Biochemical Pharmacology, Vol. 35, No. 22, pp. 3983–3987, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

DIFFERENTIAL EFFECTS OF ETHANOL ON PLASMA CATECHOLAMINE LEVELS IN RATS

WOLFGANG H. VOGEL,* KATHRYN DETURCK and JOANNE M. MILLER
Departments of Pharmacology and Psychiatry and Human Behavior, Thomas Jefferson University, Philadelphia, PA 19107, U.S.A.

(*Received* 6 *September* 1985; *accepted* 14 *April* 1986)

Abstract—Acute ethanol administration (1–4 g/kg, i.p.) had no effect on plasma catecholamine levels in nonstressed animals except at the highest dose where levels of both catecholamines increased. In animals stressed for 30 min, the higher doses had a biphasic effect on plasma catecholamines; at earlier times during stress a reduction in stress-induced increases in both catecholamines was seen, whereas later during stress or after release from stress an increase was noted. Semi-chronic ethanol administration (0.5 and 2 g/kg/day, i.p.) had no significant effect on plasma catecholamine levels in nonstressed rats. In stressed rats, ethanol reduced stress-induced catecholamine increases but these reductions were less than those seen after acute administration. Although ethanol reduced the gross behavioral stress response, no correlation between gross behavioral and biochemical responses was detected. These data show that ethanol can indeed reduce the behavioral and biochemical stress responses in rats but that effects seen depend on the state (nonstressed vs stressed) of the animal, the dose of ethanol (low vs high) used, the length of ethanol administration (acute vs semi-chronic), and the time of measurement of the catecholamine level after ethanol administration.

Recent studies have shown that ethanol can affect plasma biogenic amine levels differently in nonstressed and stressed rats. A low dose of ethanol (0.5 g/kg) given i.p. had no effect on plasma levels of norepinephrine and epinephrine in nonstressed rats but antagonized significantly stress-induced increases in both plasma catecholamines [1]. Similar studies on the action of ethanol on plasma levels of corticosterone have also shown that ethanol can differently affect the levels of this steroid in nonstressed and stressed rats [2–5]. In addition, these studies showed that the effect of ethanol on this steroid is dose dependent. Low doses (less than 0.5 g/kg, i.p.) had no effect on plasma corticosterone levels, whereas larger doses of ethanol (2 g/kg, i.p.) increased the levels of the steroid in nonstressed rats. In stressed rats, low doses (0.5 g/kg, i.p.) of ethanol attenuated the stress-induced increases, whereas higher doses (2 g/kg, i.p.) potentiated the stress-induced increases of corticosterone.

To see whether or not ethanol given acutely or semi-chronically over a period of 2 weeks would also produce a dose-dependent effect on plasma catecholamines in both nonstressed and stressed rats, animals were injected with different doses of ethanol. Plasma norepinephrine and epinephrine levels were then measured at rest and during restraint stress. Results obtained indeed showed that the effects of ethanol on plasma catecholamine levels depended strongly on the state (stressed vs nonstressed) of the animal, the dose of ethanol (low vs high) used, the length of ethanol administration prior to restraint, and the time of measurement of the catecholamines after ethanol administration.

* To whom all correspondence should be sent.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats (Perfection Breeders, Douglassville, PA), weighing about 250 g, were used in all experiments. The animals were housed three per cage for a 1-week acclimation period, were kept on a 12-hr light/dark cycle, and were allowed free access to food and water.

Surgery. To obtain blood samples, animals were anesthetized with ketamine/pentobarbital and fitted with an indwelling, chronic jugular catheter as described previously [1, 6, 7]. The rats were then kept in individual cages and used after 1–2 days which had been shown previously to be sufficient for recovery from surgery [1, 7]. Samples of 0.2 to 0.3 ml of blood were drawn during the experiments. Repeated blood sampling (up to eight samples) over a 2-hr period of time had been shown previously to be without effect on hematocrit or catecholamine levels [1, 7].

