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Effects of Acetaldehyde on Responses of Rabbit Corpus Cavernosal Smooth Muscle

Ethanol has various effects on male sexual activity under the influence of direct and indirect, in acute and chronic alcohol ingestion. However, whether acetaldehyde, a principal metabolite of ethanol, may affect penile erection directly has still not been elucidated. This present study was, therefore, designed to clarify the pharmacologic effects of the acetaldehyde on corpus cavernosal smooth muscle. Corpus cavernosal strips were prepared from rabbit penises. Isometric tension changes of rabbit corpus cavernosal strips to various drugs and electrical field stimulation (EFS) in an organ chamber were recorded with a pressure transducer after active muscle tone had been induced by phenylephrine (10^{-5} mol/L). At the concentrations employed, acetaldehyde had no effect on the pH of the bathing medium. Acetaldehyde in each concentration did not significantly affect resting tone of the smooth muscle during 30 min incubation. Acetaldehyde suppressed contractility induced by phenylephrine and KCl at 10^{-4} mol/L, and relaxation induced by EFS and bethanechol at 10^{-3} mol/L and 10^{-4} mol/L respectively, but acetaldehyde enhanced relaxation induced by ATP at high acetaldehyde level. Sodium nitroprusside-induced relaxation was not affected at any employed acetaldehyde concentration. This suggests that increasing the acetaldehyde level may contribute to male erectile dysfunction mainly by the inhibition of nitric oxide formation.

Key Words: *Acetaldehyde; Corpus Cavernosum; Muscle, Smooth*

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INTRODUCTION

Ethanol has various effects on male sexual activity. Ethanol-induced male sexual dysfunction is caused by ethanol's effect on the central nervous system mainly through acute ethanol ingestion and chronic alcoholism, in decreased testosterone and increased estrogen levels, and alcoholic polyneuropathy (1). Recently in an in vitro study, ethanol had significant effect on both contraction and relaxation of rabbit corpus cavernosal smooth muscle (CCSM) (2). Following ingestion, ethanol metabolized to acetaldehyde, acetate and CO_2 and H_2O, sequentially (3, 4). The negligible blood concentration of acetaldehyde found after ingestion of alcohol in normal subjects under normal condition suggests that acetaldehyde was of interest only when acetaldehyde dehydrogenase was inhibited. However in approximately 50% of Oriental subjects, there was a deficiency of aldehyde dehydrogenase, resulting in increasing acetaldehyde level (normal group: 2.1 μmol/L, aldehyde dehydrogenase deficiency group: 35.4 μmol/L of acetaldehyde) (5). Also in chronic alcoholism acetaldehyde level was increased (6). Therefore in subjects with aldehyde dehydrogenase deficiency and chronic alcoholics, acetaldehyde may play an important role in physiologic action. Ethanol seems to inhibit smooth muscle contraction (7, 8), however, the effects of acetaldehyde on responses of the corpus cavernosal smooth muscle have not been elucidated. This present study was designed to clarify the pharmacologic effects of acetaldehyde on corpus cavernosal smooth muscle relaxation.

MATERIALS AND METHODS

Male New Zealand White rabbits weighing 2.5 to 3 kg were used. Total penectomy was performed under general anesthesia by intravenous pentobarbital injection and exsanguination. The whole penis was sharply dissected free in 0 to 4℃ Krebs solution aerated 95% O_2 and 5% CO_2. Two strips of CCSM (2×2×10 mm) were produced from each corpus. Then each strip was mounted in organ-bath chamber containing 30 mL of a Krebs

solution with the following composition (mmol/L): NaCl 120, KCl 4.6, KH_2PO_4 1.17, $NaHCO_3$ 23.8, $CaCl_2$ 1.8, $MgSO_4$ 1.2 and glucose 10. The solution was aerated with 95% O_2 and 5% CO_2, maintaining a pH 7.4 at 37℃. During equilibration the bath solution was replaced every 10-15 min.

