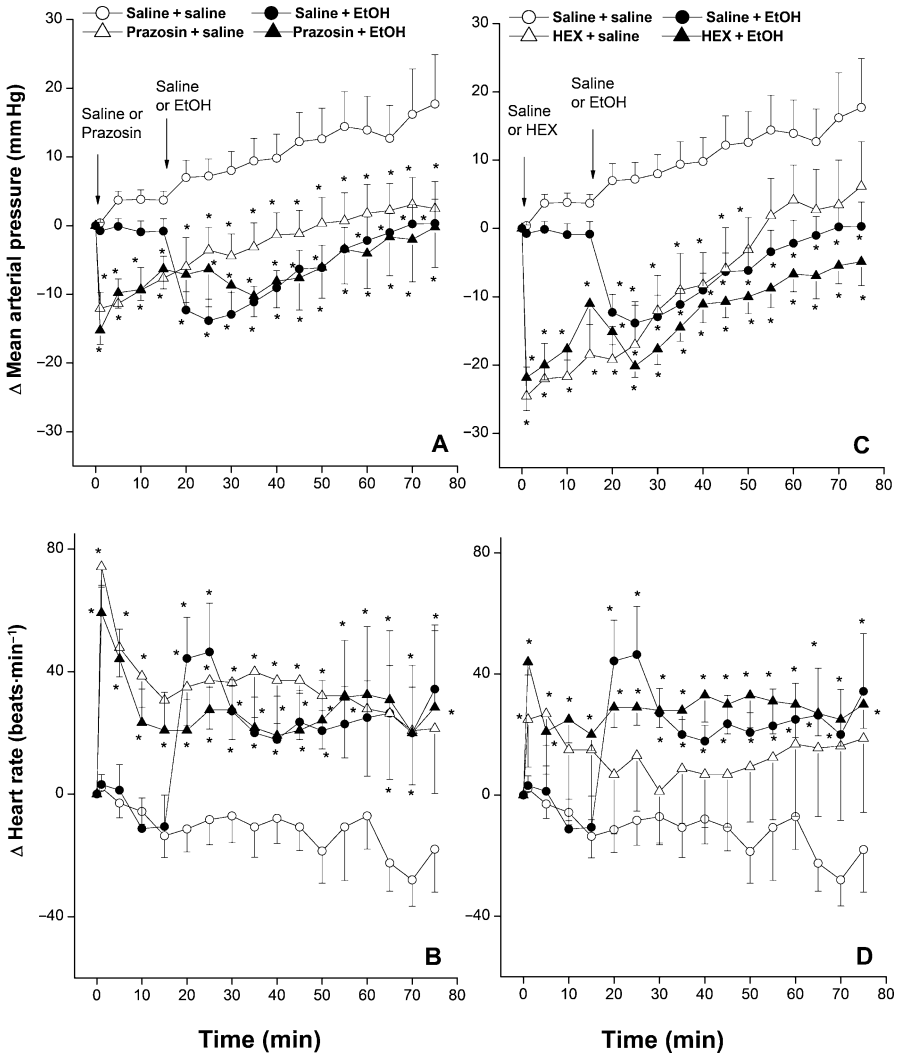

Figure 4 Effect of pretreatment with i.v. hexamethonium (nAChR antagonist, 20 mg·kg^{-1}) or prazosin α_1-adrenoceptor antagonist, 1 mg·kg^{-1}) on changes in mean arterial pressure (A and C) and heart rate (B and D) evoked by ethanol (EtOH, 1 g·kg^{-1}, i.v.) or equal volume of saline in conscious glycerol (50%, 10 mL·kg^{-1}, i.m.)-treated Wistar rats. Values are means ± SEM of six to eight observations. *$P < 0.05$ versus respective 'saline + saline' values.

concentration of haem proteins, which bind nitric oxide and reduce its systemic vasodilator capability (Zager, 1996). Moreover, the delayed expansion of extracellular and plasma volumes, which follows early tissue fluid accumulation (Vanholder *et al.*, 2000), might also underlie the hypertensive response to glycerol. Our present finding that glycerol hypertension was attenuated in the presence of sympatholytic treatments highlights a contributory role for sympathoexcitation in the hypertensive response elicited by glycerol.