Experiments. For the acute studies, blood was obtained through the catheter from resting rats in their home cages just before and at various times after the administration of saline or ethanol. In the studies using restraint, blood was obtained before the administration of saline or ethanol, and the animals were immobilized 15 min after injection for 30 min. After 30 min of immobilization, the animals were returned to the home cage. Blood was obtained at various times during immobilization and after a 30 min rest period in the home cage. For the semi-chronic studies, rats received saline or ethanol by daily injection in the morning for 14 consecutive days. Catheterization was performed in the afternoon of day 12. On day 14, the last administration of ethanol was given and the same experimental protocol as described above was performed. Injec-

Table 1. Effects of different doses of ethanol on plasma norepinephrine and epinephrine levels in nonstressed rats

	Time (min) 0	15	30	45	60
			Norepinephrine (pg/ml)		
Saline	180 ± 25	235 ± 25	238 ± 31	214 ± 12	184 ± 11
Ethanol (1 g/kg)	156	156	168	124	200
Ethanol (2 g/kg)	231 ± 42	312 ± 35	293 ± 33	323 ± 31	228 ± 21
Ethanol (4 g/kg)	240 ± 21	364 ± 40*†	403 ± 49*†	395 ± 60*†	511 ± 75*†
			Epinephrine (pg/ml)		
Saline	95 ± 4	99 ± 19	117 ± 15	93 ± 18	86 ± 12
Ethanol (1 g/kg)	90	60	108	112	70
Ethanol (2 g/kg)	142 ± 25	111 ± 16	184 ± 39	151 ± 24	121 ± 18
Ethanol (4 g/kg)	106 ± 18	269 ± 60*†	268 ± 35*†	427 ± 29*†	643 ± 100*†

Values represent mean ± S.E.M. Number of animals: five per group except for 1 g/kg where values represent the average of closely agreeing data from two animals. Injection (i.p.) of ethanol was performed following 0 time measure (baseline).

* $P < 0.05$ (comparison with 0 time).

† $P < 0.05$ (comparison with saline value).

tions of ethanol were always given in the same room and restraint was carried out in an adjacent room.

Restraint. Stress was induced by taping the four paws of the animal to the laboratory bench which immobilized or restrained the animal. All experiment were performed between 10:00 a.m. and 1:00 p.m. to minimize the effects of diurinal variations. Ethanol was administered by i.p. injection at a concentration of 20% (w/v).

Behavior. The gross behavior of the rats was evaluated by a "blind" observer during rest and stress, and vocalization, struggling, number of fecal boli and biting were recorded.

Assays. The concentrations of the catecholamines were assayed by the Cat-a-Kit assay marketed by the Upjohn Diagnostics Co., Kalamazoo, MI. The ethanol analyses were performed by Dr. E. Mezey, Baltimore City Hospital, Baltimore, MD. The area under the stress curve (AUC) represents the area under the individual time points (0–60 min) and is recorded as ng/ml × min.

Statistics. The statistical analyses used a two-way analysis of variance with repeated measurements accompanied by the Newman–Kuels test ($P < 0.05$ was accepted as significant).

RESULTS

Effect of acute ethanol in resting rats. The behavioral effects of different doses of ethanol in nonstressed rats ranged from mild (1 g/kg) to moderate (2 g/kg) to marked sedation and hypnosis (4 g/kg). A previous study using 0.5 g/kg of ethanol had shown only little sedation [1]. In this previous study, no effect on plasma catecholamine levels was seen in resting rats. The effects of higher doses of ethanol on plasma norepinephrine and epinephrine levels in nonstressed rats are shown in Table 1. Again ethanol did not cause an increase in plasma catecholamines at the lower doses, but it caused an increase after 15, 30, 45 or 60 min at the highest dose (4 g/kg).

Effect of acute ethanol on stressed rats. Previously it was found that a small dose of ethanol (0.5 g/kg, i.p.) antagonized only slightly the behavioral responses to restraint but significantly antagonized the stress-induced increases in plasma catecholamine

Table 2. Effect of ethanol on plasma norepinephrine and epinephrine levels in stressed rats

	Time (min) 0	5	15	30	60
			Norepinephrine (pg/ml)		
Saline	146 ± 21	1253 ± 219*	754 ± 82*	554 ± 65*	255 ± 35*
Ethanol (2 g/kg)	101 ± 24	421 ± 46*†	796 ± 253*	820 ± 171*	295 ± 19*
Ethanol (4 g/kg)	119 ± 22	958 ± 377*	418 ± 114*†	698 ± 119*	668 ± 184*†
			Epinephrine (pg/ml)		
Saline	49 ± 8	1182 ± 301*	792 ± 81*	563 ± 64*	84 ± 40
Ethanol (2 g/kg)	57 ± 17	297 ± 82*†	639 ± 101*	548 ± 188*	325 ± 89*†
Ethanol (4 g/kg)	56 ± 16	419 ± 87*†	577 ± 162*	1245 ± 185*†	869 ± 46*†