After 60 min equilibration, the strips of CCSM were loaded with resting tension of 1 g. Changes in isometric tension were measured using Grass FT03 transducer (Grass instruments, Quincy, MA, U.S.A.) and recorded on Grass Polygraph model 79E. Electrical field stimulation (EFS) was delivered from Grass-88 square-wave pulse-stimulator connected to two platinum electrodes parallel to the muscle strip.

The optimal isometric tension was obtained by two consecutive contractions to phenylephrine (10^{-5} mol/L) that differed by less than 10%.

The drugs used were phenylephrine hydrochloride, potassium chloride, bethanechol chloride, ATP, sodium nitroprusside, ethanol and acetaldehyde, all obtained from Sigma Chem Co (St Louis, MO, U.S.A.).

Experimental procedure

The effects of acetaldehyde on basal tension were repeated as the tension at the end of 30 min incubation in the presence of 10^{-5}, 10^{-4} or 10^{-3} mol/L acetaldehyde separately. Following each procedure, muscle strips were washed 3 times with fresh Krebs' solution, then incubated for at least 60 min.

EFS-induced relaxation of the CCSM was assessed after active muscle tone had been induced by phenylephrine (10^{-5} mol/L), with the EFS delivered at 200 mA and pulse duration of 1 ms for 10 seconds at 1, 2, 4, 8, 16 and 32 Hz. There was a 2-min interval between stimulation. After 30-min incubation with fresh Krebs solution, 10^{-5} mol/L acetaldehyde was added to each organ bath, and the tissue was incubated for 30 min; EFS was repeated and its effects observed. Subsequentially, the same procedure was performed with 10^{-4} or 10^{-3} mol/L acetaldehyde following washout and refilled with fresh Krebs solution. The above set experiment was carried with the same muscle strip.

In separate experiments, the effects of acetaldehyde on phenylephrine or KCl-stimulated contraction were determined. After 1-hr incubation at 1 g tension, 10^{-5} mol/L phenylephrine or 127 mmol/L KCl solution was added to each organ bath and peak responses observed. Each tissue was washed 3 times with fresh Krebs solution, the bath refilled and 10^{-5}, 10^{-4} or 10^{-3} mol/L acetaldehyde added to it. After 30 min incubation in acetaldehyde concentration, phenylephrine or KCl solution stimulation was repeated and their effects noted. Changes in Krebs' solution pH were measured following application of chemicals.

The effects of acetaldehyde on bethanechol, ATP or sodium nitroprusside-induced relaxation were investigated using another muscle strip. After 1-hr incubation at 1 g tension, each tissue was contracted with 10^{-5} mol/L phenylephrine. When contractions reached their plateau, 2 mmol/L ATP, 5×10^{-4} mol/L bethanechol or 10^{-4} mol/L sodium nitroprusside was added to each organ bath and their effects were noted. Each tissue was then washed 3 times with fresh Krebs solution, the bath refilled and 10^{-5}, 10^{-4} or 10^{-3} mol/L acetaldehyde added. After 30-min incubation in each acetaldehyde concentration, phenylephrine preincubation followed by ATP-, bethanechol- or sodium nitroprusside-induced relaxation was repeated and their effects were noted.

Changes in contraction and relaxation were calculated as percentage of active muscle tone induced by phenylephrine (10^{-5} mol/L); all values are presented as the mean (SEM). The significance of differences among groups were determined using Student's t-test with differences considered significant at $p<0.05$.

RESULTS

At the concentration employed, acetaldehyde had no effect on the pH of the bathing medium. Acetaldehyde in each concentration had no significant effect on the resting tone of the smooth muscle during 30-min incubation.