The present investigation showed that whereas ethanol had no effect on BP in conscious rats with intact renal function, it dose-dependently attenuated the acute progressive increase in BP in the glycerol model of ARF. This differential effect of ethanol on BP cannot be attributed to discrepancies in ethanol pharmacokinetics because similar serum ethanol concentrations were observed in the two groups of rats. The effect of ethanol on BP is known to be correlated with changes in sympathetic activity, and these two effects (BP and sympathetic activity) are influenced by factors such as the route of administration, duration and amount of ethanol consumed, as well as the animal sex, species, and strain. For example, sympathoexcitation mediates the hypertensive effect of centrally administered ethanol in spontaneously hypertensive rats (Li *et al.*, 2005). Similarly, in rats with compromised sympathetic activity, i.v. ethanol causes sympathoexcitation and hypertension (El-Mas and Abdel-Rahman, 1999c). Paradoxically, ethanol causes sympathoinhibition and hypotension in anesthetized normotensive rats (El-Mas *et al.*, 1994). Also, ethanol lowers BP in female rats partly due to inhibition of sympathetic activity, and these effects are abolished in ovariectomized animals and restored after estrogen replacement (El-Mas and Abdel-Rahman, 1999a). Here, the abolition of the hypotensive effect of ethanol in ARF rats subjected to α_1-adrenoceptor (prazosin) or ganglionic (hexamethonium) blockade clearly implicated sympatho-inhibition in the ethanol-induced hypotension.

This latter conclusion, however, deserves two comments. First, treatment with prazosin or hexamethonium caused a

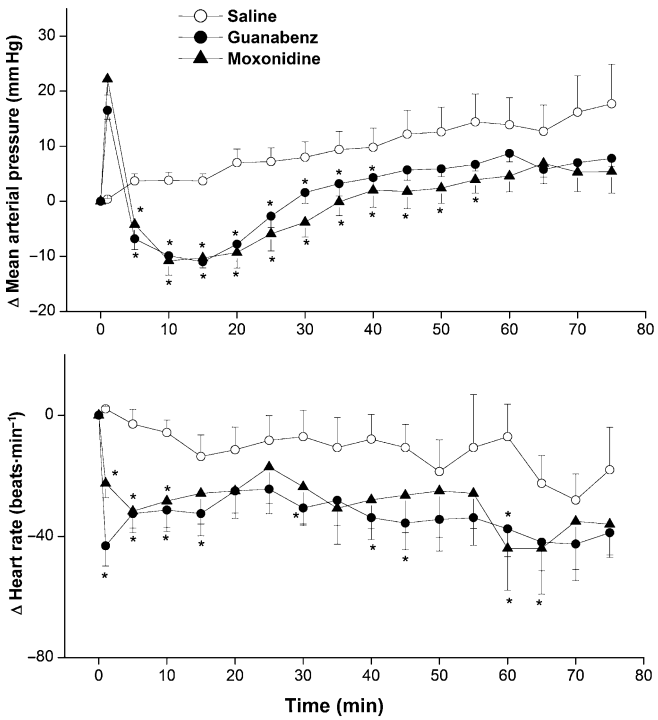

Figure 5 Changes in mean arterial pressure (A) and heart rate (B) evoked by i.v. moxonidine (I_1-site agonist, 100 μg·kg^{-1}), guanabenz (α_2-adrenoceptor agonist, 30 μg·kg^{-1}) or equal volume of saline in conscious glycerol (50%, 10 mL·kg^{-1}, i.m.)-treated Wistar rats. Values are means ± SEM of six to eight observations. *P < 0.05 versus respective 'saline' values.

