ORIGINAL

Less contribution of the nicotinic cholinergic and α_2-adrenergic action of high acetaldehyde concentration on the inhibition of intestinal ethanol absorption

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Abstract : In the present study, we investigated the effects of hexamethonium, a ganglionic nicotinic receptor blocking agents and yohimbine, an α_2-adrenergic antagonist, on reduction of ethanol absorption in presence of high acetaldehyde concentration. Hexamethonium had no effect, whereas yohimbine by itself reduced ethanol absorption, but no additional effects were observed with presence of high acetaldehyde. Propionaldehyde had an inhibitory action on intestinal 1-propanol absorption. As both yohimbine and propionaldehyde are associated with vagus nerve activation, these results indirectly support the hypothesis that a cholinergic mechanism through vagus nerve activation is responsible for the inhibition of intestinal ethanol absorption by acetaldehyde. **J. Med. Invest. 51 : 38-42, February, 2004**

Keywords : *acetaldehyde, ethanol, hexamethonium, intestinal absorption, yohimbine.*

INTRODUCTION

In some oriental populations, the activity of aldehyde dehydrogenase (ALDH), which is regulated genetically, is reduced hence high concentrations of acetaldehyde (AcH) are accumulated in blood following ethanol (EtOH) ingestion (1). AcH, which has a simple chemical structure and is the first metabolite of ethanol, has many pharmacological and physiological actions (2), which when present at high concentrations in the blood leads to adverse effects including flushing, headache and discomfort.

EtOH absorption from the intestine is performed by simple diffusion (3-5) and this is regulated by many factors, including the concentration gradient during absorption, blood flow at the absorption site, stomach emptying time, speed of EtOH ingestion and drug interactions with the gastrointestinal tract (5,6). We have previously found that accumulation of AcH in blood inhibits EtOH absorption in canines and rats with induction of intestinal secretion and reduction of intestinal blood flow (7-10). In addition, we have also clarified that such inhibition is mediated by cholinergic nerves via peripheral muscarinic receptors using atropine, bethanechol and pilocarpine (11). However, the involvement of other receptors, such as nicotinic or adrenergic receptor, has not been studied yet.

Hence the main aim of this study was to investigate the mechanism of action by examining the effects of hexamethonium (C_6), a ganglionic nicotinic receptor blocking agents and yohimbine (YO), an α_2-adrenergic antagonist, on the inhibition of intestinal EtOH absorption through the accumulation of AcH in the blood. Additionally, we wished to investigate whether other aliphatic aldehyde compounds can inhibit the absorption of their parent alcohol, using 1-propanol (PrOH) with cyanamide (CY), a potent inhibitor of ALDH.

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MATERIALS AND METHODS

Male Wistar rats (273±30g) were used in this study. The rats were housed in a temperature-controlled environment (22-24°C), with controlled humidity (50-70%) and a standard light/darkness cycle (12/12hrs). Rats were fasted for 18h prior to experiment, but had free access to water. Experimental procedures were performed following pentobarbital anesthesia (50mg/kg), as previously described (9-11). In brief, catheters (Intramedic PE-50, Becton Dickinson, Sparks, U.S.A.) were placed in the femoral artery and vein for sample collection and drug administration. A 20 cm-length of jejunum segment was prepared for perfusion of EtOH or PrOH, after a midline laparotomy. Following the surgical procedure, the jejunum segment was returned to the abdominal cavity and abdomen was closed to maintain the local temperature and humidity. Body temperature was maintained at 37±0.5°C throughout the experiment.

Experiment 1. Rats were divided into six experimental groups (5-7 rats/experimental group), as follows : pretreatment saline (control), CY, YO, CY+YO, C_6 and CY+C_6. The dose of CY, YO and C_6 used in pretreatment were 50mg/kg, 0.5mg/kg and 10mg/kg, and each were performed 60 min, 30 min and 10 min before EtOH perfusion, respectively. Each of these agents was disolved in saline(0.1 ml/100g body weight). EtOH solution (4% w/v) was perfused for 30 min(1.6g EtOH/kg) at a steady rate.

Experiment 2. Rats were divided into two experimental groups (4 rats/experimental group), as follows : pretreatment saline (control), or CY. The pretreatments of CY were the same as in Experiment1, and PrOH solution (4% w/v) was perfused for 30min (1.6g PrOH/kg) at a steady rate.

