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Chronic ethanol consumption impairs adrenoceptor- and purinoceptor-mediated relaxations of isolated rat detrusor smooth muscle

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Objective To investigate the effects of chronic ethanol consumption on the reactivity of detrusor smooth muscle.

Materials and methods Eight male rats received ethanol (7.2% v/v) in a modified liquid diet for 4 weeks. Two control groups were assessed; eight rats in one group were fed sucrose and received a liquid diet, and 12 rats in the second group received standard rat chow and water for 4 weeks. The reactivity of detrusor smooth muscle strips from ethanol-fed animals and control animals was evaluated in organ chambers.

Results The relaxation response elicited by isoprenaline or adenosine was unaffected in the both control groups while it was significantly inhibited, with decreased maximum responses and pD_2 values, in the ethanol-fed group. Contractile responses of detrusor smooth muscle to carbachol or 80 mmol/L KCl and relaxant responses to papaverine were similar in the control groups and the ethanol-fed group. There was no change in agonist potency among the groups.

Conclusion Chronic ethanol consumption impairs β-adrenoceptor- and purinoceptor-mediated relaxation but not cholinoceptor-mediated contraction of the rat detrusor smooth muscle. Thus, it appears that different regulatory mechanisms are involved in ethanol-induced alterations in β-adrenergic, purinergic and muscarinic receptors in detrusor strip.

Keywords ethanol, detrusor smooth muscle, contraction, relaxation, rat, bladder

Introduction

In animal experiments, ethanol relaxes smooth muscle, decreases contractile strength and is a potent anaesthetic for the CNS [1–4]. Further evidence suggests that chronic alcohol consumption has effects on cardiovascular function; it can also induce autonomic neuropathies, affecting both sympathetic and parasympathetic nervous systems [5]. To date, there are very few reports about the effect of chronic alcohol consumption on urinary bladder function. As alcohol is considered a serious risk factor for urinary retention in patients with BPH [6], the present study was conducted to investigate the effects of chronic alcohol consumption on the contractile and relaxant responses of detrusor smooth muscle in Wistar albino rats.

Materials and methods

Adult male Wistar rats (200–250 g) were placed in a temperature- and humidity-controlled room ($22 \pm 3^{\circ}C$ and $62 \pm 7\%$, respectively) in which a 12–12 h light-dark cycle was maintained (08.00–20.00 h light). Rats were individually housed in metal cages and divided into three groups; 12 rats in group 1 received a standard diet, and eight each in groups 2 and 3 were fed sucrose or ethanol. Rats in group 3 were fed a liquid diet fortified with vitamins to which ethanol was added in increasing amounts, i.e. 5% v/v ethanol during week 1 and 7.2% ethanol during weeks 2–4, as previously described [7]. Sucrose-fed animals received the same fortified diet except that isocalorific sucrose was substituted for ethanol. The liquid diet was prepared daily and animals received the diet at the same time of the day (11.00 hours). The rats were weighed every day, and daily ethanol intake measured and expressed as grams per kilogram per day. Rats in group 1 were given the standard rat chow but made isocalorific with the other two groups for the same period. These experiments were carried out in accordance with the Declaration of Helsinki; ethical approval was granted by the Kocaeli University Ethics Committee.

Blood ethanol concentration was determined using the head-space gas chromatography method [8], with blood samples taken by intracardiac puncture from the rats placed under very light ether anaesthesia. Glucose

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was determined in a drop of whole blood collected at the time of death, using a commercial glucose meter and glucose-sensitive dipsticks.

At the end of the 4-week period the rats were killed using an overdose of ether. The urinary bladder was removed, weighed and placed in physiological saline solution of the following composition (mmol/L): NaCl 118; KCl 4.7; $CaCl_2$ 2.5; KH_2PO_4 1.18; $MgSO_4$ 1.2; $NaHCO_3$ 24.88; glucose 5.55. After removing adhering fat and connective tissue, the bladder was opened and specimens from the anterior wall of the bladder divided into longitudinal strips. The strips were suspended in a 20-mL water-jacketed (37°C) tissue bath, containing physiological saline solution continuously gassed with 95% O_2 and 5% CO_2, resulting in a pH of 7.4. The resting tension on the tissues was maintained at 1 g, during which the solution was replaced at 15-min intervals before adding drugs. This tension was shown in preliminary experiments to be optimal for this preparation. Agonists were added directly to the organ bath.