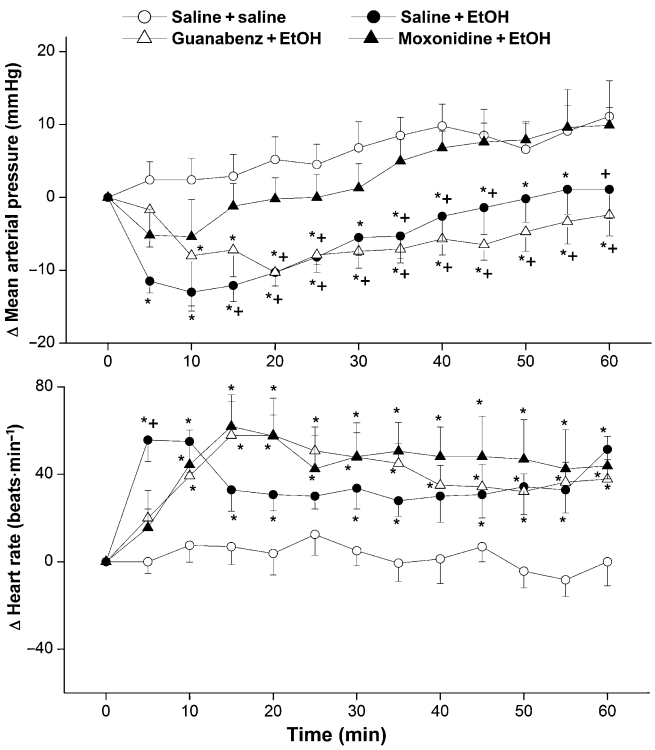

Figure 6 Effect of pretreatment with i.v. moxonidine (I_1-site agonist, 100 μg·kg^{-1}), guanabenz (α_2-adrenoceptor agonist, 30 μg·kg^{-1}) or equal volume of saline on changes in mean arterial pressure (A) and heart rate (B) evoked by ethanol (EtOH, 1 g·kg^{-1}, i.v.) in conscious glycerol (50%, 10 mL·kg^{-1}, i.m.)-treated Wistar rats. Values (means ± SEM of six to eight observations) represent haemodynamic changes demonstrated after the second treatment (i.e. ethanol or saline). *P < 0.05 versus respective 'saline + saline' values, ^{+}P < 0.05 versus respective 'moxonidine + ethanol' values.

relatively large drop in BP. Although the hypotensive response elicited by either drug was similar in rats treated subsequently with ethanol or saline (see Figure 4A,C), the impact of the initial hypotension caused by peripheral sympatholytic drugs on the interpretation of the ethanol data cannot be overlooked. Notably, the doses of prazosin and hexamethonium employed in this study were found in previous studies to effectively block α_1 adrenoceptors and ganglionic nicotinic receptors, respectively (El-Mas *et al.*, 2009). This was further confirmed by the current observation that the pressor and reflex bradycardic actions of phenylephrine were virtually abolished in the presence of prazosin. Second, because some α_1- adrenoceptor blocking-like activity has been attributed to ethanol (Zhang *et al.*, 1988), it could be argued that alterations in vascular α_1-adrenoceptor responsiveness might have contributed to the ethanol hypotension. To investigate this postulate, we assessed the effect of ethanol on abrupt increases in BP evoked by phenylephrine, α_1-adrenoceptor agonist. The results showed that the pressor effects of phenylephrine were preserved in ethanol-treated rats, thus ruling out a possible role for reduced α_1-receptor responsiveness in the ethanol-evoked hypotension.

The present finding that the hypotension caused by systemic ethanol was replicated when ethanol was administered centrally (i.c.) suggests a direct role for central neurons in the hypotensive, and possibly sympathoinhibitory, response to ethanol. These findings led us to conduct more research to determine the role of central pathways of α_2 adrenoceptors and I_1 sites in the cardiovascular actions of ethanol. Indeed, when activated, both types of receptor sites decrease central sympathetic outflow and subsequently BP (Timmermans and Van Zwieten, 1982; Bousquet *et al.*, 1984; 1992). Luckily, the availability of sympatholytic ligands with selective agonist (moxonidine and guanabenz, respectively) and antagonist (efaroxan and yohimbine, respectively) activity at α_2 adrenoceptors and I_1 sites (Timmermans and Van Zwieten, 1982; Bousquet *et al.*, 1984; 1992) enabled us to address this important issue.

Two important observations emerged from these experiments. First, prior exposure to moxonidine, but not to guanabenz, abolished the hypotensive effect of ethanol in ARF rats. The lack of effect of ethanol on BP in moxonidine-treated rats infers that both substances utilize a common pathway (i.e. I_1 sites), such that the initial activation of this pathway by moxonidine blocked interaction of the subsequently administered ethanol with the same site. The doses of moxonidine and guanabenz employed caused equipotent hypotensive effects, thereby excluding a possible role for differences in the magnitude of hypotension on the BP response to ethanol. Second, results of the antagonist studies re-affirms the selective involvement of I_1 sites in the hypotensive effect of ethanol, because the latter was abolished in presence of efaroxan but not yohimbine, selective antagonists of I_1 sites and α_2 adrenoceptors respectively.

