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Arginine-8-Vasopressin Potentiates Acute Ethanol Intoxication

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WU, P. H., J-F. LIU, S. J. MIHIC AND H. KALANT. *Arginine-8-vasopressin potentiates acute ethanol intoxication.* PHARMACOL BIOCHEM BEHAV **41**(2) 409–414, 1992. – AVP maintains ethanol (EtOH) tolerance after cessation of chronic EtOH treatment. However, the acute interaction of AVP and EtOH has not been well characterized. Rats were trained on a moving belt and the EtOH dose-response relationship (range 1.0–2.0 g/kg) was determined after pretreatment with saline, AVP (2.5–40 μg SC or 10 ng ICV), the AVP-V_1 receptor antagonist [Des-Gly^9,d(CH$_2$)$_5^1$,O-Et-Tyr^2,Val^4,Arg^8]-vasopressin (10 ng ICV), or AVP in combination with the V_1 antagonist. AVP produced a 16% decrease in the EtOH ED_{50} when given either SC or ICV; this decrease, which appears to represent true potentiation rather than additivity, was prevented by the preadministration of the V_1 antagonist. Other rats were made EtOH-tolerant by 7 daily injections of either EtOH alone (1.8 g/kg IP) or EtOH (1.5 g/kg IP) + AVP (10 μg SC), followed by a practice session on the moving belt. In both sets of tolerant animals, AVP potentiation of acute EtOH effects was still seen on day 6. The mechanism of AVP potentiation of EtOH-induced impairment is unknown, but the failure of the V_1 antagonist alone to alter the effect of EtOH suggests that endogenous AVP is not involved directly in modulating EtOH intoxication.

Ethanol Arginine vasopressin [Des-Gly^9,d(CH$_2$)$_5^1$,O-Et-Tyr^2,Val^4,Arg^8]-vasopressin Moving belt Tolerance Rats Acute Chronic Subcutaneous Intracerebroventricular

THERE is strong evidence implicating the neurohypophyseal hormone, arginine-8-vasopressin (AVP), as a neurotransmitter or neuromodulator in the central nervous system (CNS) (8). AVP has also been shown to influence behavior. For example, de Wied (6) showed that pitressin, a crude extract of posterior pituitary tissue, decreases the rate of extinction of an avoidance response in neurohypophysectomized rats, by an action unrelated to the effects of AVP on water metabolism (4). These behavioral effects of AVP have led to the suggestion that it affects memory (21,29).

AVP is able to maintain tolerance to the hypothermic and hypnotic effects of ethanol in mice (13–15) and the motor-impairing effects of ethanol in rats (22,27) after the cessation of chronic ethanol administration. Recently, Szabo et al. (29) reported that AVP maintenance of tolerance to ethanol-induced loss of righting reflex (sleep time) was mediated specifically by vasopressin V_1 receptors: a specific V_1 agonist was more potent than AVP in maintaining tolerance, and a V_1 antagonist facilitated the loss of tolerance. The mechanism by which AVP maintains tolerance remains largely unclear, but intact cerebral catecholaminergic (13) and mesolimbic serotonergic (22,27) systems appear to be required.

In an ongoing study of AVP maintenance of ethanol tolerance, we noticed that AVP-pretreated animals seemed more intoxicated than saline-pretreated animals after ethanol administration. This may suggest a functional interaction between these substances. However, very little is known about the acute interaction between AVP and ethanol. In an earlier study, Hoffman et al. (14) observed that both AVP and cyclo(Leu-Gly)oxytocin, given before an acute dose of ethanol, increased the duration of loss of righting reflex. In the present report, we demonstrate that AVP markedly enhances ethanol-induced motor impairment. We also show that intracerebroventricularly injected AVP has the same effects as those seen after subcutaneous (SC) injection, and that the AVP enhancement of the ethanol effect can be abolished by the prior administration of an AVP-V_1 antagonist.

