CHOLINERGIC NERVES MEDIATE ACETALDEHYDE ACTION IN THE GASTROINTESTINAL TRACT

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Abstract — The regulation mechanism of inhibition of intestinal ethanol absorption induced by high acetaldehyde (AcH) concentration in blood was investigated. We used atropine (AT), atropine methylbromide (ATMB), pirenzepine (PI), bethanechol (BE) and pilocarpine (PL) with or without cyanamide (CY; a potent inhibitor of aldehyde dehydrogenase, which induces high AcH concentration in blood). The $K_a$ (absorption rate constant) value after the CY-alone pretreatment was significantly lower than that in controls. In the high AcH-induced cases, the values of $K_a$ in AT and ATMB pretreatments were similar to controls, but the value of $K_a$ in PI pretreatment was lower than that in controls. The values of $K_a$ in the case of BE pretreatment with and without high AcH levels were lower than in controls. The $K_a$ value in the PL with CY was significantly lower than that with CY alone. However, its action was blocked by ATMB pretreatment. These results suggest that high blood AcH concentrations inhibit intestinal ethanol absorption through the peripheral cholinergic nerves via muscarinic receptors, except for the muscarinic M1 receptor, compared to other subtypes of muscarinic receptors.

INTRODUCTION

Acetaldehyde (AcH), the first metabolite of ethanol, has many pharmacological and physiological actions, but the details of its effects are still largely unknown (Brien and Loomis, 1983). Genetically, in some Oriental populations with a lower activity of aldehyde dehydrogenase (ALDH), high concentrations of AcH are produced in blood following ethanol (EtOH) ingestion (Enomoto et al., 1991). As EtOH has a relatively small and simple structure, it is readily absorbed from the gastrointestinal tract by a process of simple passive diffusion after oral ingestion (Kricka and Clark, 1979; Beck and Dinda, 1981; Jones, 1991). The rate of absorption of EtOH through the gastrointestinal tract may depend on many factors, such as concentration gradient during absorption, blood flow of the gastrointestinal tract, stomach emptying time, speed of consumption and drug interactions in the gastrointestinal tract (Jones, 1991; Ameno et al., 1996). High AcH levels inhibit EtOH absorption in canines and rats as described in our previous report (Shinohara et al., 1992, 1993; Kinoshita et al., 1995, 1996). It is well established that the autonomic nervous system affects intestinal absorption and gut absorption is reduced by cholinergic stimuli and increased by alpha-adrenergic stimuli (Field and McColl, 1973; Hubel, 1976; Brunsson et al., 1979). However, the regulatory mechanisms of modulation of intestinal EtOH absorption by AcH are unclear.

The aim of the present study was to investigate the effects of cholinergic blocking and stimulating agents on intestinal EtOH absorption. Using rats with high AcH concentrations induced by cyanamide (CY; a potent ALDH inhibitor) pretreatment, we compared the values of the EtOH absorption rate constant ($K_a$) in the investigation of involvement of cholinergic nerves in the reduction of EtOH absorption by high AcH concentrations.

MATERIALS AND METHODS

Sixty-seven male Wistar rats (mean weight ± SD 294 ± 31g) were used in this study and were housed in a controlled environment with respect to temperature (22–24°C), relative humidity (50–70%) and light/darkness cycle (12 h/12 h). Rats were starved for 18 h prior to experiment, but had free access to water. Experimental procedures were performed following pentobarbital anaesthesia (50 mg/kg), as previously described (Kinoshita et al., 1995, 1996). Briefly, catheters (Intramedic PE-50; Becton Dickinson, Sparks, MD, USA) were placed in the femoral artery and vein for sample collection and drug administration. A 20-cm length of jejunum was prepared for ethanol perfusion after a midline laparotomy. Following the surgical procedure, the jejunal was returned to the abdominal cavity and the abdomen was closed to maintain the local temperature and humidity. EtOH solution (4% w/v) was perfused for 30 min (1.6 g EtOH/kg) at a steady rate. Body temperature was maintained at 37 ± 0.5°C during the experiment. The $K_a$ value was calculated based on the change in the amount of EtOH before and after perfusion (Yada and Hayashi, 1985). EtOH and AcH concentrations in each sample were quantified by a head-space gas chromatographic method (Okada and Mizoi, 1982).

This study was approved by the Kagawa Medical University Animal Investigation Committee.

Experiment 1

Rats were divided into 10 experimental groups (five or six rats each), as follows: pretreatment saline (control), atropine sulphate (AT; a parasympatholytic muscarinic antagonist), atropine methylbromide (ATMB; a peripherally acting anticholinergic agent), pirenzepine dihydrochloride (PI; a muscarinic receptor antagonist with a high affinity for M1 receptors) and bethanechol chloride (BE; a choline ester with muscarinic actions mainly on smooth muscles of the gastrointestinal tract) with or without CY. The amounts of CY, AT, ATMB, PI and BE used in pretreatment were 50, 0.5, 0.5, 1.0 and 0.1 mg/kg respectively. Pretreatment of CY was performed 60 min before EtOH perfusion, whereas AT,
A TMB, PI or BE treatments were performed 10 min before EtOH perfusion.

**Experiment 2**

To investigate the participation of the muscarinic receptor, additional experimental groups, such as pilocarpine hydrochloride (PL: a cholinomimetic agent which has a dominant muscarinic action) alone, PL + CY and PL + ATMB + CY, were tested. The doses of PL and ATMB were 5 and 20 mg/kg, respectively. The dose of ATMB was determined according to the previous report (Proctor et al., 1966). ATMB and PL pretreatments were performed 40 and 5 min before EtOH perfusion, respectively, and pretreatment of CY was the same as in Experiment 1. All reagents were purchased from Sigma Chemical Co. (St Louis, MO, USA).

