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Antagonism of Ethanol-Evoked Responses by Amantadine: A Possible Clinical Application

F. S. MESSIHA

Department of Pathology and Psychopharmacology Laboratory, Department of Psychiatry
Texas Tech University School of Medicine, Lubbock, TX 79409

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MESSIHA, F. S. *Antagonism of ethanol-evoked responses by amantadine: a possible clinical application.* PHARMAC. BIOCHEM. BEHAV. 8(5) 573–577, 1978. – The behavioral and biochemical effects of amantadine hydrochloride (ATD) on some ethanol (ETOH) mediated responses in rats and mice were studied. Administration of ATD, 0.5 mM/kg, IP, prior to a narcotic dose of ETOH significantly decreased the central depressant action of ETOH, as measured by the duration of ETOH-produced narcosis in mice. The time required for the onset of ETOH-narcosis was significantly delayed in ATD-treated mice compared to controls. Analyses of whole blood and brain ETOH concentrations showed that ATD-treatment prior to ETOH significantly reduced brain content of ETOH from saline-pretreated mice at the time of onset of ETOH narcosis as well as 30 min after ETOH injection without concomitant change in blood ETOH concentrations at the respective time intervals. Administration of ATD, 0.5 mM/kg, IP, reduced voluntary intake of ETOH by rats voluntarily selecting 5% ETOH solution over water as the drinking fluid. There were no changes in cytoplasmic rat liver alcohol dehydrogenase (L–ADH) and mitochondrial aldehyde dehydrogenase (L–ALDH) activities in rats maintained on water or 5% ETOH as the drinking fluid and administered ATD, 0.5 mM/kg, IP, once or identical dose once daily for six consecutive days. However, ATD produced in vitro non-competitive inhibition of both L–ADH and L–ALDH at a concentration range between 10^{-3} M and 3×10^{-3} M assay mixture. The results indicate the potency of ATD in negating ETOH-mediated responses measured and suggest for a possible clinical trial for ATD in the management of alcoholic patients provided it is devoid of disulfiram-like reaction in man.

Alcohol-dehydrogenase Aldehyde-dehydrogenase Amantadine-hydrochloride Brain ethanol concentration Ethanol-narcosis Voluntary ethanol drinking

THE USE of rationale approach to the development of pharmacotherapeutic agents often leads to logical consequences resulting in their application in the management of symptoms other than those they were initially developed for. Amantadine hydrochloride (ATD) has been introduced as an antiviral prophylactic agent for the treatment of Asia influenza Type A_2 [7,23]. Subsequently, it has been shown in 1968 that ATD possesses antiparkinsonian activity when administered alone or concurrently with levodopa in the management of Parkinson's disease [19,20]. The implications of dopaminergic of mechanism in the pharmacologic actions of ATD [8, 10, 22] and of ethyl alcohol (ETOH) coupled with the CNS stimulating property of ATD [2,14] as contrasted with the central depressant action of ETOH provided the rationale for the present study. This report describes the effects of ATD on certain ETOH-evoked responses in rodents and evaluate ATD–ETOH interactions with the enzymes primarily involved in the metabolism of ETOH and its metabolite acetaldehyde.

MATERIALS AND METHOD

Male Sprague-Dawley rats, weighing 329–380 g and HA/ICR male mice weighing 30–35 g were obtained from Holzman Farm and Sprague-Dawley (Madison, WI), respectively. Purina chow pellet food and water were available ad lib unless otherwise indicated. Housing and behavioral experiments were performed in the same room maintained at 22°–24°C with regulated 12 hr light and dark cycles.