METHOD

Animals and Training

Male Sprague-Dawley rats weighing approximately 175 g were purchased from Charles-River Laboratories (Montréal, Québec). They were individually housed and fed standard rat chow and water ad lib. The vivarium was kept at 20 ± 1°C, with lights on from 7:00 a.m. to 7:00 p.m. daily. All animals were trained daily on a moving belt apparatus (10,20) until they reached criterion performance (less than 1.2 s off the belt in a 2-min testing period); this was usually achieved in 14–20 days.

Acute Interaction Studies

When the performance of the rats on the moving belt reached criterion, 30 of these rats were divided into five matched groups

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of 6 animals each and used in ethanol dose-response studies. On the first day of dose-response testing, all groups were given saline (0.3 ml, SC) 1 h before the administration of ethanol (1.2, 1.5, 1.8, 2.0 and 2.2 g/kg, IP) as a 9% w/v solution of ethanol in saline. The animals were tested on the moving belt in three trials starting at 2.5, 7.5 and 12.5 min after ethanol injection and the maximal time off belt for each rat in the three trials was used as its measure of alcohol-induced impairment. Seven days later, the same groups of rats were given a SC injection of 10 μg AVP (Sigma Chemical, St. Louis, MO) in 0.3 ml saline. One h after AVP injection, the rats received an IP injection of ethanol (1.0, 1.2, 1.5, 1.8, and 2.0 g/kg) and were then tested on the moving belt 2.5, 7.5 and 12.5 min after ethanol administration. Blood (50 μl) was taken from the tip of the tail for blood alcohol level (BAL) measurement immediately after the completion of the 12.5-min moving belt test (15 min after ethanol injection).

A separate experiment was carried out to clarify further the nature of the acute interaction by testing the effects of different doses of AVP on the response to the same dose of ethanol. Fourteen trained rats were divided into three groups (n = 4, 5 and 5) that were injected SC with AVP in doses of 2.5, 10 and 40 μg respectively. One hour later, each rat received a dose of 5.0 ml saline IP, followed by three tests on the moving belt apparatus beginning at 2.5, 7.5 and 12.5 min postinjection. Three days later, the same rats received the same doses of AVP, but were given 1.5 g/kg ethanol IP (in a total volume of 5.0 ml) in place of the saline, and were again tested on the moving belt as above.

Time Course of Acute Interaction

Five groups of 6 rats each were all given AVP (10 μg in 0.3 ml/rat, SC), and 3 groups of 6 animals each were given saline (0.3 ml/rat, SC). Ethanol (1.8 g/kg IP) was then administered 0, 0.5, 1, 2 or 4 h after AVP (one group for each time), and 0, 1 and 4 h after saline. Performance of each rat on the moving belt was measured in trials starting at 2.5, 7.5 and 12.5 min after ethanol injection. The maximal time off belt for each rat in the three trials was used as its measure of alcohol-induced impairment.

Intracerebroventricular (ICV) Injection of AVP

Rats were trained on the moving belt until they reached criterion performance. Each animal received ethanol (1.8 g/kg IP), and its motor performance was assessed on the moving belt 2.5, 7.5 and 12.5 min after ethanol injection. One week later, the rats were anaesthetized with pentobarbital (50 mg/kg IP) and an intracerebroventricular cannula (Plastic Products Co., Roanoke, VA) was implanted in the right lateral ventricle, using stereotaxic coordinates provided by Paxinos and Watson (25). Rats were allowed to recover from surgery for two weeks; during this period, the cannulae were flushed with 5 μl of saline every second day to avoid blockage. The rats were tested for their motor performance on the moving belt as described above, before and after an ICV injection of saline (3 μl) followed by ethanol (1.8 g/kg IP). They were left in the vivarium for two weeks to recover from all treatments. The animals were then randomly assigned to separate groups that were given saline, AVP alone (10 ng in 3 μl saline), the V_1 antagonist [Des-Gly^9,d(CH$_2$)$_5$^{1},O-Et-D-Tyr^2,Val^4,Arg^8]-vasopressin alone (10 ng in 3 μl saline), or the V_1 antagonist plus AVP, all followed 30 min later by ethanol (1.2, 1.5, 1.8, and 2 g/kg IP). Motor performance on the moving belt was measured as described above.