Each strip was connected to a force-displacement transducer for measuring isometric force, which was continuously displayed and recorded on-line on a computer via a four-channel transducer data acquisition system using appropriate software.

In a series of preliminary experiments, muscle strips were stimulated with 80 mmol/L KCl to determine the optimum length for force development. To assess the contractile response to the muscarinic agonist carbachol ($10^{-9}-10^{-4}$ mol/L), cumulative concentration-response curves (CRCs) were constructed in a stepwise manner after the response to the previous concentration had reached a plateau. Alternatively, the muscle strip was contracted with 1 μmol/L carbachol, and after the tonic force had reached a stable plateau, cumulative CRCs to isoprenaline ($10^{-9}-10^{-4}$ mol/L) or adenosine ($10^{-10}-10^{-4}$ mol/L) were obtained.

In the high K^+ solution NaCl was exchanged for equimolar amounts of KCl. Fresh solutions were prepared on the day of the experiments. The following drugs were all obtained from Sigma Chemical Co., St Louis, MO USA: carbachol chloride, isoprenaline hydrochloride, adenosine and papaverine hydrochloride. All drugs were dissolved in distilled water and freshly prepared on the day of the experiments

All data are expressed as the mean (SEM); contractile responses to carbachol were calculated as a percentage of the maximal contraction caused by KCl (80 mmol/L), with the relaxant effects of isoprenaline and adenosine expressed as a percentage of the contraction caused by carbachol. To evaluate the effects of agonists, maximum responses (E_m) and pD_2 values ($-\log ED_{50}$) were calculated. The CRC data obtained in each individual experiment were plotted as the response/concentration against the response, to produce a straight-line relationship for each experiment, as predicted from the Scatchard equation for drug–receptor interaction:

$$\text{response/concentration} = (\text{response}/EC_{50}) + (E_m/EC_{50})$$

Agonist pD_2 values were calculated from each agonist CRC by linear regression of the linear part of the curve and taken as a measure of the sensitivity of the tissues to each agonist. The significance of differences was tested by one-way ANOVA with a posthoc Tukey's Kramer test and $P<0.05$ considered to indicate significance.

Table 1 Characteristics of rats in the three groups, and the results from agonist studies on contraction in strips of detrusor muscle

Mean (SEM) variable	*Group*		
	1 (standard)	*2 (sucrose)*	*3 (ethanol)*
Body weight (g)	249.3 (19.6)	245.0 (12.1)	258.5 (16.5)
Bladder weight (g)	0.13 (0.07)	0.14 (0.03)	0.14 (0.02)
Strip weight (mg)	37.93 (2.55)	44.84 (9.96)	49.60 (4.84)
Blood glucose (g/L)	1.24 (0.047)	1.28 (0.174)	1.21 (0.114)
Detrusor strips:			
Tension with carbachol (g)	4.1 (0.6)	4.5 (0.6)	4.6 (0.1)
E_m (% of KCl response):			
Carbachol	220 (15.7)	250 (22.1)	240 (12.2)
Isoprenaline	88.6 (6.7)	90.8 (10.5)	65 (3.6)*
Adenosine	77 (8.2)	81 (10.2)	53.8 (12.4)*
Papaverine	98.6 (1.5)	97.4 (1.2)	96.2 (3.1)
PD_2			
Carbachol	6.25 (0.25)	6.16 (0.53)	6.14 (0.23)
Isoprenaline	8.15 (0.44)	7.96 (1.07)	6.75 (1.71)*
Adenosine	7.45 (0.35)	7.51 (0.92)	5.72 (0.89)*
Papaverine	4.27 (0.06)	4.30 (0.05)	4.28 (0.06)

Values are from 8–12 observations in each group; *$P<0.05$ comparing group 3 with 1 and 2.

Results

There were no significant differences among the body or bladder weights of the rats in all groups (Table 1). The daily ethanol consumption of rats in group 3 was 12.3–18.3 g/kg and the mean blood ethanol level 2.936 (0.052) g/L ($n=5$). The blood glucose levels in each group are also shown in Table 1 and were not significantly different.