Studies on ETOH–Narcosis

Mice used for the ETOH-narcosis experiments were caged in groups of six for at least three weeks prior to testing and were fasted for 22–24 hr before the experiments. Mice were administered saline or ATD 0.5 mM/kg of body weight 30 min before a narcotic dose of 25% ETOH, 5.0 g/kg. ETOH solution was administered intraperitoneally (IP) and prepared from 95% ETOH by dilution with saline to 25% concentration (w/w). Volumes of solution injected did not exceed 0.33 ml to minimize experimental variables [15]. The onset of narcosis was considered as the duration of time (sec) between the injection of ETOH and the loss of the righting reflex. The time (min) from the loss to the regaining of the righting reflex was recorded as the duration of ETOH-narcosis provided the animal righted itself at least twice within a 2 min period. In a separate series of experiments, groups of mice, 6 per group, were sacrificed by decapitation im-

mediately after the onset of narcosis, 5, 30, 45 or 90 min of ETOH injection. Whole blood specimens were collected over anticoagulant and the brains of the animals were quickly removed, washed with 0.1 M phosphate buffer pH 6.8, plotted dry and weighed. The brains were individually homogenized in ice-cold 0.4 M $HC10_4$ perchloric acid and centrifuged at 5,000 × g for 15 min. Blood samples were deproteinized with 6% trichloroacetic acid and similarly centrifuged. Aliquots from the supernatant fluids were assayed for both blood and brain content of ETOH [5]. The results of ETOH concentrations are expressed as mg/L for blood and as mg/kg of wet brain tissue.

Studies on Voluntary Drinking of ETOH Solution by the Rat

The rats were housed individually and were offered 5% (w/w) ETOH solution as the only drinking fluid for a two consecutive week habitation period. Then each home cage was supplied with three drinking bottles, one containing the 5% ETOH solution, the other distilled water and a third empty bottle. Their positions were changed once daily to avoid position preference. The amounts of water and ETOH intake were determined by weight, once daily at 10:30 a.m. Animals who established preference for ETOH drinking over water were used to test the effect of ATD on their voluntary drinking of ETOH. ATD was dissolved in saline and injected 0.5 mM/kg, IP.

Studies on Rat Liver Alcohol–(L–ADH) and Aldehyde Dehydrogenase (L–ALDH)

In a separate set of experiments, rats were given water or 5% ETOH solution as the sole drinking fluid for four weeks prior to the administration of saline or ATD, 0.5 mM/kg, IP, and their subsequent decapitation 16 hr after drug injection. Their livers were removed and rinsed with 0.1 KCl solution and homogenized with the ice-cold KCl solution by Waring blender. These homogenates were subjected to differential centrifugation to obtain the mitochondrial and cytoplasmic fractions required for the enzymatic assays of ADH and ALDH, respectively. Liver homogenates were centrifuged at 600 × g for 20 min and the resulting supernatants were recentrifuged at 4,500 × g for 20 min to obtain the mitochondrial pellet. These were resuspended in the KCl solution by means of motor-driven teflon-glass homogenizer and recentrifuged at 4,500 × g. This mitochrondrial washing procedure was repeated twice prior to solubilization of the mitochondrial pellet by 2% sodium desoxycholate solution to obtain the mitochondrial preparations. The 4,500 × g supernatants were recentrifuged for 90 min at 22,000 × g to obtain the cytoplasmic supernatant fluids. The analytical procedures for ADH and ALDH were made according to the methods of Blair and Vallee [3] and Blair and Bodley [4], respectively. The enzymes reaction was measured at pH 9.6 and the biuret procedure was used for the determinations of the protein content of the liver fractions. The results are expressed as specific activity nM/min/mg protein measured at 30°C. Line-weaver and Burk plots were performed to determine the type of inhibition and the kinetic data of the enzymes studied. The results are given for mean ± SE and the data were evaluated statistically by student *t* test for independent means.

RESULTS

Figure 1 shows the effect of ATD on the central depressant action of ETOH, as measured by ETOH-narcosis test. Administration of ATD 0.5 mM/kg, IP, 30 min prior to narcotic dose of ETOH, 5 g/kg, significantly ($p<0.05$) delayed the onset of the central depressant action of ETOH to 126.1 ± 10.3 sec from 98 ± 5.8 sec of the controls (left panel). Preadministration of ATD shortened ETOH-narcosis to 40.2 ± 2.8 min from 62.7 ± 5.5 min of controls (right panel). This difference was statistically significant ($p<0.005$).