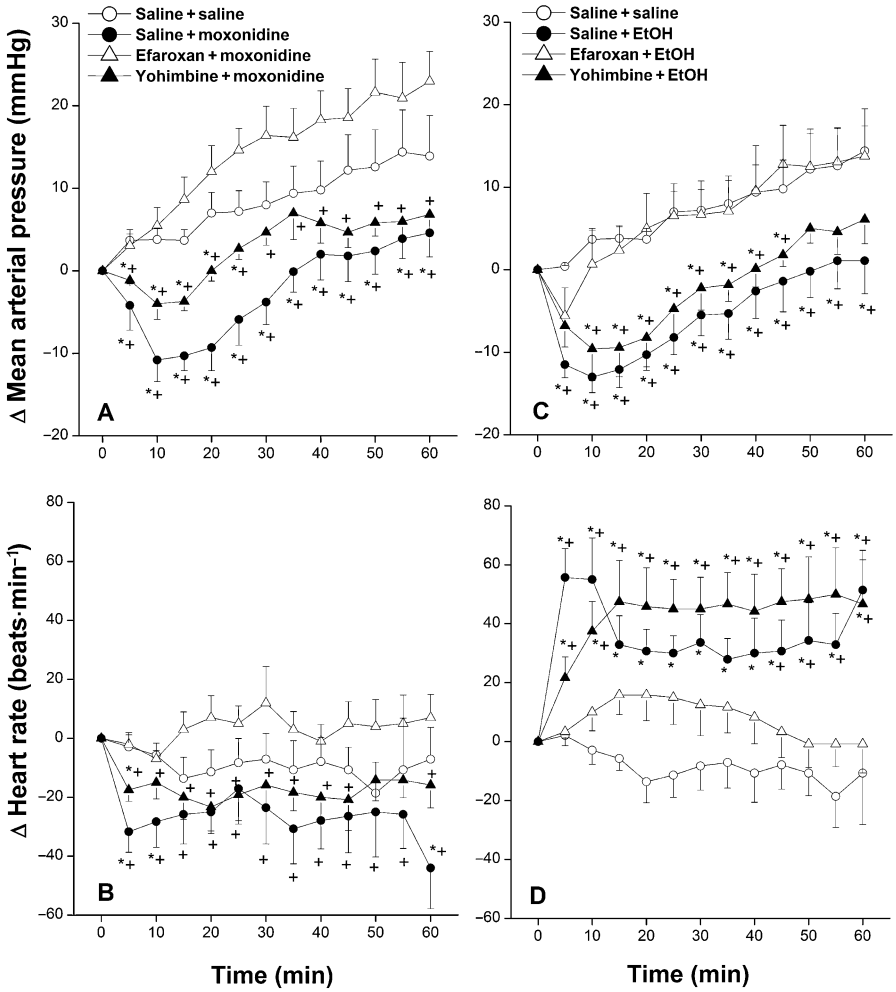

Figure 7 Effect of pretreatment with i.v. efaroxan (I_1-site antagonist, 1 mg·kg^{-1}) or yohimbine (α_2-adrenoceptor antagonist, 1 mg·kg^{-1}) on changes in mean arterial pressure (A and C) and heart rate (B and D) evoked by moxonidine (100 μg·kg^{-1}, left panels) or ethanol (EtOH, 1 g·kg^{-1}, right panels) in conscious glycerol (50%, 10 mL·kg^{-1}, i.m.)-treated Wistar rats. Values (means ± SEM of six to eight observations) represent haemodynamic changes demonstrated after the second treatment (i.e. moxonidine or ethanol). *P < 0.05 versus 'saline + saline' values; ^{+}P < 0.05 versus respective 'efaroxan + moxonidine' or 'efaroxan + ethanol' values.

Because efaroxan and yohimbine were administered systemically, a potential role for peripheral mechanisms (Szabo, 2002) in their interaction with ethanol cannot be ruled out. Future experiments in our laboratory will employ microinjection studies into discrete brainstem areas to directly involve central sympathoinhibitory pathways in the ethanol-evoked hypotension. Collectively, studies performed in this investigation underscore the importance of central I_1-site activation in the BP lowering effect of ethanol in ARF rats. With that said, the notion should be emphasized that evidence obtained from pharmacological, receptor binding, and molecular studies regarding the existence of I_1 sites and their involvement in BP control is not conclusive (Szabo, 2002; Parkin *et al.*, 2003). In effect, our finding that yohimbine significantly attenuated the hypotensive effect of moxonidine highlights a contributory role for α_2 adrenoceptors in the moxonidine effect.