The cumulative addition of carbachol produced concentration-dependent contractions of the detrusor strips; the contractility was no different among the groups (Fig. 1) and as shown in Table 1, there were no significant differences among the E_m or pD_2 values of detrusor strips from the groups. The contractions elicited by 80 mmol/L KCl were also similar in the three groups (data not shown).

Isoprenaline, adenosine and papaverine produced concentration-dependent relaxation in submaximally (55–60% of maximal contraction) pre-contracted (1 μmol/L carbachol) detrusor strips obtained from each group. When tissues were contracted with carbachol to assess responses to relaxant agonists, the tension induced was similar in the three groups (Table 1). The relaxation in response to isoprenaline and adenosine was significantly less in strips from rats in group 3 than in those from control rats; the CRC for isoprenaline or adenosine was shifted to the right, with significantly lower E_m and pD_2 values ($P<0.05$) (Figs 2 and 3). However, responses to isoprenaline or adenosine were similar in the two control groups. The relaxation elicited by papaverine was similar in all groups and there were no significant changes in the pD_2 values (Table 1); nor were there significant differences in the mass of the strips used for the contractility studies (Table 1).

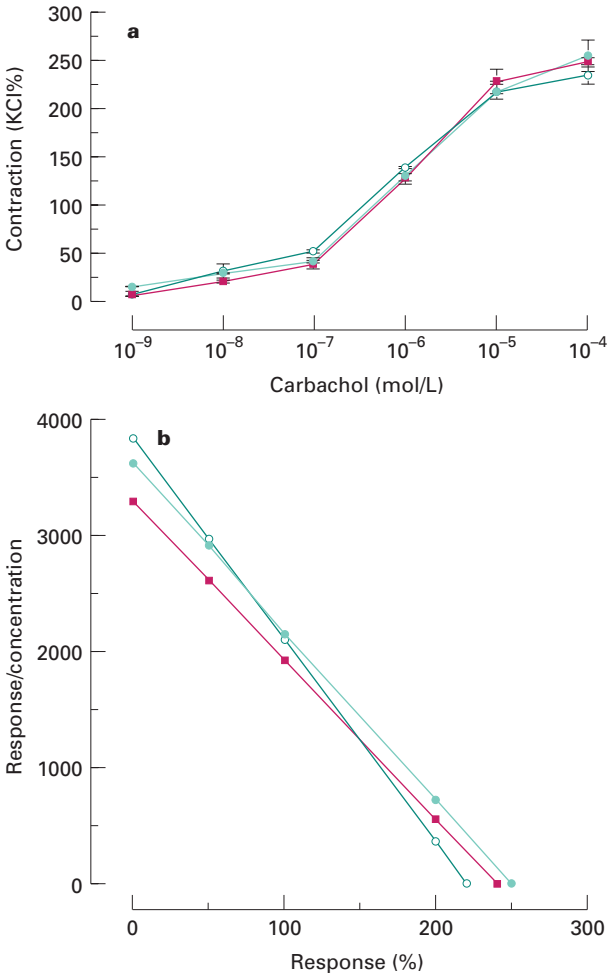


Fig. 1. a, Carbachol CRCs in isolated rat detrusor strips. Each point is expressed as a percentage of the contraction induced by 80 mmol/L KCl and is the mean (SEM). Green open circles, group 1 (standard diet, 12 strips); light green closed circles, group 2 (sucrose-fed, eight strips); red squares, group 3, (ethanol-fed, eight strips). **b,** Scatchard analysis; the E_m and pD_2 values are shown in Table 1. $r=0.71$, 0.74 and 0.65 for groups 1–3, respectively.