Values represent means ± S.E.M. Number of animals: six per group. Animals received saline or ethanol after the first blood drawing (0) and were immobilized 15 min later for 30 min with drawings at 5, 15 and 30 min during this period. After immobilization, animals were returned to the home cage and blood was drawn after 30 min of rest or recovery (60 min).

* $P < 0.05$ (comparison with 0 time).

† $P < 0.05$ (comparison with stress/saline value).

Table 3. Effect of semi-chronically administered ethanol on plasma norepinephrine and epinephrine levels in stressed rats

	Time (min)				
	0	5	15	30	60
			Norepinephrine (pg/ml)		
Saline	188 ± 29	1256 ± 44*	916 ± 171*	642 ± 27*	363 ± 24*
Ethanol (0.5 g/kg)	199 ± 13	957 ± 137*	839 ± 72*	557 ± 62*	365 ± 60*
Ethanol (2 g/kg)	205 ± 31	863 ± 91*†	791 ± 71*	696 ± 91*	412 ± 51*
			Epinephrine (pg/ml)		
Saline	94 ± 28	1213 ± 144*	874 ± 40*	665 ± 88*	94 ± 40
Ethanol (0.5 g/kg)	101 ± 14	915 ± 54*	638 ± 88*†	407 ± 33*†	237 ± 42*†
Ethanol (2 g/kg)	112 ± 12	935 ± 76*	731 ± 71*	541 ± 61*	471 ± 53*†

Values represent means ± S.E.M. Number of animals: six per group. Animals received saline or ethanol in the morning for 2 weeks. On the day of the experiment, saline or ethanol was administered after the first blood drawing (0), and the rats were immobilized 15 min later for 30 min (5, 15, 30). After immobilization, animals were returned to the home cage and blood was drawn after 30 min of rest or recovery (60).

* $P < 0.05$ (comparison with 0 time).

† $P < 0.05$ (comparison with saline value).

levels [1]. With the higher doses used here, the animals showed a marked reduction in the gross behavioral response to restraint. They vocalized and struggled considerably less and at 4 g/kg appeared completely sedated and behaviorally "unaffected" by restraint. The effects of these higher doses of ethanol on plasma norepinephrine and epinephrine levels in these stressed rats are shown in Table 2. As can be seen, effects of ethanol on stress-induced rises in plasma catecholamines were time and dose dependent. Stress-induced levels of norepinephrine were depressed significantly by 2 g/kg at 5 min and by 4 g/kg at 15 min. After recovery for 30 min (i.e. at the 60-min measure), norepinephrine levels were still elevated in rats that had received 4 g/kg. Stress-induced increases of epinephrine were depressed significantly by both doses at 5 min, but were increased by the highest dose of ethanol at the end of the stress period (30 min) and were elevated after both doses after 30 min of recovery (60 min). The AUC was decreased significantly for the 2 g/kg but not the 4 g/kg dose (Norepinephrine: stress = 26.2 ± 2.1, 2 g/kg of ethanol = 18.4 ± 2.9, 4 g/kg of ethanol = 23.2 ± 3.1; epinephrine: stress = 24.7 ± 2.5, 2 g/kg of ethanol = 20.1 ± 3.3, 4 g/kg of ethanol = 29.1 ± 4.4.)

Effect of semi-chronic ethanol on nonstressed and stressed rats. For the semi-chronic studies, nonstressed and stressed rats showed the same gross behavioral responses to ethanol as rats exposed to their first dose of ethanol. Although catecholamine levels tended to be higher in the semi-chronically treated animals, no significant effects of 0.5 or 2 g/kg/day for 14 days on plasma catecholamine levels were observed in nonstressed rats following the last dose. Catecholamine responses to semi-chronically administered ethanol are shown in Table 3. During stress, semi-chronic administration of a low dose of ethanol (0.5 g/kg) caused only a slight reduction in stress-induced epinephrine values (whereas an acute injection of ethanol caused a reduction in both norepinephrine and epinephrine; Ref. 1). Semi-chronic administration of a higher dose of ethanol (2 g/kg) caused a slight reduction in stress-induced norepinephrine values (whereas an acute injection reduced both norepinephrine and epinephrine; Table 2).