EtOH, AcH, PrOH and propionaldehyde (PrCHO) concentrations in each sample were quantitated by the head-space GC method (12). The value of Ka was calculated according to the previous report (9-11, 13). AcH was purchased from Merck (Munchen, Germany). All reagents except AcH were purchased from Wako Pure Chemical (Osaka, Japan.).

Data were expressed as means±SD. Statistical analysis of the data was performed using the student's t-test. Values of $p<0.05$ were accepted as representing significant differences. This study was approved by the Kagawa Medical University Animal Investigation Committee.

RESULTS

Figures 1 and 2 show the Ka values and peak AcH concentrations, respectively from Experiment 1. The value of the control group and CY group have been published previously (11). The Ka value in the YO group was significantly lower than that of the control, without the high concentration of AcH. No additional decrease in the value of Ka in the CY+YO group was observed in comparison with that of the YO-alone group, but was significantly lower compared to the CY group. The Ka values were not significantly different between C_6 and the control, CY+C_6 and CY, respectively.

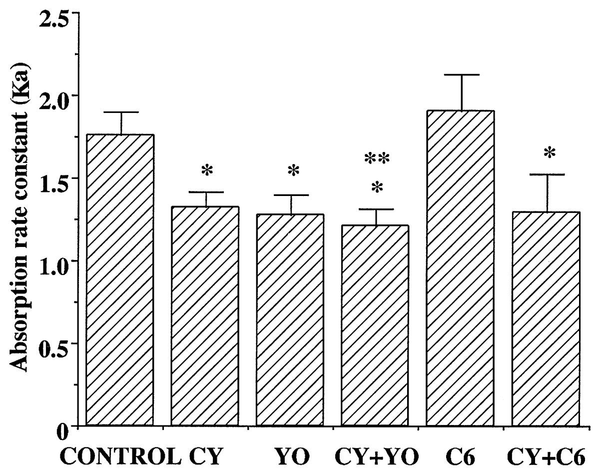

Figure 1. Values of absorption rate constant (Ka).
CONTROL : control group, CY : cyanamide pretreated group, YO: yohimbine hydrochloride pretreated group, CY+YO : cyanamide with yohimbine hydrochloride pretreated group, C_6 : hexamethonium bromide pretreated group, CY+C_6 : cyanamide with hexamethonium bromide pretreated group. The values are means±SD (n=5 or 7). *p<0.05 compared with control. ** p<0.05 compared with CY.

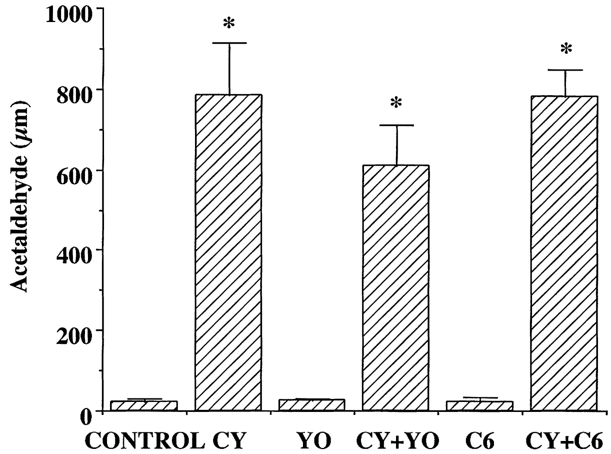

Figure 2. Peak blood acetaldehyde concentration for each treatment group.
All abbreviations are same as in Figure 1.
The values are means±SD (n=5 or 7). *p<0.01 compared with control.

Table 1. Peak blood concentration of 1-propanol and propionaldehyde in each group of Experiment 2 (n=4).

	1-propanol (mM)	propionaldehyde (μM)
Control	22.5±3.2	3.9±1.0
Cyanamide	17.6±3.0	303.9±40.6*

*p<0.001 compared with control. Values are means±SD.

Table 1 shows peak concentration of PrOH and PrCHO for each group in Experiment 2. Mean blood concentrations of PrCHO pretreated with CY groups were increased markedly compared to the control group and the value of Ka in the CY group was significantly lower than in the control (Figure 3).