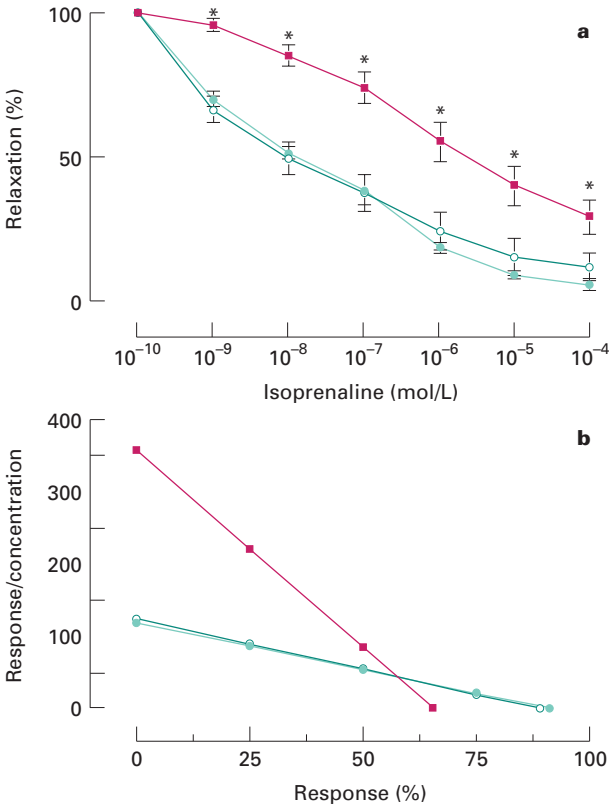


Fig. 2. a, Isoprenaline CRCs in isolated rat detrusor strips precontracted with 1 μmmol/L carbachol. Each point is expressed as a percentage of the contraction induced by carbachol and is given as the mean (SEM). Symbols are as for Fig. 1. *$P<0.05$, statistically different from the response of strips from controls. **b,** Scatchard analysis; the E_m and pD_2 values are shown in Table 1. $r=0.64$, 0.88 and 0.60 for groups 1–3, respectively.

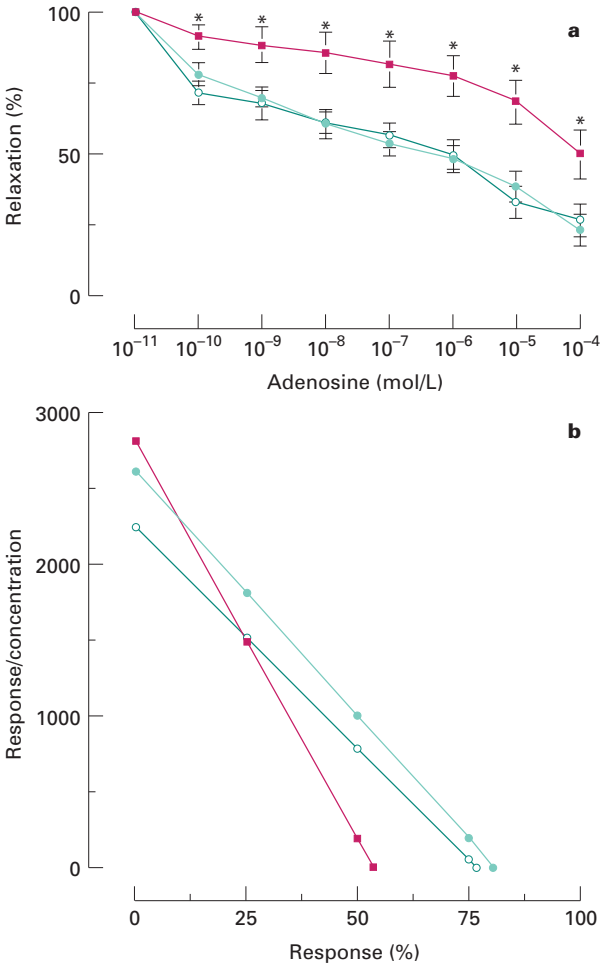


Fig. 3. a, Adenosine CRCs in isolated rat detrusor strips precontracted with 1 μmol/L carbachol. Each point is expressed as a percentage of the contraction induced by carbachol and is given as the mean (SEM). Symbols are as for Fig. 1. *$P<0.05$, statistically different from the response of strips from controls. **b,** Scatchard analysis; the E_m and pD_2 values are shown in Table 1. $r=0.88$, 0.72 and 0.41 for groups 1–3, respectively.

Discussion

In this study there were significant differences in the response to β-adrenergic and purinergic stimulation as a function of ethanol consumption, whereas there were no ethanol-related changes in the response to cholinergic or generalized depolarization with KCl in rat detrusor muscle.

