Pharmacology Biochemistry and Behavior, Vol. 42, pp. 831-842, 1992

0091-3057/92 $5.00 + .00

Daily Dose of Ethanol and the Development and Decay of Acute and Chronic Tolerance and Physical Dependence in Rats

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Received 22 April 1991

POHORECKY, L. A. AND P. ROBERTS. *Daily dose of ethanol and the development and decay of acute and chronic tolerance and physical dependence in rats.* PHARMACOL BIOCHEM BEHAV 42(4) 831-842, 1992.—Using behavioral and physiological measures, we compared the rates of development and decay of acute and chronic tolerance to ethanol (ET) and the severity of the withdrawal syndrome. Male rats were treated with 6, 9, or 12 g/kg/day ET or equicaloric dextrin maltose, delivered intragastrically. Although treatment duration varied, the total dose of ET was kept constant at 162 g/kg/rat for the three groups. The effects of a cumulative test dose of ET or equicaloric dextrin maltose, after exposure to a total of 0, 42, 83, 126, and 162 g/kg ET, and at 3, 5, and 7 days after termination of the chronic treatments, were evaluated on rectal temperature, dowel performance, and tail-flick and startle responses. After the initial five tolerance tests, chronic treatments were discontinued and rats were tested in a modified open-field apparatus and for their startle response to an auditory stimulus at 8, 12, 16, 20, 32, and 40 h later. With all measures, little tolerance developed in the 6-g/kg/day group. On the other hand, development of chronic tolerance was fastest in rats treated with the 12-g/kg/dose of ET. Chronic tolerance did not develop to ET's depressant effect on the startle response. Acute tolerance declined with chronicity of treatment in animals given the largest daily dose of ET. During withdrawal, and in contrast to the dextrin maltose-treated animals, there was impairment in all measures taken during the modified open-field test and hypersensitivity of the startle response for all three chronic ET-treated animals. Greatest behavioral impairment occurred in animals treated with 12 g/kg/day, and some impairment was still evident 40 h after the last dose of ET. Thus, the severity of the withdrawal syndrome was greatest in the group displaying the most acute and chronic tolerance.

Chronic ethanol Acute tolerance Chronic tolerance Ethanol withdrawal Rectal temperature
Open-field test Startle response Dowel test Tail-flick test

TOLERANCE is generally defined as the loss of sensitivity or responsiveness produced by the repeated exposure to a drug (9). Operationally, several types of tolerances to ethanol (ET) have been described (24). *Acute* tolerance develops during the course of exposure to a single administration of ET. It is defined as the change in response to a given concentration of ET during the rising compared to falling aspects of the blood-ET curve. For example, behavioral impairment in both humans and experimental animals is less severe while ET levels are falling compared to when they are rising (8,11). While the time course for the development of acute tolerance by definition is short, in the range of minutes to hours, its other characteristics are largely unknown. For example, how long does it take to reach peak acute tolerance after a single dose of ET, is it dose dependent, and is it affected by repeated exposures to ET? By contrast, long-term or *chronic* tolerance comes into play upon repeated exposure to ET. Chronic tolerance has a gradual onset and rate of development and reaches maximum levels generally between 14-21 days (4,11).

The neurochemical basis of tolerance is unknown, although the involvement of several neurotransmitters have been proposed. Our working hypothesis has been that different mechanisms underlie the expression of acute and chronic tolerance to ET. If this is the case, the rates of development and decay of the two forms of tolerances are most probably different. Since both acute and chronic tolerance can be evaluated using the same measures, the two can probably be differentiated only by careful comparison of their respective

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characteristics. For example, acute and chronic tolerance probably differ with respect to the rates of development and decay, as well as their threshold dose and maximal level. Furthermore, it is also not known whether acute tolerance changes in chronically ET-treated animals.

The scarcity of data on acute compared to chronic tolerance may reflect the difficulties in assessing it: Two to three tests should be performed before peak blood ET concentration (BEC) is reached (i.e., within 10 min of an intraperitoneal injection) for meaningful comparison with tests carried out when BEC is falling. Because of this time constraint, it is essential to employ tests that are brief as well as sensitive, but unfortunately few available tests meet both criteria.

To answer some of the issues mentioned above, we investigated the development and decay of tolerance to ET in rats treated with different daily doses of ET using a cumulative dose-response procedure (25). Previous research indicated that development of acute tolerance is sensitive to increasing BEC. To maximize the development of acute tolerance, we employed a cumulative dose that allows slow incremental elevation of BEC. To achieve a slow rise in BEC, the cumulative testing dose was given intragastrically, which produces a slower rise in BEC. Also, three distinct daily treatment dose levels—6, 9, and 12 g/kg/day—were used to evaluate the dose dependence of both acute and chronic tolerances. Each subject was evaluated using behavioral (dowel performance and startle response) and physiological (rectal temperature) measures were determined on each subject since development of chronic tolerance can vary with different measures (18).

