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Adenosinergic modulation of the imidazoline I_1-receptor-dependent hypotensive effect of ethanol in acute renal failure

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ABSTRACT

We reported that inhibition of central sympathetic pools of imidazoline I_1 receptors abolishes the hypotensive effect of ethanol in rats with glycerol-induced acute renal failure (ARF). This study investigated whether adenosine receptors modulate the ethanol-I_1-receptor interaction. The effect of selective blockade of adenosine A_1, A_{2A}, or A_{2B} receptors on hemodynamic responses to ethanol in the absence and presence of the I_1-receptor agonist moxonidine was determined in ARF rats. Ethanol (1 g/kg i.v.) decreased and increased blood pressure (BP) and heart rate (HR), respectively. Pretreatment with moxonidine abolished the hypotensive but not the tachycardic effect of ethanol. The hypotensive effect of ethanol remained unaltered after selective blockade of A_1, A_{2A}, or A_{2B} receptors with 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and 8-(3-chlorostyryl) caffeine (CSC) and alloxazine, respectively. Neither was ethanol hypotension affected after inhibition of adenosine uptake by dipyridamole (DPY). Alternatively, the ability of moxonidine to abolish ethanol hypotension was still evident in presence of alloxazine whereas it disappeared or weakened in rats pretreated with CSC and DPCPX, respectively. These findings implicate adenosine A_{2A} receptors in the moxonidine-evoked inhibition of the hypotensive action of ethanol. A modulatory role for adenosine A_1 site in the ethanol-I_1-receptor interaction is also possible through as yet unidentified mechanism.

1. Introduction

In a recent experimental study (El-Mas et al., 2009), we reported for the first time on the hemodynamic effects of ethanol in the glycerol model of acute renal failure (ARF). In this model, which serves as a surrogate for studies of human ARF, glycerol causes rhabdomyolysis and acute tubular necrosis together with rapid myoglobinuria, reduced glomerular filtration rate and oliguria (Wolfert and Oken, 1989; Rodrigo et al., 2004). The most important observations of our study were: (i) ethanol, administered systemically or intracisternally, caused dose-related falls in BP, (ii) perturbations (activation or inhibition by moxonidine and efaroxan, respectively) of central imidazoline I_1 receptors abolished the hypotensive effect of subsequently administered ethanol, whereas similar manipulations of α_2-receptors failed to do so, and (iii) central inhibition of ERK 1/2 and p38 mitogen-activated protein kinses (MAPK) abolished the hypotension caused by ethanol or moxonidine (El-Mas et al., 2009). It is concluded that sympathoinhibition caused by enhanced central ERK/p38 MAPK pathway, downstream products of I_1 receptors (Zhang et al., 2001), underlies the hypotensive action of ethanol in ARF rats (El-Mas et al., 2009). Moreover, the initial activation of I_1/$ERK_{1/2}$/p38 signaling by moxonidine seems to offset a similar facilitatory action for ethanol on the same signaling cascade (El-Mas et al., 2009).

The neurobiological effects of ethanol including anxiolytic, locomotor, motivational (Houchi et al., 2008; Ichinose et al., 2009), hypnotic (El Yacoubi et al., 2003), and reinforcing (Batista et al., 2005) properties are modulated by signaling pathways coupled to adenosine receptors. The cardiovascular actions of ethanol are also controlled by adenosinergic mechanisms, possibly via modifying the neural pools related to the chemoreceptor and baroreceptor reflex responses (Mullane and Williams, 1990; Timmers et al., 2004; Ichinose et al., 2009). Adenosine A_{2A} receptors (A_{2A}AR) are implicated in ethanol-induced arteriolar dilation (Nagata et al., 1996). Reports from our laboratory also suggest a causal relationship between endothelial adenosine receptor/nitric oxide cascade and ethanol-evoked hypotension in spontaneously hypertensive rats (Rekik et al., 2002). Indeed, increased nitric oxide bioavailability mediates the facilitatory effects of both ethanol (Baraona et al., 2002) and adenosine (Abebe et al., 1995) on blood flow.