In recent years the increased risk of urinary retention caused by acute alcohol ingestion in patients with BPH has been recognized as a serious problem [6] but little is known of the mechanisms responsible for this bladder dysfunction. In a previous study, the responsiveness of the rabbit lower urinary tract was significantly reduced by exposure to ethanol [9]. Yokoi *et al.* [10] showed that ethanol significantly impaired detrusor contractility in the rat *in vivo* and *in vitro*, and it was also reported that muscarinic receptor levels decreased in bladders from alcohol-preferring rats [11]. In contrast, Knight and Burnstock [12] showed that chronic ethanol consumption significantly augmented cholinoceptor- and purinoceptor-mediated contractions of the isolated rat bladder. The discrepancy between these results might be attributable to differences in treatment protocols, animal sex or the duration of alcohol consumption in the animals at the time of the various studies. Extrapolating to the *in vivo* environment, these *in vitro* observations are consistent with the hypothesis that the aetiology of urinary retention in many patients may be related to impaired detrusor smooth muscle contractility or relaxation.

The impairment of β-adrenoceptor-mediated relaxation is reportedly associated with elevated glucose levels and diabetes mellitus [13,14]; it might be argued that the decreased relaxation in ethanol-fed rats is a result of increased glucose levels. However, this possibility is unlikely, because the blood glucose levels in the present rats did not differ significantly among the three groups.

Androgens and oestrogens are known to affect the contractile responses and receptor kinetics of some urogenital smooth muscles [15–17], and previous investigations established that testosterone levels are decreased during chronic ethanol consumption [18,19]. Although it is well established that testosterone levels affect contractile responses of androgen-sensitive tissues such as the vas deferens and seminal vesicles, a previous study suggested that castration has no effect on the contractile responses or β-adrenoceptor-mediated relaxant responses of the urinary bladder [20]. Therefore, because contractile responses of the bladder are not testosterone-dependent, it appears unlikely that the androgen status of the ethanol-fed rats used in the present study had an effect on the contractile or relaxant responses of the urinary bladder.

Furthermore, because the levels of the pre-contractile response to carbachol were comparable among the three groups, the decreased relaxation in ethanol-fed rats is unlikely to be caused by differences in that level. Thus, the decreased relaxation is most likely caused by the effect of ethanol itself on the detrusor smooth muscle.

Endogenous adenosine may modulate bladder neurotransmission by acting both prejunctionally (A_1 receptors) and postjunctionally (A_2 receptors). In the rat bladder, Nicholls *et al.* [21] suggested that the P_1 purinoceptors inhibiting contractions were of the A_2 type (A_{2b} subclass). Adenosine increases cAMP levels

by activating adenylate cyclase in smooth muscle cells [22]. cAMP has also been shown to be a second messenger in β-adrenoceptor-mediated relaxation in most types of smooth muscles, including urinary bladder [23,24]. The decreased responsiveness to β-adrenoceptor or purinergic stimulation in alcoholic tissues might be assumed to be attributable to impaired signal transduction in addition to the decreased number of β-adrenoceptors or purinergic receptors in alcoholic tissues, respectively. However, more recently adenylate cyclase activity was reported to be increased in the presence of ethanol [25]. As an adenylate cyclase activator was not used in the present study (e.g. forskolin), we cannot draw any conclusion at present. The role of changes in G-protein, another intermediate indispensable for signal transduction between β-adrenoceptors and adenylate cyclase, remains to be investigated.

In conclusion, the present results indicate that the contractile response to carbachol did not change significantly among the three groups of rats. Therefore, the urinary retention by chronic alcohol consumption is unlikely to be caused by changes in the properties of the muscarinic receptor-mediated contraction of detrusor muscle. Furthermore, the relaxant responses of strips obtained from rat detrusor smooth muscle to isoprenaline and adenosine were decreased by chronic ethanol consumption. These impaired β-adrenoceptor and/or purinoceptor-mediated relaxant responses, the mechanism of which has yet to be elucidated, may be important in urinary retention after alcohol ingestion in patients with BPH.

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Abbreviations: **CRC**, concentration–response curve.