METHOD

Subjects

Male Sprague-Dawley rats (Charles River Laboratories) were individually housed in a room controlled for temperature (21.0 ± 1.0°C) and light (12 L : 12 D cycle, lights off at 0900 h). All subjects were implanted with gastric cannulae made of polyethylene PE-100 as previously described (18). Animals were connected via a liquid swivel to syringes housed in a Harvard Multiple Infusion Pump (Harvard Apparatus, Inc., South Natick, MA). The PE cannula connection as it exited the animal was protected by a lightweight metal spring; this assembly did not in any way restrict the movements of subjects. Solutions were delivered by the infusion pump, over a 10-min period, simultaneously to all subjects of the study (18). Subjects were randomly assigned to one of six chronic treatment groups—chronic ET (6, 9, and 12 g/kg/day) and corresponding equicaloric dextrin maltose (DM)—and one acute ET group. ET treatments were initiated at 6 g/kg/day and gradually raised to 9 or 12 g/kg/day. The daily treatment dose was administered in three infusions (each 2, 3, or 4 g/kg for the 6-, 9-, or 12-g/kg/day treatment groups) 8 h apart (0800, 1600, and 2400 h). The total treatment dose was 162 g/kg ET or equicaloric DM. To enable simultaneous comparison of the withdrawal syndrome in all three treatment groups, chronic treatment began according to the schedule shown in Table 1. The tolerance testing was carried out after exposure to approximately the same total dose of ET for all three treatment groups, namely, at 0, 42, 84, 126, and 162 g/kg ET (Table 1A). The equicaloric DM-treated rats were tested alongside ET-treated rats. On day 28 of the study, chronic treatments were discontinued and rats were observed for withdrawal over the next 2 days, as described below. On days 30, 32, and 34 (tests 6-8), all previously chronically treated subjects, plus an additional set of naive rats, were again tested for tolerance. The naive group was added to determine if the repeated testing during the chronic treatment and/or the withdrawal experience modified the response to the test dose of ET. The experiment was run in three identical replications, each consisting of two to three rats per experimental group.

Tolerance Testing

A cumulative testing procedure was used to evaluate tolerance to the hypothermic and motor-incoordinating effects of ET. The schedule for the tolerance testing is shown in Table 1B and was carried out in the indicated order at each time period. ET was administered intragastrically immediately after the test for a given time period was carried out. Previously [(17); Pohorecky and Roberts, unpublished data)], we determined the rise in BEC after administration of cumulative test doses of ET via the intragastric route. The startle and tail-flick responses were determined only at the 60-min test period.

Withdrawal Testing

At 8, 12, 16, 20, 32, 36, and 40 h after the last dose of ET or DM, rats were tested in a modified open field (16,20). The elevated floor of the open field (divided into four quadrants) contained four 3-cm diameter holes per quadrant. Head-pokes, believed to reflect exploration, were counted when the animal inserted its head into the holes in the floor below eye level. Crossover activity, a measure of locomotion, was counted when the rat crossed with all four legs from one quadrant to another. Rearing behavior was counted when a rat reared on its hindlimbs and the forelimbs were completely off the floor. The behavioral testing was carried out as described before (13,21). Briefly, the onset, frequency, and total duration of the following behaviors were evaluated: crossover activity, rearing, and head-poke. These behaviors were quantified with the aid of an IBM-XT computer equipped with an interface during a 1-min period every 5 min for a total of 3 min of observation over a 15-min period; total scores for the three tests are reported.

Motor Coordination Test

This test was a modified version (18) of a procedure originally described for mice (3). Subjects were required to balance on an elevated rotating cylinder for up to 60 s. Prior to the experiment, all subjects were given a training session to familiarize them with the apparatus.

Rectal Temperature

Temperature was measured using a Data Precision Digital Multimeter (Mansfield, MA) and a Yellow Springs Instruments (Yellow Springs, OH) rectal probe. The probe was lubricated with mineral oil and gently inserted into the rectum to a depth of 4.5 cm. Once the reading stabilized, about 60 s, the temperature was recorded and the probe removed.

Auditory Startle Response

The startle response was measured as previously described (18) using a linear accelerometer attached to the cage holding the test animal; the apparatus was housed in a sound-attenuating chamber. The tone (108 dB, 90-ms duration) was provided by an Altec speaker and an audio generator (Electro-Voice, Buchanan, MI). The output of the accelerometer was

TABLE 1

A. SCHEDULE FOR CHRONIC ET TREATMENT AND TOLERANCE TESTING

Experimental Group (g/kg/day)	Days of Chronic Treatment	Tolerance Testing	Withdrawal Testing	Postwithdrawal Tolerance Testing
6	1-27	1, 8, 14, 21, 27	28/29	30, 32, 34
9	10-27	10, 14, 18, 22, 27	28/29	30, 32, 34
12	14-27	14, 18, 21, 24, 27	28/29	30, 32, 34

B. SCHEDULE FOR THE CUMULATIVE TESTING PROCEDURE

Time (min)	Parameters Measured/Injection	Injection Dose (g/kg)
−5	Rectal temperature, dowel, tail-flick startle response	–
0	Injection	0.3
10	Dowel, BEC, injection	0.6
20	Rectal temperature, dowel, BEC, injection	0.9
50	Rectal temperature, dowel, BEC, injection	1.5
60	Tail-flick, startle response	–
90	Rectal temperature, dowel, BEC	–
150	Rectal temperature, dowel, BEC	–

digitalized and fed into a microprocessor; the data is expressed as response amplitude.

Tail-Flick Response

The tail-flick response was measured as previously described (19). Each animal was briefly restrained in a holder and a Grass Instruments (Quincy, MA) force-displacement transducer was used to measure the displacement of the tip of the tail in response to an electrical pulse delivered from a Grass S44 stimulator via connected pin electrodes.

Breath ET Concentration

Blood ET concentration was extrapolated from breath ET levels, which were determined using a gas chromatographic procedure. A 1-ml sample of equilibrated expired air was taken from an airtight plastic cylinder placed over the animal's nose and mouth and then immediately injected into the gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD) as previously described (15).

Data Presentation and Statistical Analysis

For rectal temperature, dowel performance, and BEC, we also calculated the total response over time by integrating the area under the curve for the given response over test time. For the first of these two measures, we also calculated an impairment index by dividing each behavioral measure or physiologic response by the corresponding blood ET level at each test time (21). Therefore, this index takes into consideration possible variations in individual blood ET levels due to various causes (e.g., differences in absorption due to differences in stomach food content) and is an accurate representation of the changes in behavior or physiology produced by ET at each test time.