Cell culture studies including our own demonstrate that the activation of I_1 sites results in the accumulation of phosphatidylcholine-specific phospholipase C and downstream phosphorylation of ERK 1/2 (Zhang *et al.*, 2001; Li *et al.*, 2006). Functional studies also implicate brainstem ERK 1/2 in the hypotensive response to I_1, but not α_2 adrenoceptor activation (Zhang and Abdel-Rahman, 2005). This view was confirmed by the present observation that inhibition of ERK 1/2 by PD98059 attenuated the hypotensive effect of moxonidine. In addition, the attenuation of moxonidine hypotension by SB203580 provides the first experimental evidence that p38 MAPK activation in cardiovascular nuclei of the brainstem might also be pivotal for the I_1-site-mediated BP control. More importantly, pharmacological targeting of MAPK signalling revealed the interesting finding that concurrent central administration of PD98059 and SB203580 produced additive inhibitory effects on the ethanol-induced hypotension. The similarity in the BP response to ethanol and moxonidine, together with the abolition of the hypotensive effect of ethanol after pharmacological elimination of I_1 sites or their downstream signalling

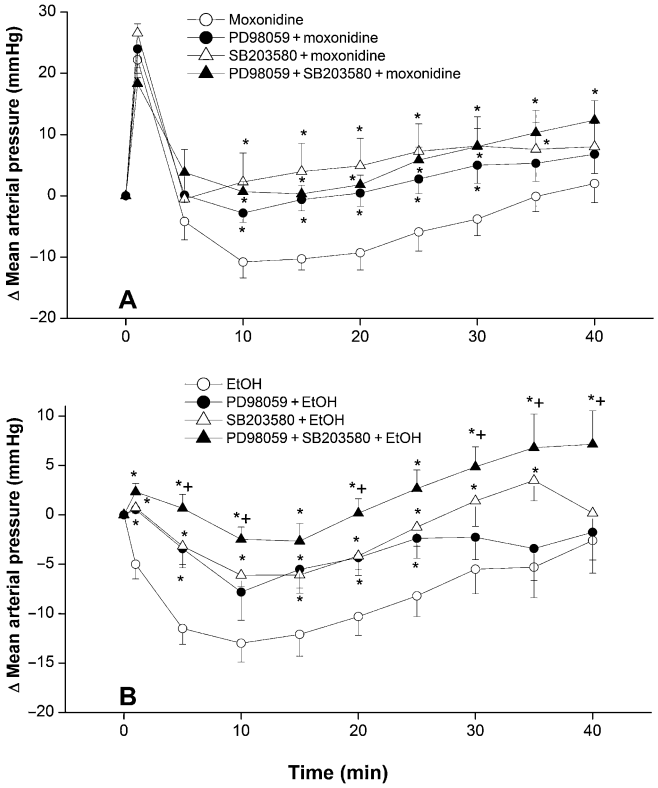

Figure 8 Effect of pretreatment with i.c. PD98059 (ERK 1/2 inhibitor, 10 μg per rat), SB203580 (p38 MAPK inhibitor, 10 μg per rat) or their combination on changes in mean arterial pressure evoked by i.v. moxonidine (100 $\mu g \cdot kg^{-1}$, A) or ethanol (EtOH, 1 $g \cdot kg^{-1}$, B) in conscious glycerol (50%, 10 $mL \cdot kg^{-1}$, i.m.)-treated Wistar rats. Values (means ± SEM of six to eight observations) represent haemodynamic changes demonstrated after the last treatment (moxonidine or ethanol). $^*P < 0.05$ versus moxonidine (A) or ethanol (B) values; $^+P < 0.05$ versus 'PD98059 + ethanol' or 'SB203580 + ethanol' values.

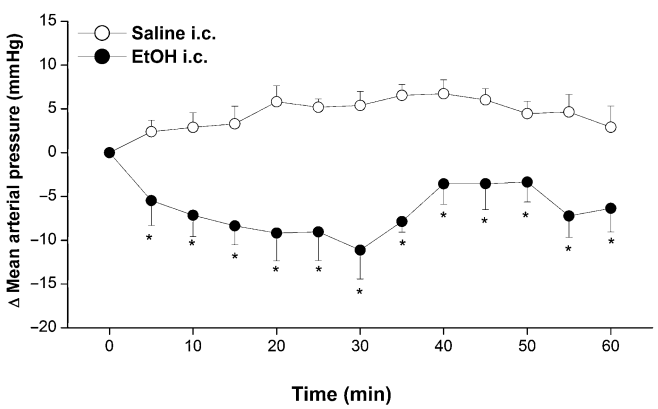

Figure 9 Changes in mean arterial pressure evoked by i.c administration of ethanol (EtOH, 7.5 mg per rat) or equal volume of saline in conscious glycerol (50%, 10 $mL \cdot kg^{-1}$, i.m.)-treated Wistar rats. Values are means ± SEM of six to eight observations. $^*P < 0.05$ versus saline values.

events (MAPKs) provide convincing evidence that implicates central circuits of I_1 sites in the ethanol-induced hypotension.