To study the time course of AVP effect on ethanol-induced motor impairment, 5 groups of trained rats (6 per group) were given ICV injection of saline (3 μl) or AVP (10 ng in 3 μl) followed immediately, 30, 60 and 120 min later by an IP injection of ethanol (1.8 g/kg). The ethanol-induced motor incoordination was measured according to the method described above.

Chronic Interaction Studies

Rats (n = 48) were tested for the acute interaction of saline (SC)-ethanol and, one week later, for the effects of AVP (10 μg, SC)-ethanol, using a procedure identical to that described in the acute interaction studies. These rats were then divided into two matched groups of 24 rats each, and treated for 7 consecutive days with SC injections of either saline or AVP (10 μg/rat) in saline, followed 1 hour later by ethanol (IP) at a dose which produced 50% impairment (ED_{50}) in the acute interaction study. The acute ED_{50} for the group pretreated with AVP in the initial experiment was 1.5 g/kg, compared to 1.8 g/kg in the group pretreated with saline. Both groups of animals were treated and tested daily on the moving belt at 2.5, 7.5 and 12.5 min after administration of their respective doses of ethanol; i.e., the experiment employed an intoxicated-practice paradigm (23).

Ethanol dose-response curves showing impairment on the moving belt task were generated on the eighth day. To accomplish this, subgroups (6 animals each) of the two treatment groups received pretreatment with saline, followed 1 h later by ethanol (1.5, 1.8, 2.0, or 2.2 g/kg, IP). Impairment tests on the moving belt were carried out 2.5, 7.5 and 12.5 min after the ethanol injection. On the next day (the ninth day of treatment), the same rats were given a SC injection of AVP (10 μg/rat), followed 1 h later by ethanol (1.5, 1.8, 2.0, or 2.2 g/kg, IP). Impairment was again tested on the moving belt using the the same procedure. A 50 μl blood sample was then taken from the tail-tip of each rat at the completion of the final test on the moving belt.

Blood Alcohol Measurement

Blood alcohol levels (BAL) were measured using the method of Hawkins et al. (12). Briefly, 50 μl of venous blood was collected from the tip of the tail and mixed with 10 ml of 0.45% $ZnSO_4$–0.1 N NaOH solution. Protein was removed by centrifugation, and 2 ml of the resultant supernatant was then used for analysis of ethanol concentration by the alcohol dehydrogenase method.

RESULTS

Acute Interaction Studies

An acute dose of AVP (10 μg, SC) or saline did not have any effect on moving belt performance tested either 10 min or 1 h later. Ethanol administration produced a dose-dependent increase in maximal impairment, $F(3,40) = 44.48$, $p = 0.000$. The motor impairment scores after ethanol were significantly higher in rats pretreated with AVP than in those pretreated with saline, $F(1,40) = 11.49$, $p < 0.002$; Fig. 1A. The ethanol ED_{50} values were 1.5 g/kg for the AVP-pretreated and 1.8 g/kg for the saline-pretreated groups. As expected, the BALs measured immediately after the last behavioral test (15 min after ethanol administration) were dependent on the dose of ethanol administered, $F(3,40) = 16.40$, $p = 0.000$. The average BAL was slightly, but not significantly, $F(1,40) = 2.499$, $p > 0.120$, lower in the AVP-pretreated group (Fig. 1B).