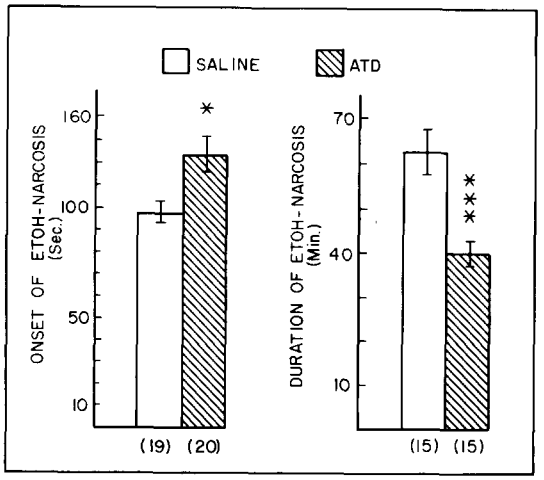

FIG. 1. Effect of Amantadine hydrochloride (ATD) on ethanol (ETOH) narcosis in mice. Left panel shows the time required for onset of ETOH-produced narcosis expressed as mean ± in seconds. Right panel shows the duration of ETOH–mediated narcosis given as mean ± SE in minutes. The controls were injected with saline and the treatment group with ATD 0.5 mM/kg, IP, 30 min prior to the administration of a narcotic dose of 25% ETOH solution, 5 g/kg, IP.

Figure 2 shows the whole blood and brain content of exogenously administered ETOH at the time of onset of narcosis, i.e., the loss of righting reflex (OST), 5, 30, 45 and 90 min after ETOH injection. ATD-treated mice showed no difference in their ETOH blood concentrations from controls. There was a concomitant moderate decrease of 16.5% ($p<0.05$) and 12% reduction ($p<0.02$) in the whole brain content of ETOH in ATD-treated mice at OST and 30 min intervals compared to controls.

Figure 3 shows the in vitro effects of ATD, in molar concentrations/assay mixture, on specific activities of rat liver ADH (upper panel) and ALDH (lower panel). The dose-enzyme response relationship indicates that the mitochondrial L–ALDH was more sensitive to ATD-produced inhibition than the cytoplasmic ADH. At a concentration of 10^{-2} M ATD inhibited L–ADH and L–ALDH by approximately 31% ($p<0.02$) and 55% ($p<0.001$) from controls, respectively. ATD at 3×10^{-3} M assay mixture exerted a significant inhibition only on L–ALDH. The Line-weaver and Burk plots of the in vitro specific activities of rat liver ADH and ALDH in the presence of and absence of ATD are shown in Figs. 4 and 5, respectively. The type of ATD-produced inhibition on both enzymes was of the non-competitive type with a V max 6.0 and Km 9 μM