Ethanol plasma levels in nonstressed and stressed rats. To compare catecholamine levels with those of ethanol under identical conditions and to assure that ethanol levels were unaffected by stress, a separate group of animals from the same supplier and of the same weight and the same strain was injected with ethanol, and blood was drawn for ethanol determinations as indicated. Table 4 shows these results. Although levels were initially somewhat higher in stressed animals, no significant differences were apparent between nonstressed and stressed rats.

DISCUSSION

In agreement with a previous study [1], results from this study shown quite clearly that ethanol affected peripheral catecholamine levels differently

Table 4. Blood levels of ethanol in non-stressed and stressed rats

	Ethanol (mg/100 ml)						
	Time (min)						
	0	15	30	60	100	150	200
Nonstressed (5)	0	197 ± 21	175 ± 22	145 ± 19	137 ± 13	92 ± 8	42 ± 6
Stressed (5)	0	255 ± 57	215 ± 18	186 ± 19	158 ± 16	99 ± 16	31 ± 17

Values represent means ± S.E.M.; number of animals is given in parentheses. Resting rats received 2 g/kg, i.p., after 0 time. Stressed rats received 2 g/kg, i.p. after 0 time and were immediately restrained for 60 min (15, 30, 60) and were then released into their home cages (100, 150, 200).

in nonstressed and stressed animals. In addition, this study shows that the effect of ethanol on plasma catecholamines is dependent on the dose of ethanol used, the length of its administration, and the time of measurement of the biogenic amine levels after ethanol administration.

In nonstressed rats, a low dose of ethanol (0.5 g/kg) has no effect on behavior or on plasma norepinephrine and epinephrine levels [1]. In this study, mild to strong sedation was observed after the higher doses of ethanol. No effects of ethanol on plasma catecholamines were observed at doses of 1 and 2 g/kg. However, a dose of 4 g/kg increased plasma norepinephrine and epinephrine levels significantly after 30–60 min, indicative of an ethanol-induced stress response. In rats treated for 14 days with ethanol (0.5 and 2 g/kg/day) no major effects were seen on catecholamine levels after the last dose, although levels of both amines tended to be consistently, but not significantly, higher.

In stressed rats, plasma catecholamine levels rise sharply at the beginning of restraint (5 min), decrease over the remainder of the stress period (15 and 30 min), and reach almost baseline levels 30 min after the animals have been released [1, 7]. A single low dose of ethanol (0.5 g/kg) has been found to reduce these stress-induced increases in both catecholamines in most animals at 5 and 15 min, to have no effect on later times, and to reduce the area under the stress curve significantly [1]. In this study with higher doses, an acute dose of 2 and 4 g/kg initially reduced the stress-induced increases in both catecholamines but increased plasma levels of these biogenic amines later in the stress period or recovery period. This could be interpreted that ethanol reduces at lower doses but shifts the time–course to the right and only delays the response to stress at higher doses as a result of a stress-reducing and stress-inducing effect. The area under the curve was decreased significantly for both norepinephrine and epinephrine at the 2 g/kg dose but no significant changes were seen at the 4 g/kg dose. After treatment with ethanol for 2 weeks, ethanol also reduced the stress-induced increases in catecholamines but the reductions were somewhat less than those seen after a single administration of ethanol. Although we do not have blood levels in semi-chronically treated animals, the reduced effect was probably not due to increased ethanol metabolism since ethanol was only given once a day and since only doses much higher than those given in this study (32% of total diet) for 24 days increase ethanol metabolism significantly [8]. This reduction probably represents development of tolerance to the stress-reducing effects of ethanol since saline-injected animals still showed the same high stress-response as did the acutely saline-treated rats.