The SC injection of AVP in doses of 2.5 to 40 μg, followed by IP saline, did not produce any impairment on the moving belt test (Fig. 2). The same doses of AVP combined with 1.5 g/kg

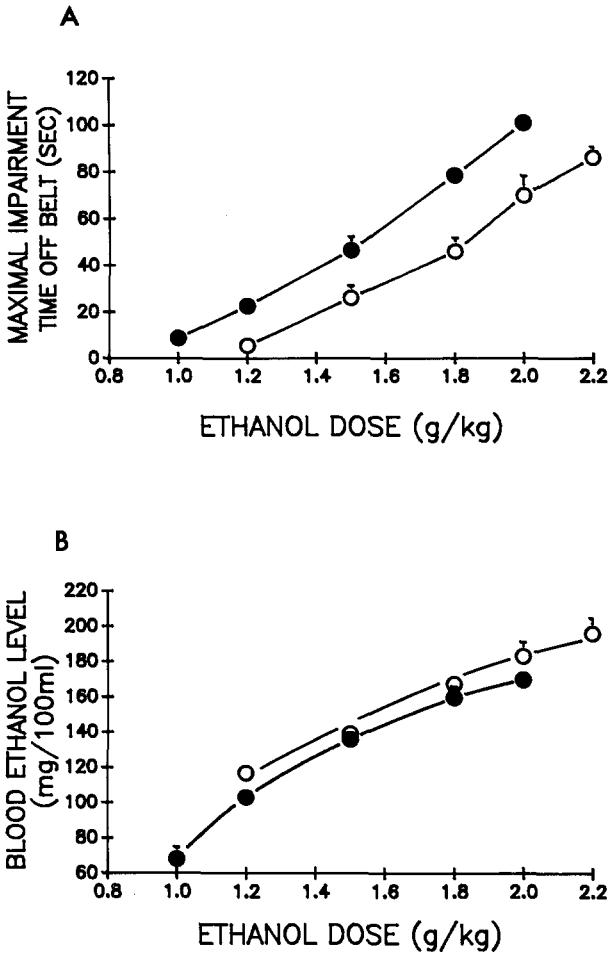

FIG. 1. Effects of subcutaneously injected AVP on ethanol-induced motor impairment. (A) Rats were pretreated with saline (○) or AVP (●) 1 h before an IP injection of ethanol. The motor impairment was measured, and dose-response curves were constructed. Results are expressed as mean ± S.E.M. of 6 rats per treatment condition and dose. (B) At the end of motor impairment measurement, blood was taken from the tip of the tail, and blood alcohol levels were determined. AVP (●) pretreatment did not significantly affect blood alcohol levels compared to those in saline (○)-pretreated animals. Results are expressed as mean ± S.E.M. of 6 rats per treatment condition.

ethanol caused a dose-dependent increase in the ethanol-induced impairment, $F(2,13) = 7.79$, $p<0.0078$. The lowest dose of AVP did not increase the ethanol effect, whereas 10 μg produced an almost maximal increase; Fisher's LSD test for a post hoc comparison of the effects of 10 and 40 μg of AVP showed no significant difference at 0.05 level. AVP (10 ng in 3 μl/rat), injected intracerebroventricularly 30 min before the administration of ethanol, also potentiated the effect of ethanol on motor incoordination, $F(1,40) = 10.78$, $p<0.002$. The ED_{50} value in the saline-ethanol-treated rats was 1.88 g/kg while it was 1.7 g/kg in the AVP (ICV)-ethanol-treated rats (Fig. 3). Intracerebroventricular injection of AVP, the V_1 antagonist or saline did not produce any motor impairment on their own or in combination, and pretreatment with the V_1-antagonist alone did not attenuate ethanol-induced motor incoordination. However, the V_1-antagonist, injected ICV 30 min before the ICV injection of