Results were analyzed with the SAS statistical package (SAS, Cary, NC). For each outcome measure, a 2 × 3 (chronic drug treatment × acute ET dose) one-way repeated-measures analysis of variance (ANOVA) for unequal number of subjects was carried out, with day and testing time as nested repeated variables, using the SAS general-linear model procedure. Planned-comparison F tests were then made between treatment groups using the SAS contrast procedure for general linear models (SAS user's guide: Statistics, version 5 edition, 1985, p. 144). Startle- and tail-flick responses, which were tested at two time points only, were analyzed with a paired-comparisons t-test (SAS User's Guide, p. 799). Criterion for significance was set at $p \leq 0.05$.

RESULTS

Development and Decay of Tolerance

Since the responses of the three groups of rats chronically treated with DM did not differ statistically on any of the measures employed in these studies, these data were combined into a single mean DM value as shown in the figures. However, all statistical analyses were carried out using the separate data for the three DM-treated control groups. Although there were no mortalities from the surgical or treatment procedures, sporadically throughout the study the cannulae of one or two subjects would be blocked temporarily, and occasionally permanently, resulting in the loss of that subject's data on that test day. All animals gained body weight during the course of the study. The changes in body weight, from the first to the last day of drug treatment before withdrawal, for the 6-, 9-, and 12-g/kg/day groups were the following: 52.8 ± 2.95, 115.2 ± 53.22, and 66.7 ± 21.79 for the DM-treated groups and 62.4 ± 10.7, 37.9 ± 5.0, and 10.83 ± 7.6 for the ET-treated groups. Thus, of the ET-treated groups only the lowest dose group gained as much weight as did DM-treated animals [contrast of 6- vs. 9-g/kg/day groups, $F(1, 16) = 3.88$, $p = 0.060$; contrast of 6- vs. 12-g/kg/day groups, $F(1, 16) = 7.93$, $p = 0.012$]. It should be noted that when some individual ET-treated animals appeared not to gain weight their daily

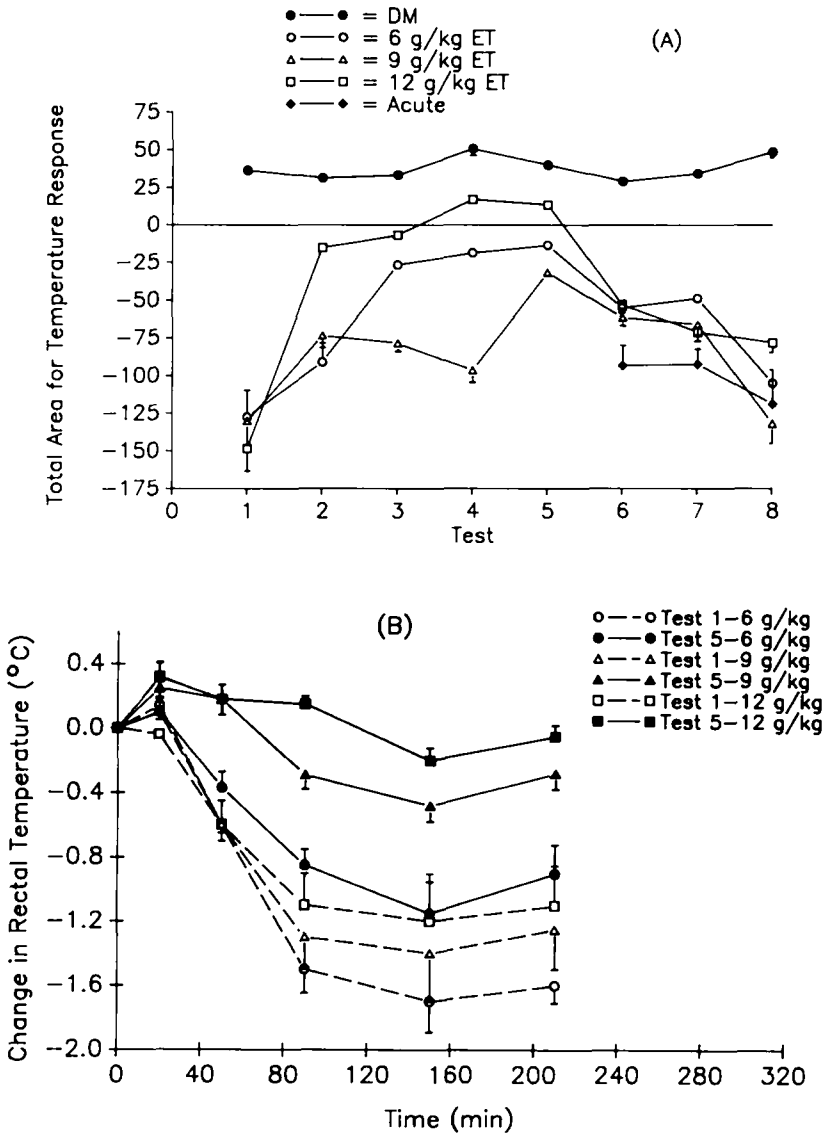

FIG. 1. Change in rectal temperature after challenge with ethanol of chronically ethanol-treated animals. Groups of eight animals each were treated chronically with 6, 9, or 12 g/kg/day ethanol or equicaloric dextrin maltose (DM) intragastrically. The three DM-treated groups did not differ statistically; therefore, in this and all subsequent figures the combined mean DM value for the three treatment groups is presented. Rats were tested prior to and at 20, 50, 90, and 150 min after a cumulative test dose of 3.3 g/kg ethanol (or equicaloric DM) following a total treatment exposure of 3.3 (test 1), 42 (test 2), 84 (test 3), 126 (test 4), and 162 g/kg (test 5) ET (or equicaloric DM). (A) Total change in rectal temperature. (B) Time course data for tests 1 and 5. The results are expressed as the mean ± SEM for the difference in temperature from the baseline value taken prior to each cumulative treatment.

dose was decreased by 10% for a day or two until they began to gain weight again.