**Statistics**

Data are expressed as means ± SD. Statistical analysis of the data was performed using Student’s t-test. Values of $P < 0.05$ were accepted as representing significant differences.

**RESULTS**

Figure 1 shows peak blood concentrations of AcH for each group in Experiment 1. Mean blood concentrations of AcH in the groups pretreated with CY were strikingly higher than those of the control and experimental groups without CY pretreatment.

Figure 2 shows the $K_a$ values in Experiment 1. The $K_a$ value of the CY-alone group was significantly lower than that of the control group. The $K_a$ of the AT + CY group was similar to that of the AT-alone group, despite the higher blood AcH concentrations. By contrast, the $K_a$ value of the BE-alone group was significantly lower than that of the control group and this decrease was not modified by the high AcH concentration in the BE + CY group. The ATMB + CY treatment did not change the $K_a$, compared to controls, as was the case for the AT + CY group, whereas the PI + CY group had a lower $K_a$. ATMB-alone and PI-alone did not have any effects on EtOH absorption.

Table 1 shows the $K_a$ values and peak AcH concentrations in Experiment 2, control and CY. The $K_a$ values in the PL-alone and CY-alone groups were significantly lower than that of the controls. The $K_a$ in the PL + CY group was significantly lower than that in the CY-alone group. However, the PL + ATMB + CY group did not have a $K_a$ different from that of the CY-alone group.

**DISCUSSION**

Gastrointestinal function is generally regulated by antagonistic double innervation of the sympathetic and parasympathetic nervous systems with the latter acting dominantly at an effector site in the intestine by cholinergic neurotransmission (Taylor, 1990b). Many pharmacological and physiological actions of AcH have been reported previously (Brien and Loomis, 1983). However, there are no reports describing AcH itself as having a parasympathomimetic action.
Since cholinergic stimuli generally reduce gut absorption (Hubel, 1976), we postulated that a high AcH concentration has the possibility to act as an inhibitor of intestinal EtOH absorption through cholinergic nerves. Our hypothesis was supported by AT, one of the parasympatholytic drugs which acts through a non-selective blocking action of the muscarinic receptor in the gastrointestinal tract (Brown, 1990), which was demonstrated to block entirely the inhibition of intestinal EtOH absorption, with $K_v$ values remaining at control levels. A similar inhibition of intestinal EtOH absorption was induced by BE-alone, which is a choline ester with muscarinic actions mainly on the smooth muscles of the gastrointestinal tract (Taylor, 1990a). There was no evidence of $K_v$ level inhibition in combination with a high AcH concentration. This regulation may therefore be controlled by peripheral nerves because the value of $K_v$ did not decline in the ATMB + CY pretreatment group, similar to the AT pretreatment in our study. ATMB, an anticholinergic agent, which is unable to cross the blood–brain barrier, acts mainly through the peripheral nerves. As observed in Experiment 2, both $K_v$ values of PL-alone and when combined with CY were significantly lower than that of CY, but there was no significant difference in $K_v$ values between PL-alone and PL + CY. PL itself, which acts at both the central and peripheral nervous systems as a muscarinic receptor stimulant, has a potent inhibitory action of EtOH absorption. Therefore, additional inhibition on EtOH absorption induced by a high AcH level may be masked by PL. The decline in $K_v$ after PL + CY was partially overcome by pretreatment with ATMB, which has peripheral effects alone. These results thus suggest that a high AcH level reduces the intestinal EtOH absorption through peripheral cholinergic nerves via muscarinic receptors.

It has been known that muscarinic receptors may be divided into five subtypes including M1, M2, M3, M4 and M5 (Lefkowitz et al., 1990). PI has a high affinity for the M1 receptor and acts as a muscarinic M1-receptor-selective blocking agent. This has similar pharmacological actions to AT, including reduced motility and inhibition of intestinal secretion (Brown, 1990). PI pretreatment, however, had no effect on the reduction of intestinal EtOH absorption by high AcH concentrations and there was also no increase in intestinal EtOH absorption in the PI-alone-treated group.

In the intestine, activation of cholinergic nerves, which is a powerful vasodilator as well as a stimulator of intestinal constriction, acts dominantly, but a decrease of intestinal blood flow is observed. The reason for this is that intestinal blood flow is regulated, not only by vascular tone, but also by spontaneous intestinal contraction (Ching, 1989). This agrees with our previous observation that a high AcH concentration reduces portal blood flow in canines (Shinohara et al., 1993). AT has the effect of increasing the absorptive site blood flow following intravenous administration (Mailman, 1984). In our studies, $K_v$ did not fall when CY was employed with AT and this may account for an increase of intestinal blood flow. In the gastrointestinal tract, AT acts to decrease secretion and BE or PL act to increase secretion via respectively a blocking or stimulating action of the muscarinic receptor. Less secretion in AT + CY group and more secretion in the BE or PL group, which are capable of affecting intestinal absorption, may have occurred (Shinohara et al., 1993).

Alternatively, it has been reported that EtOH absorption in humans following oral ingestion is retarded by AT. One explanation is that AT acts to delay the stomach emptying time (Rinkel and Myerson, 1941). In our experiments, however, AT pretreatment alone induced neither an increase nor a decrease in intestinal EtOH absorption, presumably because EtOH was directly administered into the intestinal segment.

In conclusion, these observations clearly indicate that a high AcH concentration in blood stimulates cholinergic nerves via peripheral muscarinic receptors and we further speculate that the peripheral M1 receptor has little effect compared to other subtypes of muscarinic receptors.

REFERENCES