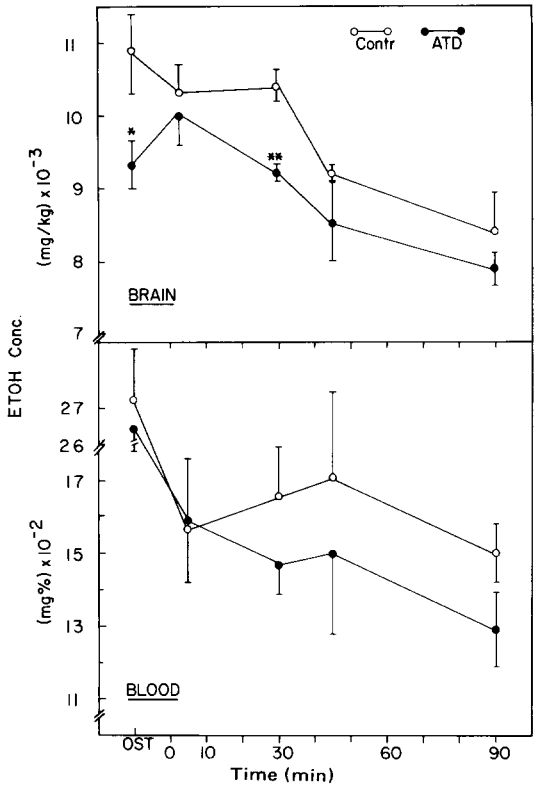

FIG. 2. Effect of amantadine hydrochloride (ATD) on whole blood (lower panel), and brain (upper panel) content of exogenously administered narcotic dose of ethanol (ETOH), 5 g/kg, IP. Injections of saline o——o or ATD, 0.5 mM/kg, IP, were given 30 min prior to administration of ETOH. Mice were sacrificed, in groups of 6, at onset of ETOH–narcosis (OST) at 5, 30, 45 and 90 min post ETOH injection. The mean ± SE of ETOH concentrations expressed as mg/L and mg/kg wet tissue of whole blood and brain, respectively. $^{**}p<0.02$, $^{*}p<0.05$.

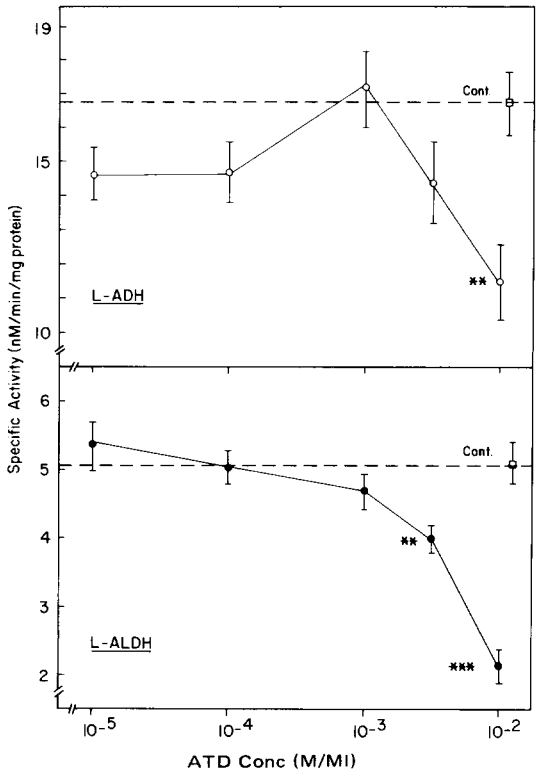

FIG. 3. The in vitro dose response effects of amantadine hydrochloride (ATD) on specific activities (nM/min/mg protein) of rat liver alcohol dehydrogenase (L–ADH) upper panel and mitochondrial liver aldehyde dehydrogenase (L–ALDH), lower panel.

values for L–ADH compared to a V max 4.0 and Km 170 μM for L–ALDH occurring by 2×10^{-3} M concentration of ATD.

Figure 6 shows the effect of a single dose administration of ATD, 0.5 mM/kg, IP on voluntary intake of ETOH solution by rat. There was a marked and significant ($p<0.02$) reduction in ETOH intake for the 24 hr period following ATD injection.

The results of the in vivo effects of acute, single dose of ATD 0.5 mM/kg, or semi-chronic injection of ATD, 0.5 mM/kg, IP, once daily for six consecutive days, on specific activities of cytoplasmic rat L–ADH and mitochondrial L–ALDH are listed in Table 1. Rats were maintained on water or 5% ETOH solution for 21 days ad lib and were sacrificed 15–16 hr after last dose injection of saline or ATD. Both acute and semi-chronic administration of ATD produced little changes in specific activities of both enzymes in rats maintained on water or 5% ETOH solution as the drinking fluid. The slight decrease in specific activity of L–ADH from controls in acutely ATD–treated rats maintained on water was not statistically significant ($p<0.1$) nor were the changes in L–ALDH activity of semi-chronically ATD-treated rats ($p<0.1$).