Pre-incubation with 10^{-3} mol/L acetaldehyde significantly changed the contractile or relaxed responses in all the stimulation methods employed except sodium nitro-

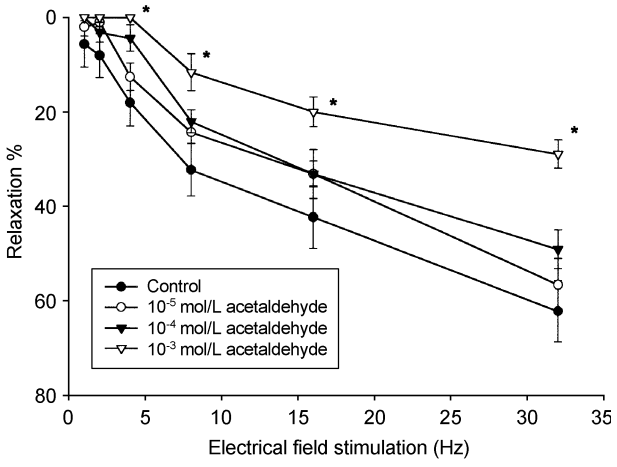

Fig. 1. Field stimulation-induced relaxation under acetaldehyde. Field stimulation-induced relaxation is suppressed by pre-treatment with 10^{-3} mol/L actaldehyde. *$p<0.01$ compared with the respective control; n=7 in each group.

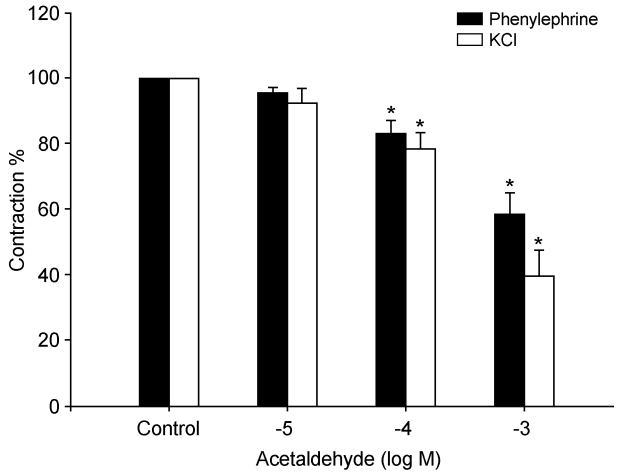

Fig. 2. Effects of acetaldehyde on phenylephrine or KCl-induced contraction. Contractions are significantly suppressed at 10^{-4} mol/L acetaldehyde. *$p<0.05$ compared with the respective control; n=7 in each group.

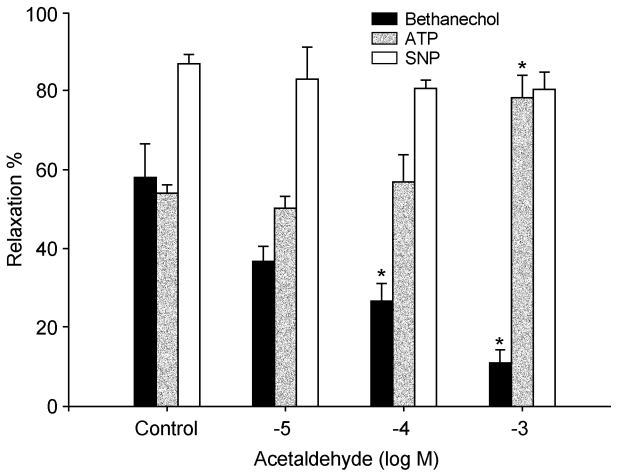

Fig. 3. Effects of bethanechol-, ATP- and sodium nitroprusside (SNP)-induced relaxation under acetaldehyde. Bethanechol-induced relaxation is suppressed at 10^{-4} mol/L, ATP-induced relaxation is enhanced at 10^{-3} mol/L acetaldehyde and SNP-induced relaxation is not affected by pretreatment with acetaldehyde. *$p<0.05$ compared with respective control; n=7 in each group.

prusside-induced relaxation. The response to field stimulation was suppressed by this concentration of acetaldehyde proportionally, and lower concentrations of acetaldehyde had little significant effect (Fig. 1). Responses to phenylephrine (10^{-5} mol/L) and KCl (127 mmol/L) were significantly suppressed at concentrations above 10^{-4} mol/L acetaldehyde (Fig. 2). Responses to bethanechol (5×10^{-4} mol/L) were significantly suppressed by all concentrations of acetaldehyde used. Responses of relaxation to ATP (2 mmol/L) were significantly increased only by concentrations of 10^{-3} mol/L acetaldehyde. Acetaldehyde had no effect on responses to sodium nitroprusside-induced relaxation (Fig. 3).