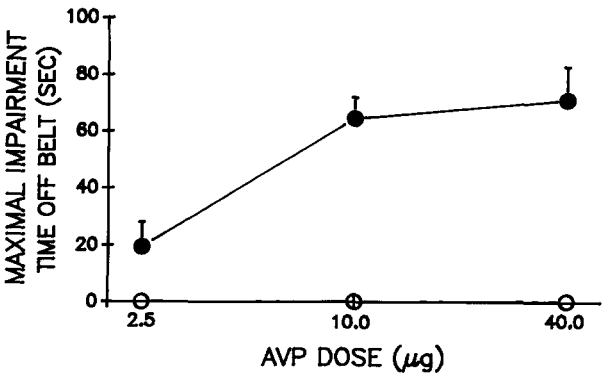

FIG. 2. Effects of different doses of AVP on ethanol-induced motor impairment. Fourteen trained rats were divided into three groups (n = 4, 5 and 5) and were given AVP (2.5, 10 or 40 μg, SC) 1 h before the IP injection of saline or ethanol (1.5 g/kg). AVP plus saline (○) did not produce any motor impairment. In contrast, AVP produced a dose-dependent potentiation of ethanol-induced motor impairment (●). Results are expressed as mean ± S.E.M.

AVP, blocked the AVP enhancement of the ethanol effect, $F(1,40) = 9.64$, $p<0.003$ (see Fig. 3).

Time Course of Acute AVP-Ethanol Interaction

The acute interaction between AVP and ethanol occurs very rapidly, since it was even seen within the 12.5-min test period following an ethanol injection that was given immediately after AVP (SC). Potentiation of the ethanol effect by AVP, $F(1,45) = 16.21$, $p = 0.000$, was greatest when ethanol was given 2 h after AVP, but was no longer evident when ethanol was given 4 h after AVP (Fig. 4A). Saline pretreatment did not have any effects on ethanol-induced motor impairment at any time points tested. AVP enhancement of the ethanol effect occurred immediately after an ICV injection of 100 ng of AVP in 3 μl of saline, $F(1,21) = 10.24$, $p<0.004$. The peak effect of an ICV AVP injection was seen 30 min later and it disappeared within 1 h (Fig. 4B).

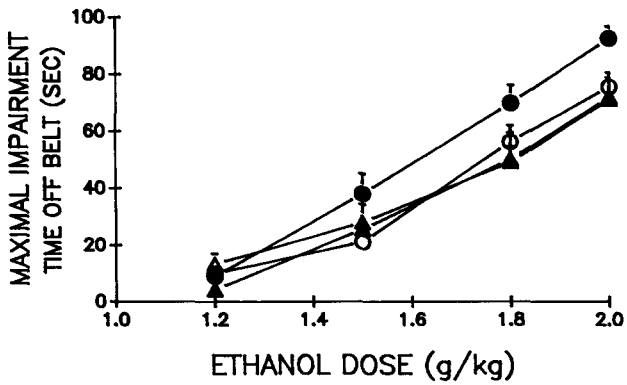

FIG. 3. Intracerebroventricular injection of AVP potentiates ethanol-induced motor impairment. Saline (○), AVP plus V_1 receptor antagonist (▲), AVP alone (●) or V_1 antagonist alone (△) was injected intracerebroventricularly 30 min before IP administration of ethanol. Ethanol-induced motor impairment was measured on the moving belt as described in the text. Results are expressed as mean ± S.E.M. of 6 animals per treatment condition and dose.