Given the importance of central sympathoinhibition in the hypotensive response caused by the activation of medullary I_1 sites or α_2 adrenoceptors (Timmermans and Van Zwieten, 1982; Bousquet *et al.*, 1984; 1992; El-Mas and Abdel-Rahman, 1999b), the reason why ethanol facilitated responsiveness to one receptor site (I_1) and not the other (α_2) is not clear. It is reasonable, however, to speculate that the differential interaction of ethanol with I_1 and α_2 receptors might be accounted for by anatomical and functional differences between the two sites. Whereas the rostral ventrolateral medulla is the major site for the sympathoinhibitory and hypotensive actions of imidazolines (Ernsberger *et al.*, 1990; Bousquet *et al.*, 1992), the nucleus of the solitary tract and the nucleus reticularis gigantocellularis play more critical roles in cardiovascular actions of non-imidazoline centrally acting drugs such as α-methyldopa and guanabenz (Bousquet *et al.*, 1984; Head and Burke, 1998). An alternative explanation may be a non-uniformity in peripheral cardiovascular structures affected by the sympathoinhibitory action of centrally acting sympatholytics (Ramage and Wilkinson, 1989; Moreira *et al.*, 2004). Accordingly, the sympathoinhibitory and hypotensive effects of ethanol might be mediated via medullary neuronal projections that are synaptically connected to I_1, but not α_2 adrenoceptor pathways. More studies are clearly needed to address this issue.

Consistent with previous reports in which the individual cardiovascular effects of glycerol and ethanol were investigated (Rodrigo *et al.*, 2004; El-Mas *et al.*, 2008), the current study showed that the changes in HR caused by glycerol and subsequent ethanol administration were directionally opposite to the changes in BP. The arterial baroreflex activity appears to mediate the HR responses because the changes in HR caused by consecutive glycerol and ethanol administration were proportional to and coincided with the changes in BP. More support for the involvement of baroreflex activity arose from the observations that (i) the tachycardia after ethanol did not occur in the presence of prazosin, hexamethonium or efaroxan, pharmacological interventions that abolished the hypotensive effect of ethanol; and (ii) neither the hypotensive nor the tachycardic effect of ethanol was affected by the α_2-adrenoceptor antagonist yohimbine.

Although the present study was not intended to investigate the impact of ethanol on the glycerol-induced ARF, it is important to note that the elevations in serum levels of urea and creatinine, biomarkers of renal damage, caused by glycerol disappeared after ethanol administration. While our finding is consistent with reported renoprotective effect of ethanol in some models of renal failure (Rodrigo *et al.*, 2004; Bertelli *et al.*, 2005), it contrasts with other studies in which ethanol exerted no effect (Peters *et al.*, 2003) or even aggravated the progression of renal failure (Cecchin and De Marchi, 1996; Vamvakas *et al.*, 1998). Interestingly, a similar renoprotective effect for guanabenz or moxonidine in ARF rats was evident as suggested by the ability of these drugs to normalize serum creatinine and urea. Renal sympathoinhibition and subsequent vasodilation are one possible mechanism by which imidazolines might guard against acute renal insult (Tsutsui *et al.*, 2009). More studies are obviously needed to provide more insights into the mechanism(s) of the protective effect of ethanol or sympatholytic drugs in the glycerol model of ARF, and whether it involves renal vasodilation and/or amelioration of histopathological tubular defects. Further,

because our studies employed a model of ARF, the issue remains unresolved whether the beneficial effects of ethanol can also be seen in chronic renal failure. These important questions will be addressed in future studies from our laboratory.

In conclusion, the present study established the first evidence that sympathoinhibition resulting from the facilitation of central I_1 site/ERK/p38 MAPK signalling is responsible for the hypotensive action of ethanol in ARF rats. Clinically, because ethanol potentiated the hypotensive effect of guanabenz and not moxonidine, the use of the second-generation centrally acting sympatholytics such as moxonidine for BP control in alcoholic hypertensive patients might be advantageous. Notably, the dose of ethanol used in the present study produced blood ethanol concentrations comparable to those attained in humans following consumption of moderate amounts of ethanol (Ireland *et al.*, 1984).

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