DISCUSSION

In chronic alcoholism, male sexual dysfunction may be caused from decreased testosterone and increased estrogen levels, and alcoholic polyneuropathy (1). In acute ethanol ingestion, it may be caused mainly central nervous system depression. It is known that acute ethanol intoxication can cause urinary retention in benign prostatic hyperplasia (9). In animal studies, the mechanism of urinary retention induced by ethanol partly showed that ethanol significantly impaired detrusor contractility in vivo and in vitro (8, 10, 11). Also in an in vitro study, ethanol had significant effect on both contractility and relaxation of rabbit corpus cavernosal smooth muscle (CCSM) (2), suggesting ethanol affected the physiologic function of genitourinary smooth muscle. The primary enzymatic steps in ethanol metabolism to acetaldehyde, acetate and CO_2 and H_2O have been known for many decades, however, only a few implications were recognized in acetaldehyde's effect on smooth muscle activity (4).

Ethanol-related flushing, which occurs in approximately 50% of Oriental subjects after ingestion of alcohol, is caused by a deficiency of mitochondrial acetaldehyde dehydrogenase (ALDH2) activity, resulting in increasing acetaldehyde levels (5, 12). Acetaldehyde is the principal metabolic by-product of ethanol metabolism, causing cytotoxicity (13). In animal studies, chronic acetaldehyde exposure leads to impairment in the inotropic response to membrane depolarization in endothelium-denuded preparation, resulting in depressed responsiveness. Acute acetaldehyde exposure significantly reduced both norepinephrine- and potassium chloride-induced contractile response in the rat aorta (14), but low concentrations of acetaldehyde enhanced spontaneous phasic contractile activity (15). Under normal circumstances acetaldehyde produced as a result of ethanol metabolism is rapidly oxidized to acetate by aldehyde dehydrogenase as well as by other microsomal enzyme system (16). However, there are several conditions in which the plasma concentration of acetaldehyde is significantly elevated, following ethanol ingestion, such as alcoholics (6) or subjects undergoing therapy with monoamine oxidase inhibitors (17). Therefore, following alcohol ingestion, acetaldehyde may affect CCSM relaxation, but the effects of acetaldehyde on CCSM are still elusive.

This study was designed to investigate the effects of acetaldehyde on isolated CCSM, thus eliminating the

effects of innervation. In this study ethanol did not affect CCSM function in the physiologic concentration, 0.1 to 0.5% (data not shown).

In this study, phenylephrine (10^{-5} mol/L) and KCl (127 mmol/L) induced corporal contractions were significantly suppressed at concentrations above 10^{-4} mol/L acetaldehyde, these might be the underlying mechanism that acetaldehyde induced inhibition of extracellular calcium ion influx into smooth muscle (14). Also both field-stimulated and bethanechol-induced corporal relaxation were significantly more suppressed in acetaldehyde-induced inhibition than ATP- or sodium nitroprusside-induced relaxation. These findings are consistent with ethanol inhibition of endothelial-derived relaxing factor (EDRF) function (18). EDRF has been proposed to form nitric oxide (NO) from the terminal guanidine of L-arginine (19). The enzyme responsible for NO synthase shows similarity to cytochrome P-450 reductase, resulting in competitive inhibition of NO formation (20). Therefore, acetaldehyde seems to affect NO formation. But further studies were needed to elucidate the action mechanism of acetaldehyde on response of ATP to CCSM.

In this study, acetaldehyde had no effect on basal tension, but depressed contractility or relaxation of CCSM in various stimulation methods employed except for nitroprusside-induced relaxation. This suggests that increasing the acetaldehyde level may affect to function of CCSM through the mechanisms of inhibition of extracellular calcium ion influx and NO formation. However, in the clinical aspect of erectile dysfunction, the inhibition of NO formation may be a major cause to need to be investigated with in vivo study.

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