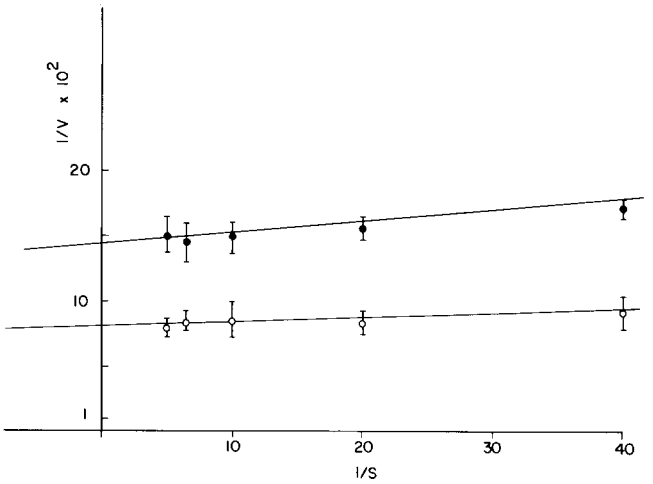

FIG. 4. Lineweaver-Burk plot(s) of rat liver alcohol dehydrogenase (ADH) activity at various ethanol concentrations with •——• and without o——o the addition of amantadine hydrochloride (ATD), at final concentration of 2×10^{-3} M. Each point represents the mean ± SE of values from at least six independent determinations.

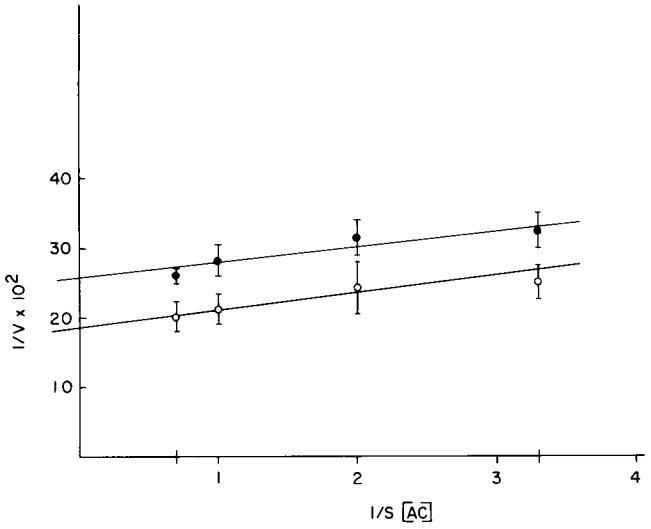

FIG. 5. Lineweaver-Burk plots of rat liver alcohol dehydrogenase (ALDH) activity at various ethanol concentrations with ●——● and without ○——○ the addition of amantadine hydrochloride (ATD), at final concentration of 2×10^{-3} M. Each point represents the mean ± SE of values from at least six independent determinations.

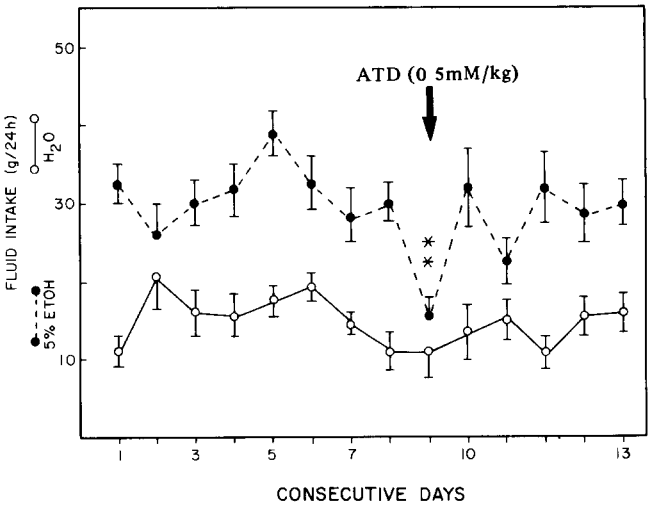

FIG. 6. Effect of amantadine hydrochloride (ATD) on voluntary intake of ethanol (ETOH) solution by the rat. Values are for mean ± SE of six rats. Arrow indicates time of injection of ATD 0.5 mM/kg, IP.