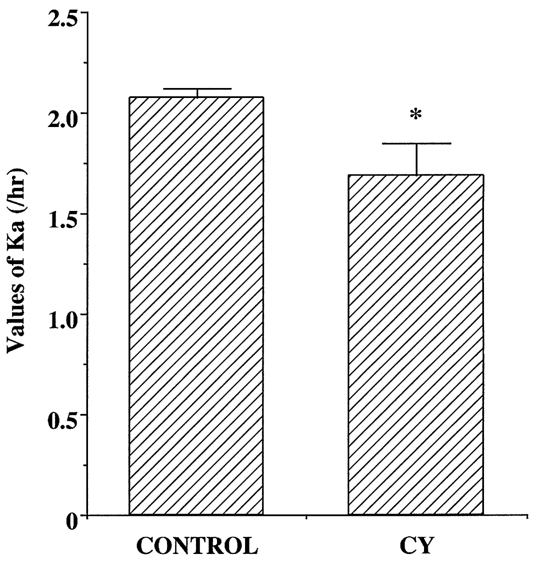

Figure 3. Values of absorption rate constant (Ka) in each group of Experiment 2.
The values are means±SD (n=4).
CY : cyanamide pretreated group, *p<0.01compared with control.

DISCUSSION

It is generally accepted that gastrointestinal function is regulated by the autonomic nervous systems, divided into three major parts including the sympathetic, the parasympathetic and the enteric nervous system (ENS) (14). Stimulation of the sympathetic nervous system increases intestinal water and electrolyte absorption (15, 16). The sympathetic nervous system exerts tonic control on intestinal fluid transport and its effect is mainly a direct action through peripheral α_2-adrenergic receptors on enterocytes (17). Therefore, YO, a selective α_2-adrenergic blocking agent, decreases the basal absorption rate of the jejunum. In the present study, YO singularly demonstrated an anti-absorptive action of ethanol. The value of Ka in CY with YO did not significantly decrease compared to YO alone, but additional inhibition of EtOH absorption was observed in the CY+YO group, compared to the CY group. AcH activity may therefore be masked by the action of YO, owing to its pharmacological effects and being potent under our experimental conditions.

The α_2-adrenergic binding sites have been identified in the dorsal motor nucleus of the vagus and in the nucleus tractus solitarii, associated with gastrointestinal efferent and afferent fibers, respectively (18). YO can easily enter the central nervous system (19) and may act directly with both peripheral α_2-adrenergic receptors on enterocytes and central α_2-adrenergic receptors leading to decreased intestinal absorption (17). Inhibition of intestinal EtOH absorption, due to YO, may be mediated through vagus nerve stimulation.

The PrCHO, an aliphatic aldehydes compound similar to AcH, suppresses intestinal absorption of PrOH. In low concentration of PrCHO, it has sympathomimetic properties similar to AcH (2, 20-22). It also has a pressor effects and releases catecholamines from the adrenal medulla and other tissues (20, 21, 23, 24). In high dose (40mg/kg of PrCHO or AcH) administration, the vagal stimulatory component may overpower the sympathomimetic action and it is diminished by atropine administration or abolished by vagotomy (25). This also supports the concept that AcH action may be due to vagal stimulation since the intestinal action of AcH is abolished by atropine pretreatment (11).

It has been reported that ENS, the nerves of mucosal surfaces, may play an important role with the pathophysiology of the intestinal tract and that ENS may contain adrenergic, cholinergic and non-adrenergic non-cholinergic neurons (14). ENS participates in the control of fluid and electrolyte movement over the intestinal mucosa and hence may be influenced by two common final pathways such as the cholinergic and non-cholinergic pathways (14). In our previous report, accumulation of AcH in blood stimulates cholinergic neurons (11), but effects on the ganglionic nicotinic receptor have not investigated yet. EtOH absorption was not affected by pretreatment with C_6, an antagonist of the nicotinic ganglionic receptor, as reported previously (26). The administration of C_6 with CY also did not prevent the decrease in EtOH absorption, suggesting that the nicotinic receptor does not mediate AcH effects.

In conclusion, these observations indicate that a high AcH concentration in blood may stimulate the vagus nerve and both nicotinic-receptors and α_2-adrenoceptors have little effect. Hence these data indirectly support the hypothesis that cholinergic nerves mediate AcH action in the gastrointestinal tract, via vagus nerve stimulation.

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