Rectal temperature. Figure 1A illustrates the change in the total hypothermic response to the cumulative test doses of ET and DM. Chronic ET treatment had a significant effect on the rectal temperature response, $F(1, 32) = 26.22, p < 0.001$, an effect that was test time, $F(4, 128) = 36.23, p < 0.001$, and test number, $F(4, 128) = 3.90, p = 0.005$, dependent. Overall, the effect of ET on body temperature was influenced by the drug dose and treatment duration, as well as the postinjection test time, $F(32, 128) = 1.71, p = 0.01$. The hyperthermic response of DM-treated rats was similar in all five tests. As expected, all ET-treated groups displayed a hypothermic response in test 1, with a nadir at 150-min and some recovery by 210 min (Fig. 1B). By the third test, 9- and 12-g/kg/day animals showed less hypothermia than the 6-g/kg/day group, a trend that continued on the fourth test (data not shown), $F(2, 16) = 5.77, p = 0.013$. By the fifth test, the hypothermic response of the 9- and 12-g/kg/day groups was negligible, while the 6-g/kg/day group still evidenced a hypo-

thermic response [6- vs. 9-g/kg/day groups, $F(1, 16) = 10.42$, $p = 0.05$; 9- vs. 12-g/kg/day groups, $F(1, 16) = 6.66$, $p = 0.02$] (Fig. 1B).

When chronic treatment with ET was terminated, there was a gradual recovery of the hypothermic response (Fig. 1A). To assess the recovery of sensitivity to ET, a seventh experimental group, consisting of naive animals, was tested concomitantly with chronically treated animals. ANOVA indicated a significant effect of drug treatment, $F(1, 44) = 1.550$, $p = 0.010$, which was dependent upon the test number, $F(2, 88) = 9.770$, $p < 0.001$. The triple interaction of drug × dose × test number was statistically significant, $F(4, 88) = 2.540$, $p = 0.045$, indicating that the effect of ET on rectal temperature depended upon the prior daily treatment dose and the postwithdrawal day. In the sixth test, subjects were still tolerant to ET, but by the eighth test only the 12-g/kg/day group differed from the 6-g/kg/day group [day × 6- vs. 12-g/kg/day groups, $F(2, 60) = 4.05$, $p = 0.022$, and test time × 6- vs. 12-g/kg/day groups, $F(5, 300) = 3.47$, $p = 0.005$]. Thus, with rectal temperature the decay in tolerance to ET was slower with the 12-g/kg/daily treatment dose.

As a possible index of acute and chronic tolerance, we calculated the impairment ratio for rectal temperature and dowel performance to the corresponding BEC at each test. The lower the index, the greater the ET-induced hypothermia or behavioral impairment. The change in impairment index within a session may reflect acute tolerance, while the change in the impairment index over tests, especially of its nadir, reflects chronic tolerance. With respect to rectal temperature, the impairment index was generally lowest at the 90-min period (Table 2). In test 1, the within-session changes in impairment index were similar for all three groups (61–74% decrease), whereas for the 6-g/kg/day group the impairment index within each test session did not change after the first test day; it decreased from 74 to 54% from the first to the fifth test for the 12-g/kg/day group. As for between-session tolerance, the lowest impairment index within each test did not change with the duration of treatment with the 6-g/kg/day treatment, indicating no development of chronic tolerance. On the other hand, with the 12-g/kg/day dose the lowest impairment index increased with duration of treatment, indicating development of chronic tolerance. Thus, the 12-g/kg/day treatment resulted in a 20% decline in within-test tolerance and a 56% increase in between test tolerance. With the 9-g/kg/day dose, acute and chronic tolerance were evident only in test 5. After withdrawal from ET, the impairment index reverted to levels equivalent to those in test 1.

Dowel performance. Because all DM-treated rats had perfect scores on all tests, their data were omitted from Fig. 2. Figure 2A illustrates the total dowel response to the cumulative test dose for the duration of the treatment. The effect of chronic drug treatment on dowel performance was dependent upon treatment dose, $F(2, 16) = 5.000$, $p = 0.020$ (Fig. 2B). Overall, there was little development of tolerance in rats treated with the lowest dose of ET. The 12-g/kg/day group showed significantly greater impairment of dowel performance compared to the 6-g/kg/day, $F(1, 16) = 8.97$, $p = 0.009$, and 9-g/kg/day, $F(1, 16) = 5.22$, $p = 0.036$, treatment groups. For all ET-treated groups, motor impairment was slow to develop in the first test and maximal impairment was evident at 90 min. With the highest dose of ET, the onset of motor impairment, as well as its recovery, occurred sooner [interaction of day × test time × dose for the contrast of 6- vs. 9-g/kg/day groups, $F(24, 384) = 1.60$, $p = 0.038$; and for 6- vs. 12-g/k/day groups, $F(24, 384) = 3.84$, $p = 0.0001$; for 9- vs. 12-g/k/day groups, $F(24, 384) = 1.71$, $p = 0.02$]. In addition, beginning with test 3 and as illustrated by the response in test 5 (Fig. 2B), dowel performance was impaired