The present study, which extends our previous work (El-Mas et al., 2009), tested the hypothesis that the hemodynamic

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interaction of ethanol with central circuits of I_1-receptors in ARF rats is modulated by adenosinergic pathways. The likelihood of this assumption is bolstered by the observations that central adenosine receptors regulate central sympathetic tone (Timmers et al., 2004) and contribute to the hypotensive action of ethanol (Nagata et al., 1996; Rekik et al., 2002) or centrally acting antihypertensive imidazolines (Nassar and Abdel-Rahman, 2006). Further, the vasodilatory response to A_{2A}AR and I_1-receptor activation involves similar biochemical, $MAPK_{p42/44}$ activation (Zhang et al., 2001; Ribé et al., 2008), and anatomical, brainstem (Thomas et al., 2000; Nassar and Abdel-Rahman, 2008, 2009), modalities. Studies were conducted in conscious ARF rats to investigate the effects of selective pharmacologic blockade of adenosine receptor subtypes or inhibition of adenosine uptake on hemodynamic responses elicited by individual or combined exposure to moxonidine and ethanol.

2. Materials and methods

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

2.1. Intravascular cannulation

The method described in our previous studies (El-Mas and Abdel-Rahman, 1993, 1997; El-Mas, 1998) for intravascular cannulation and measurement of BP and HR in conscious rats was adopted. Experiments started 2 days later in conscious rats.

2.2. Induction of ARF

The glycerol model of ARF (Rodrigo et al., 2004; El-Mas et al., 2009) was employed. Rats were deprived of drinking water for 24 h. A single dose of glycerol (50% in saline, 10 ml/kg) was injected into the thigh muscle of both hind limbs of rats 20–30 min prior to ethanol or other pharmacologic interventions.

2.3. Blood analyses

A blood sample was collected from each rat at the beginning of the experiment for the measurement of serum urea and creatinine. Blood samples were centrifuged at 1200*g* for 10 min and serum was aspirated and stored at −20 °C till analyzed. Serum urea was measured by the kinetic urease test (Randox Laboratories Ltd., United Kingdom; Hitachi 902 analyzer, Hitachinaka-Shi, Japan) and creatinine by kinetic Jaffe method (Audit Diagnostics, Ireland; Hitachi 902 analyzer, Hitachinaka-Shi, Japan).

2.4. Protocols and experimental groups

2.4.1. Moxonidine-ethanol cardiovascular interaction in ARF rats

Three groups of rats (n = 8 each, Table 1) were used to investigate the cardiovascular actions of ethanol in conscious freely moving ARF rats. On the day of the experiment, the arterial catheter was connected to a pressure transducer for measurement of BP and HR. A period of at least 30 min was allowed at the beginning of each experiment for hemodynamic stabilization. Subsequently, each rat received i.m. glycerol (50%, 10 ml/kg) and 30 min later rats were randomly allocated to receive one of the following consecutive intravenous treatments: (i) saline + saline, (ii) saline + ethanol (1 g/kg), or (iii) moxonidine (100 μg/kg) + ethanol. The hypotensive response of the 100 μg/kg dose of moxonidine in the glycerol model of ARF has been shown to be abolished in the presence of efaroxan and not yohimbine, selective blockers of I_1 and α_2 receptors, respectively (El-Mas et al., 2009), thus implicating central I_1 receptors in moxonidine hypotension. Ethanol was administered as 95% in a volume of 1.3 ml/kg (El-Mas and Abdel-Rahman, 1998, 1999). Ethanol or its vehicle (saline) was administered slowly over 3 min. The BP and HR values before and after different drug regimens were measured and peak changes in both variables were computed.

2.4.2. Adenosinergic modulation of the ethanol-moxonidine interaction

A total of 16 groups of glycerol-treated rats (n = 6–8 each) were employed in this experiment to determine the effect of inhibition of adenosine uptake with dipyridamole (DPY, 5 mg/kg) or selective blockade of adenosine A_1, A_{2A}, or A_{2B} receptors with DPCPX (0.1 mg/kg) and CSC (2 mg/kg) and alloxazine (2 mg/kg), respectively, on the hemodynamic effects elicited by ethanol (1 g/kg), moxonidine (100 μg/kg), or their combination in ARF rats. The effect of nonselective blockade of adenosine receptors with 8-phenyltheophylline (8-PT, 1 mg/kg) (Yilmaz et al., 2008) on the hypotensive action of ethanol was also investigated. Twenty min after glycerol administration, rats were randomly assigned to receive one of the following i.v. regimens (i) DMSO + saline, (ii) DMSO + ethanol, (iii) 8-PT + ethanol, (iv) CSC + ethanol, (v) alloxazine + ethanol, (vi) DPCPX + ethanol, (vii) DPY + ethanol, (viii) DMSO + moxonidine, (ix) CSC + moxonidine, (x) alloxazine + moxonidine, (xi) DPCPX + moxonidine, (xii) DPY + moxonidine, (xiii) DPCPX + DPY + moxonidine, (xiv) CSC + moxonidine + ethanol, (xv) alloxazine + moxonidine + ethanol, (xvi) DPCPX + moxonidine + ethanol, or (xvii) DPY + moxonidine + ethanol. BP and HR were monitored for 60 min after the last treatment of each regimen.