DISCUSSION

In the present study, the injection of ATD before a narcotic dose of ETOH decreased the central depressant action of ETOH as evident by the significant decrease in the duration of ETOH-produced narcosis in mice. This effect is unlikely due to interference of ETOH absorption by ATD because the noted delay in the onset of ETOH-mediated sleep, as a function of pretreatment with ATD, was not associated with decreased blood ETOH concentration compared to controls. Moreover, administration of ATD prior to ETOH resulted in a moderate but significant decrease in the amounts of ETOH penetrating the brain from the corresponding controls. These results suggest a central antagonistic action of ATD on the hypnotic effects of ETOH and a possible interference of ATD in the penetration of ETOH into the brain possibly by altering the blood barrier permeability to ETOH. ATD has a pk of 9.0 and therefore exists as an ammonium ion at physiological PH. It is known that ATD, like certain

TABLE 1

THE *IN VIVO* EFFECTS OF AMANTADINE HYDROCHLORIDE (ATD) ON SPECIFIC ACTIVITIES OF RAT LIVER ALCOHOL — (L-ADH) AND ALDEHYDE DEHYDROGENASE (ALDH)

Drinking Fluid	ATD Treatment		(n)	L-ADH (nM/min/mg protein)	L-ALDH (nM/min/mg protein)
Water	Acute	Contr.	(14)	15.6 ± 0.5	4.5 ± 0.3
		ATD	(14)	14.3 ± 0.7	6.3 ± 0.2*
	Semi-chronic	Contr.	(5)	26.5 ± 0.8	8.7 ± 0.6
		ATD	(5)	24.5 ± 1.6	7.7 ± 0.7
5% ETOH (21 days)	Acute	Contr.	(4)	20.8 ± 1.9	5.6 ± 1.0
		ATD	(4)	19.7 ± 1.7	4.9 ± 0.7
	Semi-chronic	Contr.	(6)	11.6 ± 1.8	5.5 ± 0.8
		ATD	(6)	13.5 ± 1.6	4.9 ± 0.6

ATD was administered once 0.5mM/kg, ip, or 0.5mM/kg/day for six consecutive days for the acute and semi-chronic treatment, respectively. The control animals received saline injection at the coresponding time intervals.

Values are for mean ± SE for the number of animals shown (n).

*$p<0.1$.

ammonium salts, prevents the penetration into cells of several RNA virus, a mechanism which has been ascribed to its antiviral action. On the other hand, ATD has been shown [19,20] to possess antiparkinsonian properties. The mechanism of action of ATD in parkinsonism remains controversial and might be ascribed to its stimulation and/or its interaction with the dopaminergic systems in the peripheral and central nervous system and possibly by counteracting parkinsonian-related extrapyramidal inhibition [8–12, 21, 22]. Therefore, it is conceivable that ATD antagonism to ETOH-mediated responses and to morphine-elicited actions [13] might be due to ATD interference both in the biochemical mechanism underlying the pharmacologically evoked responses of these addictive agents in addition to the possible interference in cellular membrane permeability.

In another behavioral test, ATD injection reduced the voluntary intake of ETOH by rats preferring ETOH solution over water without a concomitant change in water intake. This ATD-produced effect on voluntary ETOH drinking is not due to inhibition of the L–ADH or L–ALDH as evident by the lack of inhibition of these enzymes in vivo studies when ATD was injected acutely or semi-chronically to rats drinking water or 5% ETOH solution. The results of the present study shows, however, an in vitro non-competitive inhibition of L–ADH and L–ALDH by a non-physiological concentrations of ATD which is unlikely to occur in vivo. It seems, therefore, that the in vitro inhibition of these liver enzymes by ATD may be due to a nonspecific protein binding. However, it is conceivable that chronic administration of ATD, i.e., in Parkinson's disease, may result in a slower build up of the drug. If this occurs in vivo, then it is conceivable that continued administration of ATD with l–DOPA, i.e., in parkinsonian patients undergoing such therapy, may enhance the formation of tetrahydorisoquinolines [17], a condensation product of dopa-derived dopamine with its unreacted aldehyde metabolite, which may act as false transmitters [6] and may provide a biochemical explanation [16] of the persistant l–DOPA produced side effects with this type of drug combination. In conclusion, the modification of ETOH-mediated responses by ATD and the non-toxic effects of ATD when given during ETOH intake [1] suggests a clinical trial of ATD in alcohol detoxification and/or its evaluation as a prophylactic agent in the management of alcohol abuse.

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