TABLE 2

RATIO OF RECTAL TEMPERATURE TO BEC IN CHRONICALLY ETHANOL-TREATED RATS

Ethanol Treatment	Minutes After Injection	Test 1	2	3	4	5	WD	6	7	8
Chronic, 6 g/kg/day	20	0.65	0.93	0.99	0.94	0.78		0.70	0.65	0.63
	50	0.26	0.31	0.25	0.43	0.29		0.28	0.43	0.39
	90	0.18	0.19	0.24	0.23	0.20		0.14	0.24	0.19
	150	0.23	0.21	0.21	0.21	0.27		0.14	0.23	0.26
	210	0.45	0.40	0.39	0.50	0.41		0.30	0.47	0.51
% Change*		72	79	76	78	74		80	65	70
Chronic, 9 g/kg/day	20	0.65	0.76	0.63	0.77	0.65		0.61	0.67	0.68
	50	0.34	0.38	0.26	0.40	0.51		0.33	0.36	0.39
	90	0.31	0.33	0.21	0.29	0.29		0.15	0.19	0.18
	150	0.20	0.21	0.19	0.31	0.39		0.19	0.19	0.18
	210	0.40	0.38	0.42	0.65	0.50		0.38	0.43	0.39
% Change*		69	72	67	62	52		77	72	74
Chronic, 12 g/kg/day	20	0.87	1.06	0.60	0.83	0.80		0.63	0.71	0.67
	50	0.34	0.39	0.39	0.43	0.47		0.25	0.49	0.31
	90	0.23	0.37	0.36	0.31	0.36		0.13	0.18	0.17
	150	0.26	0.24	0.23	0.32	0.35		0.33	0.26	0.18
	210	0.49	0.53	0.56	0.69	0.65		0.61	0.55	0.49
% Change*		74	77	62	63	55		75	75	75

*Percent decrease in the ratio from the 20-min test to that of the lowest ratio within each tolerance test.

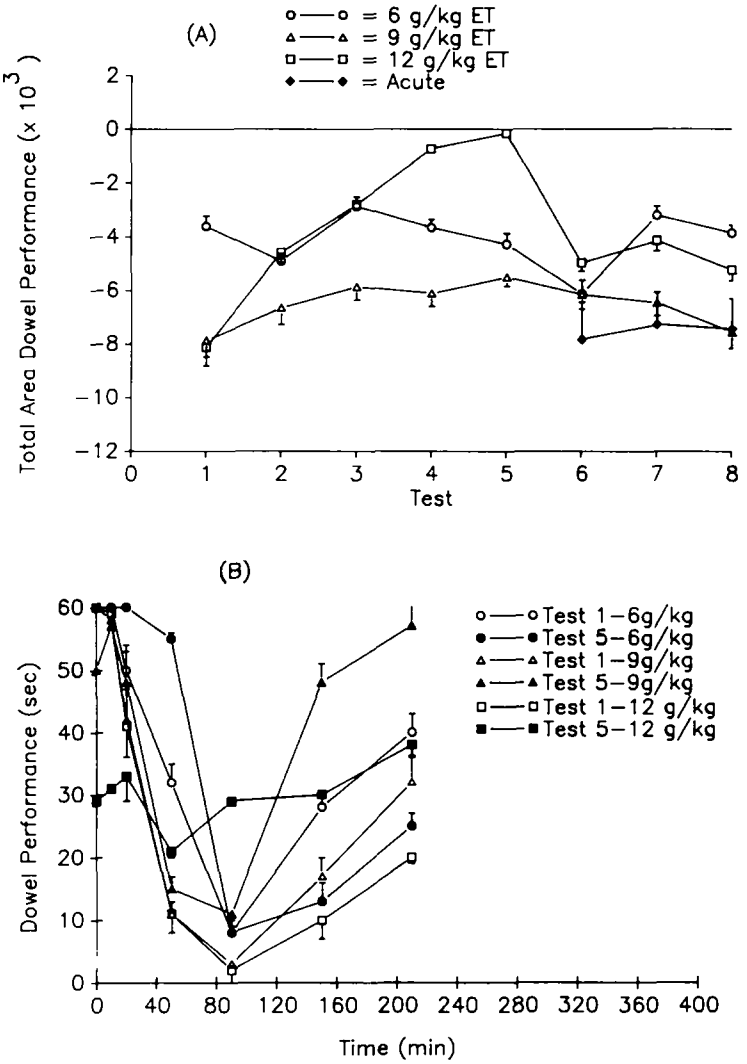

FIG. 2. Performance on the dowel apparatus after challenge with ethanol of chronically ethanol-treated rats. Rats were treated chronically with 6, 9, or 12 g/kg ET or equivalent doses of dextrin maltose (DM). Rats were tested after exposure to 3.3 (test 1), 42 (test 2), 84 (test 3), 126 (test 4), and 162 g/kg (test 5) ET or equicaloric DM. Data for DM rats is omitted from the figure since these animals had a perfect score of 60 on every test. Performance was evaluated prior to and at 10, 20, 50, 90, and 150 min after the cumulative test dose of 3.3 g/kg ethanol or equicaloric dextrin maltose. (A) Total dowel response. (B) Time course data for tests 1 and 5. Results (in seconds) for groups of eight animals each are present as the mean ± SEM of the time animals remained on the dowel.

at pretest (zero time) [contrast of 6- vs. 12-g/k/day groups, $F(1, 2) = 4.18$, $p = 0.058$]. This impairment at pretest was related to the duration of treatment since it was greatest in tests 4 and 5 [$F(2, 16) = 10.20$, $p = 0.001$, and $F(2, 16) = 9.59$, $p = 0.002$, respectively]. Thus, rats treated with the 12-g/kg/day dose of ET differed from the other two chronically ET-treated groups in several respects. First, as just mentioned, they were behaviorally impaired at pretest. Second, compared to pretest, and except for test 1, peak impairment occurred earlier (50 vs. 90 min). Third, the impairment produced by the cumulative test dose of ET was smaller the longer the duration of treatment with ET, that is, rats treated with this daily dose regimen clearly developed chronic tolerance to ET.