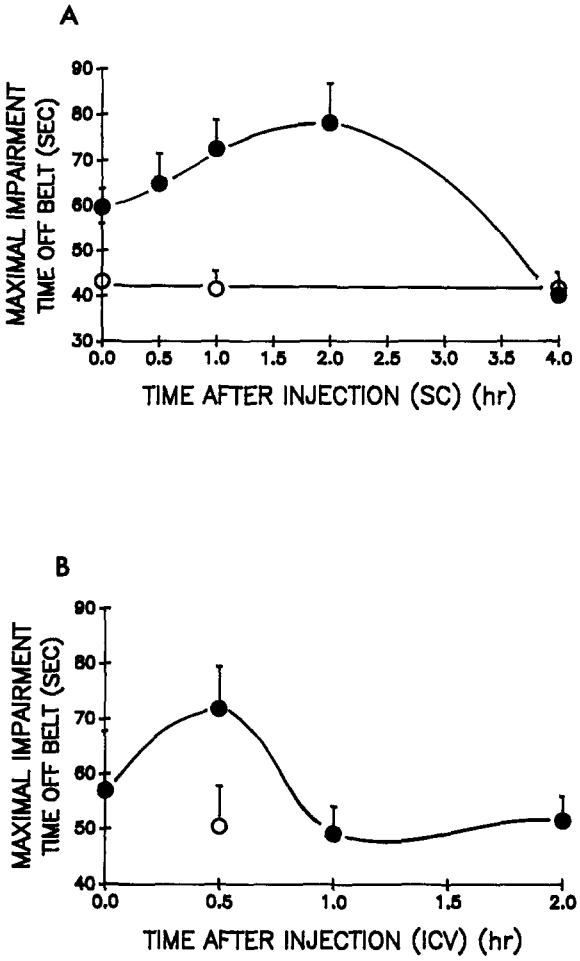

FIG. 4. Time course of the AVP-ethanol interaction. (A) Rats were pretreated with AVP (10 μg SC ●) or saline (○), and ethanol was administered to both groups at different times after the pretreatment. Results are expressed as mean ± S.E.M. of 6 animals per treatment condition. (B) Saline (○) or AVP (10 ng, ●) was given to the rats by ICV injection. Ethanol (IP) was administered at different times following saline or AVP pretreatment. Results are mean ± S.E.M of 6 animals per condition.