Table 1
Baseline values of mean arterial pressure (MAP, mmHg) and heart rate (HR, beats/min) in rats with glycerol (50%, 10 ml/kg i.m.)-induced acute renal failure.

Group	n	MAP	HR
Saline + saline	8	111 ± 7	390 ± 17
Saline + ethanol	8	114 ± 7	382 ± 27
Moxonidine + ethanol	8	106 ± 6	371 ± 22
DMSO + saline	6	100 ± 10	355 ± 10
DMSO + ethanol	7	104 ± 5	389 ± 15
8-PT + ethanol	6	105 ± 5	378 ± 18
CSC + ethanol	7	111 ± 6	364 ± 26
Alloxazine + ethanol	7	107 ± 6	353 ± 16
DPCPX + ethanol	7	108 ± 4	340 ± 13
Dipyridamole + ethanol	7	100 ± 5	384 ± 15
DMSO + moxonidine	8	104 ± 5	401 ± 16
CSC + moxonidine	7	109 ± 5	371 ± 11
Alloxazine + moxonidine	7	119 ± 3	384 ± 26
DPCPX + moxonidine	7	101 ± 4	370 ± 19
Dipyridamole + moxonidine	7	117 ± 4	348 ± 11
Dipyridamole + DPCPX + moxonidine	7	99 ± 4	406 ± 10
CSC + moxonidine + ethanol	8	117 ± 3	359 ± 11
Alloxazine + moxonidine + ethanol	8	107 ± 3	382 ± 10
DPCPX + moxonidine + ethanol	7	114 ± 6	346 ± 15
Dipyridamole + moxonidine + ethanol	7	110 ± 7	371 ± 14

Values are means ± SEM.

2.4.3. Effect of ethanol on BP in other models of renal failure

This experiment determined whether the hypotensive effect of ethanol in glycerol-treated rats could be replicated in other models of acute (cisplatin, single dose of 10 mg/kg i.p.; Santos et al., 2007) or chronic renal failure (cyclosporine, 25 mg/kg/day for 3 weeks s.c.; Tufro-McReddie et al., 1993). Four groups of rats (two cisplatin and two cyclosporine, n = 6 each) were used and allocated to receive i.v. ethanol (1 g/kg) or equal volume of saline. Intravascular cannulation was performed 1 and 19 days after the start of cisplatin and cyclosporine administration, respectively. Two days later, rats were treated with ethanol or saline and BP was monitored for 1 h.

2.5. Drugs

8-Cyclopentyl-1,3-dipropylxanthine, 8-(3-chlorostyryl) caffeine, alloxazine and dipyridamole (St. Louis, MO, USA), thiopental (Thiopental®, Sandoz, Germany), glycerol (Chemajet, Egypt), cisplatin (Mylan S.A.S, Saint-Priest, France), ethanol (Alamia®, Egypt), povidone-iodine solution (Betadine, Nile Pharmaceutical Co., Cairo, Egypt) and Penicid (Cid Pharmaceutical Co., Cairo, Egypt) were purchased from commercial vendors. Moxonidine (a gift from Solvay Pharmaceuticals GmbH, Germany) was dissolved in saline after adding few drops of 1 M HCl; the pH was then adjusted to 7.4 using 1 M NaOH. DPCPX, CSC, alloxazine, and dipyridamole were dissolved in DMSO. Cyclosporine (a gift from Novartis Pharma, AG, Basel, Switzerland) was dissolved in olive oil.

2.6. Data analysis and statistics

Data are expressed as means ± S.E.M. Mean arterial pressure (MAP) was calculated as diastolic pressure + one third (systolic pressure – diastolic pressures). The repeated measures analysis of variance (ANOVA) followed by a Newman–Keuls post hoc test was used to test for statistical significance. These analyses were performed using GraphPad Instat, software Release 3.05. Probability levels less than 0.05 were considered significant.

3. Results

The baseline values of MAP and HR measured in all experimental groups prior to any drug treatment were not statistically different (Table 1).