After termination of ET treatment, the chronic treatment dose affected the recovery of sensitivity to the motor-incoordinating effects of ET, $F(2, 35) = 6.125$, $p = 0.006$. Furthermore, the interaction of drug × dose × test number × test time was significant, $F(24, 729) = 1.713$, $p = 0.019$, indicating that the effect of ET on dowel performance was influenced by the time after withdrawal and by the time after the cumulative test dose, $F(24, 729) = 1.748$, $p = 0.016$. In tests 6 and 7, the 12-g/kg/day group showed only minor non-significant impairment of dowel performance at pretest and none in test 8 (data not shown).

In test 1, the decline in impairment index with the dowel test was greater than that with rectal temperature (Table 3). The impairment index for all three treatment groups was lowest at 90 min and had partially recovered by 210 min. This pattern of response was maintained over all tests for the 6-

TABLE 3

RATIO OF DOWEL TO BEC IN CHRONICALLY ETHANOL-TREATED RATS

Ethanol Treatment	Minutes After Injection	Test 1	2	3	4	5	WD	6	7	8
Chronic, 6 g/kg/day	20	0.82	0.92	1.17	1.12	1.08		0.85	1.15	0.83
	50	0.28	0.36	0.33	0.32	0.33		0.40	0.60	0.50
	90	0.05	0.01	0.14	0.07	0.03		0.06	0.20	0.10
	150	0.18	0.12	0.18	0.16	0.16		0.04	0.11	0.18
	210	0.28	0.10	0.29	0.28	0.10		0.08	0.19	0.28
% Change*		96	99	88	94	97		95	90	85
Chronic, 9 g/kg/day	20	0.71	0.81	0.94	1.51	1.21		0.63	1.01	1.28
	50	0.20	0.08	0.14	0.24	0.23		0.44	0.48	0.38
	90	0.03	0.06	0.05	0.08	0.10		0.02	0.03	0.03
	150	0.14	0.23	0.18	0.20	0.25		0.06	0.11	0.05
	210	0.24	0.43	0.43	0.46	0.61		0.14	0.18	0.12
% Change*		95	93	95	93	90		97	97	98
Chronic, 12 g/kg/day	20	0.62	0.70	0.60	0.87	0.65		1.25	0.79	1.45
	50	0.29	0.06	0.15	0.18	0.27		0.30	0.77	0.37
	90	0.04	0.10	0.21	0.19	0.26		0.06	0.08	0.12
	150	0.17	0.22	0.23	0.24	0.43		0.13	0.14	0.09
	210	0.31	0.39	0.46	0.53	0.98		0.29	0.25	0.20
% Change*		93	91	75	78	60		95	90	92

*Percent decrease in the ratio from the 20-min test to that of the lowest ratio within each tolerance test.

and 9-g/kg/day treatment groups, but for the 12-g/kg/day group the nadir in the impairment index shifted to 50 min from the third to the fifth tests. In addition, with the 12-g/kg/day group there was a decline in acute tolerance over tests. By contrast, chronic tolerance, as reflected by a change in the lowest impairment index over tests, increased by 2.3- and by 5.5-fold for the 9- and the 12-g/kg/day groups, respectively. For the 6-g/kg/day group, there was no change over tests in acute tolerance, nor was there development of chronic tolerance. Thus, tolerance developed to the motor-incoordinating effects of ET with the 12-g/kg/day regimen but not with the 6-g/kg/day treatment. After withdrawal, the impairment index for the 9- and 12-g/kg/day groups reverted to levels comparable to those on test 1.

Tail-flick response. DM treatment had no significant effect on the tail-flick response, which varied from −17 to +9% from baseline (Fig. 3A). Chronic drug treatment had a significant effect on the tail-flick response, $F(1, 32) = 152.33$, $p < 0.001$. In test 1, the tail-flick response was depressed approximately 80% 60 min after the cumulative dose of ET, $F(5, 37) = 11.51$, $p = 0.001$. Depression of the tail-flick response was approximately the same over the next two tests [$F(5, 37) = 12.10$, $p < 0.001$, and $F(5, 27) = 13.91$, $p < 0.001$, for tests 2 and 3, respectively]. By the fourth test, there was evidence for the development of tolerance in the 9- and 12-g/kg/day groups [$F(5, 37) = 2.82$, $p = 0.0001$, and $F(5, 37) = 7.49$, $p = 0.0001$, for tests 4 and 5, respectively]; tolerance was maximal by the fourth and fifth tests for the 9- and 12-g/kg/day treatment groups. The 6-g/kg/day group did not develop significant tolerance.

After discontinuation of chronic treatment with ET, there was little change in the depressant effect of ET on tail-flick response compared to test 5, that is, the 12-g/kg/group showed negligible loss of the tolerance by the last test day. By contrast, the acute and 6- and 9-g/kg/day ET-treated groups were comparable at all three postwithdrawal tests, that is, there was rapid loss of tolerance in the 9-g/kg/day group.