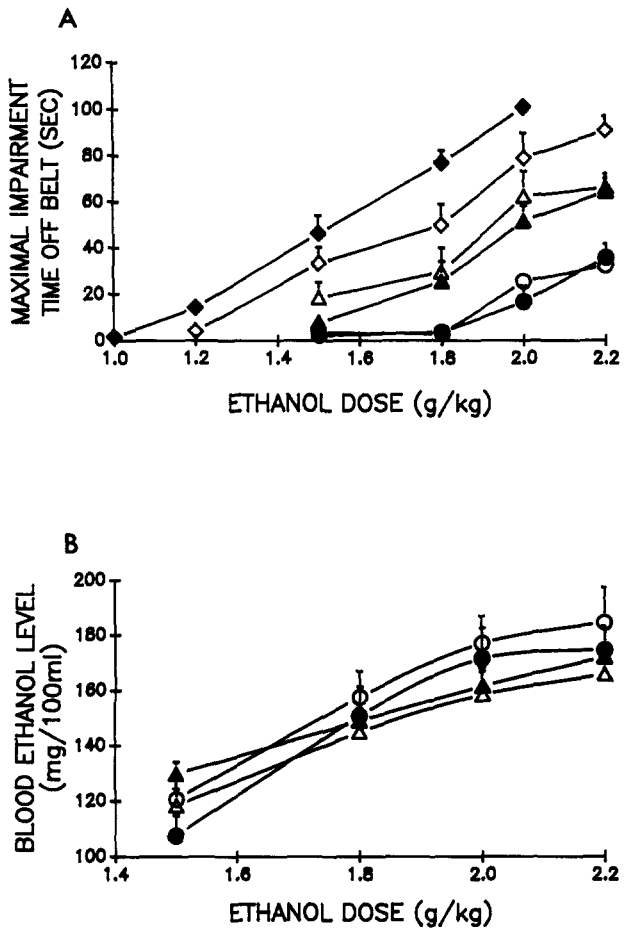

FIG. 5. AVP-ethanol interaction in rats made tolerant to ethanol. (A) Rats (n = 48) were tested with saline (SC)-ethanol (1.2, 1.5, 1.8, 2.0 and 2.2 g/kg IP ◇), and 7 days later with AVP (10 μg/rat SC)-ethanol (1.0, 1.2, 1.5, 1.8, and 2.0 g/kg IP ◆). These rats were divided into two matched groups with similar averaged ethanol impairment scores on the moving belt test. One group of the rats was treated daily with saline-ethanol (1.8 g/kg) and the other group with AVP (10 μg)-ethanol (1.5 g/kg) with intoxicated practice for 7 days. Tolerance to ethanol-induced motor impairment was tested with saline (SC)-ethanol (IP) on the 8th day in both groups of rats (after chronic treatment with saline-ethanol, ○, or AVP-ethanol, ●). On the 9th day, these two groups of rats were retested with AVP (SC)-ethanol (rats chronically treated with saline-ethanol and tested with AVP-ethanol are shown as △, and those chronically treated with AVP-ethanol and tested with AVP-ethanol are shown as ▲). Results are expressed as mean ± S.E.M. of 6 animals per ethanol dose, except that the acute interaction was done with 8 animals per ethanol dose. (B) At the end of motor impairment testing, 50 μl of blood was taken from the tip of the tail, and blood alcohol levels determined. Results are expressed as mean ± S.E.M. of 6 animals per ethanol dose.

Chronic AVP-Ethanol Interaction

Figure 5A shows a dose-dependent effect of ethanol on motor performance, $F(3,72) = 33.88$, $p = 0.000$, as well as an enhancement of this effect by AVP pretreatment, $F(1,72) = 57.29$, $p = 0.000$. After chronic treatment with either saline-ethanol (1.8 g/kg) or AVP-ethanol (1.5 g/kg), there was a shift to the right of the ethanol dose-response curves after saline pretreatment (of both groups) in the tests done on the 8th day of chronic ethanol treatment. The two groups showed almost identical dose-response curves, $F(1,72) = 1.34$, $p > 0.25$, for the motor-impairing effects of ethanol (Fig. 5A). BALs were slightly lower in the AVP-pretreated group than in the saline-pretreated group but the difference was not statistically significant, $F(1,36) = 3$, $p > 0.075$ (see Fig. 5B). Despite the fact that both groups showed tolerance to ethanol, AVP was still able to potentiate ethanol-induced motor impairment in all animals on the 9th day. AVP (10 μg/rat, SC) given 1 h before ethanol administration, to animals which acquired tolerance by daily treatment with either AVP-ethanol or saline-ethanol, produced the same degree of ethanol-induced motor impairment, $F(1,36) = 1.099$, $p > 0.3$ (Fig. 5A). Once again, the BALs were not significantly different in the AVP-ethanol and saline-ethanol groups that received the same doses of ethanol, $F(1,36) = 2.39$, $p > 0.12$ (Fig. 5B).

DISCUSSION

AVP has been shown to produce behavioral effects after peripheral or intracerebroventricular injection (1, 2, 5, 7). Although peripherally administered AVP affects blood pressure and

diuresis, the behavioral effects seen are independent of its endocrine actions (22, 27, 30). This is consistent with the finding by de Wied et al. (5) that DGAVP, an AVP analogue which is almost devoid of peripheral effects, still has the ability to modify behavior. One of the central effects of AVP is its ability to delay the extinction of acquired active and passive avoidance behaviors in normal rats (1,7). The impairment of avoidance learning seen in Brattleboro homozygous rats, which have low levels of endogenous AVP, was corrected by the subcutaneous administration of AVP or DGAVP immediately after an avoidance training session (3). This observation suggested that the exogenous AVP was able to restore memory function in the AVP-deficient rats and this notion was further strengthened by experiments showing retention deficits in rats treated with intracerebroventricular injections of AVP-antiserum (31,32).

Tolerance to ethanol and other psychoactive drugs shows many formal resemblances to learning and memory (16), and such forms of learning as intoxicated practice (11,17) and Pavlovian conditioning of stimuli from the drug-associated environment (9, 24, 26) have been shown to have a major impact on the rate of development and the magnitude of tolerance. In keeping with this link, AVP and DGAVP have been shown repeatedly to maintain ethanol tolerance after administration of ethanol is stopped, i.e., under conditions in which tolerance would otherwise extinguish (13–15, 22, 27). Despite this striking chronic interaction, the acute interaction of AVP with ethanol has scarcely been studied. Hoffman et al. (14) observed that a dose of AVP (10 μg/mouse) or of cyclo(Leu-Gly) oxytocin, an oxytocin derivative in which the C-terminal dipeptide has been cyclized, increased the duration of loss of righting reflex after acute ethanol administration, but only in the case of cyclo-(Leu-Gly) oxytocin was the effect statistically significant.