The biochemical findings on plasma catecholamine levels do not correlate well with our gross behavioral observations during stress. Animals exposed to the lower doses (0.5 g/kg) still appeared quite "stressed" and struggled and vocalized as much as the saline-treated animals. Nevertheless, the plasma catecholamine levels of these rats during stress are reduced significantly [1]. Animals receiving the moderate dose (2 g/kg) were more sedated and also showed reductions in both catecholamine levels. Animals receiving the high doses (4 g/kg) were extremely sedated and hypnotic during the restraint procedure. In spite of this fact, their plasma catecholamine reductions were less marked and they actually showed an increase in epinephrine levels at the end of the stress period. Animals receiving the acute or chronic ethanol administration showed very similar behavioral responses during restraint but they differed in the reduction of the stress-induced increases in plasma catecholamines. Thus, ethanol can significantly reduce and antagonize behavioral and biochemical responses to stress but no correlation between these gross behavioral and biochemical responses was apparent. This lack of correlation warns strongly against the extrapolation of biochemical findings to behavioral interpretations or vice versa; increased biochemical stress-responses can be seen in animals that show no behavioral stress-responses or the absence of behavioral stress-responses does not mean the absence of biochemical stress-responses.

A comparison of plasma ethanol and catecholamine levels shows the following. After the i.p. injection of 2 g/kg of ethanol into catheterized rats, peak levels were reached at about 15 min and levels declined thereafter for the next 100 min in both nonstressed and stressed rats. In nonstressed rats, ethanol had no influence on catecholamine levels except at the 4 g/kg dose after 30 or 60 min. Thus, highest levels of ethanol do not immediately affect catecholamine concentrations but catecholamine levels rise at a time when ethanol concentrations fall. In stressed rats, the most marked reductions in catecholamine concentrations were seen early when levels of ethanol were highest. This would indicate a direct effect of ethanol on stress-induced increases. However, the higher dose of 4 g/kg should produce higher levels of ethanol than would the 2 g/kg dose and would be expected to reduce stress-induced increases in both catecholamines; unfortunately, this is not so. Thus, the correlation between ethanol and its reduction of stress-induced biogenic amine levels remains unclear. Again, the increase in catecholamines observed later (60 min) occurred at a time when ethanol levels fall and are low.

In summary, the effects of ethanol on plasma catecholamine levels depended markedly on the state (nonstressed vs stressed) of the animal, the dose (low vs high) of ethanol used, the length of the administration (acute vs chronic), and the time of measurement. These findings may explain some of the conflicting results obtained with humans. In an excellent review, Pohorecky [9] showed that the effects of alcohol on human behavior and physiology are quite contradictory and that alcohol has been reported to have either no effect on certain biochemical and behavioral stress responses in man or to increase or decrease some of these responses. Apparently, the state of the subjects during testing (stressed vs nonstressed), the dose of ethanol used (low vs high), the length of alcohol exposure (chronic vs acute), the measurement selected (behavioral vs biochemical) and the time of measurement after ethanol administration (immediate vs delayed) can influence markedly the conclusion reached. Thus,

more and better controlled studies in humans are necessary to determine whether or not alcohol is indeed tension reducing [10] in man. In rats, ethanol can certainly reduce both behavioral and biochemical stress responses, although their exact relationship still needs to be elucidated.

Acknowledgement—The financial support of this study by US Public Health Service Grant AA06017 is gratefully acknowledged.

REFERENCES

1. K. H. DeTurck and W. H. Vogel, *J. Pharmac. exp. Ther.* **223**, 348 (1982).
2. J. S. Jenkins and J. Connolly, *Br. med. J.* **2**, 804 (1968).
3. F. W. Ellis, *J. Pharmac. exp. Ther.* **153**, 121 (1966).
4. L. A. Pohorecky, E. Rossi, J. M. Weiss and K. V. Michala, *Alcoholism: Clin expl. Res.* **4**, 423 (1980).
5. J. Brick and L. A. Pohorecky, in *Stress and Alcohol Use* (Eds. J. Brick and L. A. Pohorecky), p. 389. Elsevier Biomedical, New York (1983).
6. R. A. Upton, *J. pharm. Sci.* **64**, 112 (1975).
7. K. H. DeTurck and W. H. Vogel, *Pharmac. Biochem. Behav.* **13**, 129 (1980).
8. C. S. Lieber and L. M. DeCarli, *J. Pharmac. exp. Ther.* **181**, 279 (1972).
9. L. A. Pohorecky, *Neurosci. Biobehav. Rev.* **5**, 209 (1981).
10. H. Cappell and P. Herman, *Q. Jl Stud. Alcohol.* **33**, 33 (1972).