Startle response. Figure 3B illustrates the effect of ET on the startle response in rats chronically treated with ET and DM. Over the course of chronic treatment, the startle response of the control DM-treated rats varied from pretest by +15 to −18%, while treatment with ET depressed the startle response by 52–68%, $F(1, 32) = 6.472$, $p = 0.015$. This effect of ET was not altered by the chronic treatment dose of ET or duration of chronic treatment and was similar on the test after withdrawal from ET, which confirms our earlier findings (18).

BEC. Overall BECs produced by the cumulative test dose of ET were similar in the three chronic treatment groups (Fig. 4A). Although overall BECs in the three treatment groups did not differ, there was a significant interaction of test dose and treatment duration, $F(8, 56) = 3.14$, $p = 0.005$, due to the 12-g/kg/day treatment group. There was no evidence for a change in blood ET pharmacodynamics over the course of this study in the 6- and 9-g/kg/day groups. However, in the fifth test BECs for the 12-g/kg/day group were lower than those of the 6- and 9-g/kg/day groups [day × test time × 6- and 9- vs. 12-g/kg/day group comparisons, $F(16, 224) = 2.57$, $p < 0.001$]. In general, peak BECs were observed at the 90-min test in all three chronically ET-treated groups, and this pattern was maintained over the duration of the chronic treatment (Fig. 4B). In the postwithdrawal tests, the levels and time course of BECs were similar for all three chronic, as well as the acute, treatment groups.

Withdrawal

Open-field activity. After termination of the chronic ET treatment, crossover activity of drug-treated animals differed significantly from the control groups, $F(2, 42) = 15.586$, $p < 0.001$. Of the ET-treated groups, the 12-g/kg/day group in

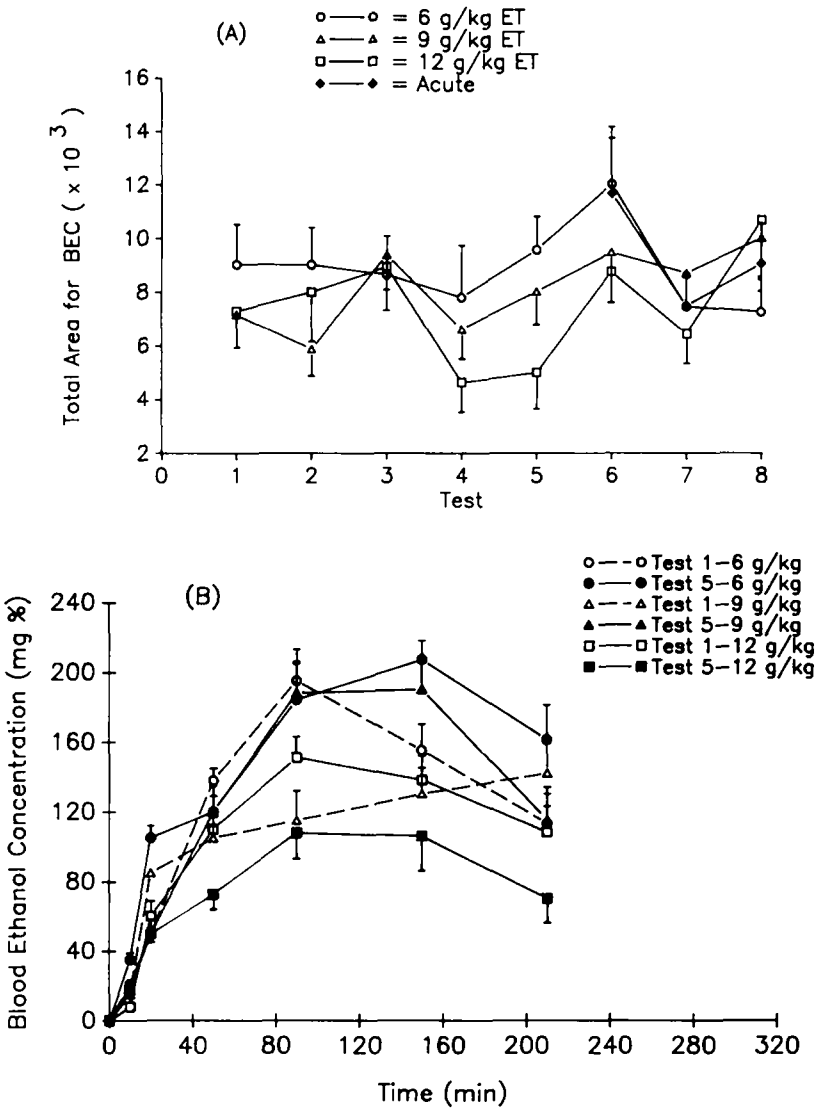

FIG. 3. Effect of cumulative challenge dose of ethanol on tail-flick and startle responses in chronically ethanol-treated rats. Groups of eight rats were each treated chronically with 6, 9, or 12 g/kg/day ethanol or equicaloric dextrin maltose (DM). Rats were tested prior to and 60 min after a cumulative test of 3.3 g/kg ET or equicaloric DM dose five times prior to withdrawal (tests 1–5) and three times (tests 6–8) postwithdrawal. Tests 1–5 (prewithdrawal) were carried out as specified in the legend of Fig. 1. Tests 6–8 were carried out on days 3, 5, and 7 after termination of chronic treatment. Data are presented as the percent change from the pretest values for both the tail-flick (A) and startle responses (B).

particular was lower than the DM-treated groups (Fig. 5A). Locomotor activity of the drug-treated group was partially recovered by the 36-h test.

Similarly, head-poke activity of ET-treated rats was lower compared to that of DM-treated rats (Fig. 5B). The effect of treatment was statistically significant, $F(2, 42) = 7.483$, $p = 0.002$, as was the interaction of treatment and time after withdrawal, $F(12, 252) = 2.185$, $p = 0.012$. In general, head-poke behavior was higher for the 6- and 9-g/kg/day groups compared to the 12-g/kg/day group. Head-poke activity of ET-treated rats had mostly recovered 36 h after withdrawal.