The moving belt test of motor incoordination offers an objective and sensitive method to measure lower degrees of ethanol intoxication (10,20) than the loss of righting reflex. We used this test to examine quantitatively the acute interaction between ethanol and AVP in the rat. AVP (SC), at a dose similar to that used by Hoffman et al. (14), potentiated the acute ethanol intoxication produced by ethanol doses of 1.0 to 2.2 g/kg. The potentiation took the form of a leftward shift of the ethanol dose-response curve. The ED_{50} value of ethanol-induced motor incoordination decreased by 16% when rats were pretreated with AVP (Figs. 1 and 3). This effect of AVP is probably not mediated by its pressor effects because maximal potentiation occurs approximately 2 h after the SC injection of AVP, at a time when the AVP-induced peripheral effects have disappeared (14). Since AVP doses as high as 40 μg produced absolutely no impairment by themselves, whereas 10 μg caused a clear increase in the ethanol-induced impairment, it seems warranted to regard the interaction as a true potentiation rather than additive effects of two independent actions. The notion of AVP potentiation of the actions of ethanol is confirmed by two more new observations: 1) AVP enhancement of the ethanol effect occurs in the absence of changes in blood ethanol levels; and 2) it is also seen in rats in which the AVP is administered intracerebroventricularly. These results suggest that AVP potentiates ethanol-induced motor incoordination by a central mechanism.

Stephen and Logan (28) demonstrated that AVP receptors in the CNS are of the V_1 subtype. Szabo et al. (29) also showed that the AVP-V_1 receptor is the one that is involved in the maintenance of ethanol tolerance. In our study, AVP potentiation of the ethanol effect was completely blocked by prior ICV administration of a V_1 antagonist, indicating that this action of AVP is also mediated by a V_1 receptor subtype. The fact that the V_1 antagonist did not attenuate ethanol-induced motor impairment on its own suggests that endogenous vasopressin may not be directly involved in mediating ethanol-induced motor incoordination, but it may play a modulatory role on systems that produce ethanol intoxication.

AVP potentiation of this ethanol effect may have influences on the development of ethanol tolerance. Our results show that rats chronically treated with AVP-ethanol (1.5 g/kg) or saline-ethanol (1.8 g/kg) developed the same final level of ethanol tolerance, i.e., their posttreatment dose-response curves were virtually identical. This is consistent with the hypothesis (17) that the stimulus to tolerance development is not the dose of drug used, but the degree of functional impairment produced. Thus the similar impairment produced by 1.5 g/kg of ethanol plus AVP and by 1.8 g/kg ethanol plus saline resulted in similar degrees of acquired tolerance. This is also consistent with other types of evidence indicating that animals which are initially more sensitive to ethanol develop more tolerance during chronic exposure (18,19). The nature of the AVP facilitation of tolerance development remains to be determined. AVP is still able to produce a similar degree of potentiation of the ethanol effect in tolerant animals, irrespective of whether they were treated with saline-ethanol or AVP-ethanol. Therefore, the tolerance that develops is to the effect of ethanol but not to the mechanism by which AVP affects the ethanol action.

In conclusion, we observed that exogenous AVP potentiates the motor incoordinating effects of ethanol through a centrally mediated action on an AVP-V_1 receptor subtype. This acute AVP potentation of ethanol-induced motor incoordination increases the stimulus for the development of ethanol tolerance. However, the lack of effect of a V_1 antagonist on acute ethanol impairment suggests that endogenous AVP does not normally modulate the degree of effect produced by ethanol.

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