ET-treated rats also exhibited significantly lower frequency of rearing than DM-treated rats, $F(2, 42) = 13.860$, $p < 0.001$ (Fig. 5C). Overall, rats withdrawn from ET reared less compared to the DM group for as long as 40 h postwithdrawal. Rearing behavior was least depressed in the 6-g/kg/day ET-treated group.

Startle response. Animals undergoing withdrawal from chronic ET treatment were hypersensitive to the auditory stimulus used in the startle response test. For ET-treated animals, there was an overall dose effect across all tests, $F(2, 29) = 4.93$, $p < 0.014$. Both the 6-, $F(1, 29) = 8.44$, $p < 0.007$, and 9-, $F(1, 29) = 6.84$, $p < 0.014$ g/kg/day groups were less hypersensitive than the 12-g/kg/day group; this difference between treatment groups extended over all test times. Even 40 h after withdrawal from ET, the 12-g/kg/day group were

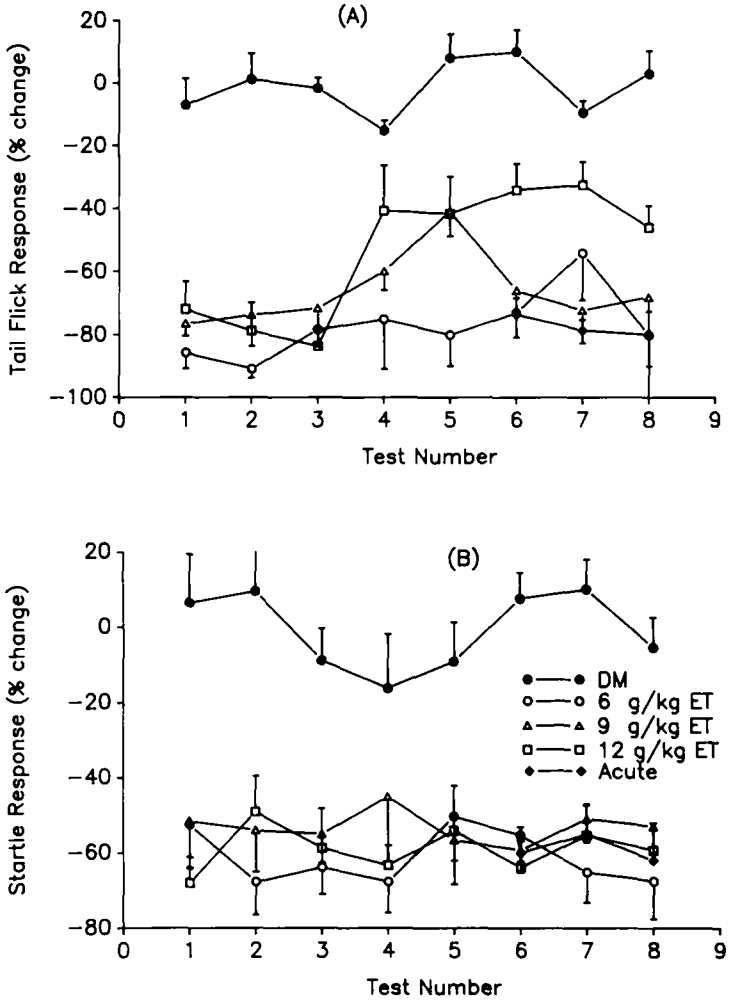

FIG. 4. Blood ethanol concentration after a cumulative challenge dose of ethanol in chronically ethanol-treated rats. Groups of eight animals were treated daily with 6, 9, or 12 g/kg/day ethanol as described in the legend of Fig. 1. Blood ethanol was determined from breath samples taken at 10, 20, 50, 90, 150, and 210 min after the cumulative challenge dose of ethanol. (A) Total change in BEC. (B) Time course data for tests 1 and 5. Results are presented as the mean ± SEM.

still hypersensitive to the startle tone (contrast of 6- and 9- vs. 12-g/kg/day groups, $F(1,29) = 5.56$, $p < 0.0253$). By contrast, the three DM-treated groups did not differ at any of the postwithdrawal tests.

DISCUSSION

Our results indicate that the development of both acute and chronic tolerance was dependent upon the daily treatment dose of ET. For instance, while BECs at 210 min were roughly equivalent to those at 50 min, dowel performance was less impaired at 210 min than at 50 min, particularly so in the 12-g/kg/day group. The development of acute and chronic tolerance is further reflected in the change in impairment index. In contrast to the 6-g/kg/day group, the impairment index for the 12-g/kg/day group was higher (less impairment) at 210 min vs. that at 50 min, that is, the 12-g/kg/day group showed a decline in within-session (acute) tolerance that was not seen with the 6-g/kg/day group. The corresponding changes for the middle dose were approximately halfway between those noted for the 6- and 9-g/kg/day treatment groups.

As expected, the greatest development of chronic tolerance occurred with the highest dose of ET. What was surprising was the low level of chronic tolerance with the 6-g/kg/day dose. Previous reports indicate that doses as low as 1.0–2.5 g/kg could induce tolerance to the hypothermic effect of ET (2,11). The reasons for this discrepancy may lie in differences in the treatment parameters and the cumulative testing procedure employed in our studies. When the method of ET treatment is stressful (e.g., daily IP injections), the resultant level of tolerance may reflect the interaction of the drug treatment with stress (2,11). Furthermore, in the absence of frequent testing manifestations of tolerance and dependence probably

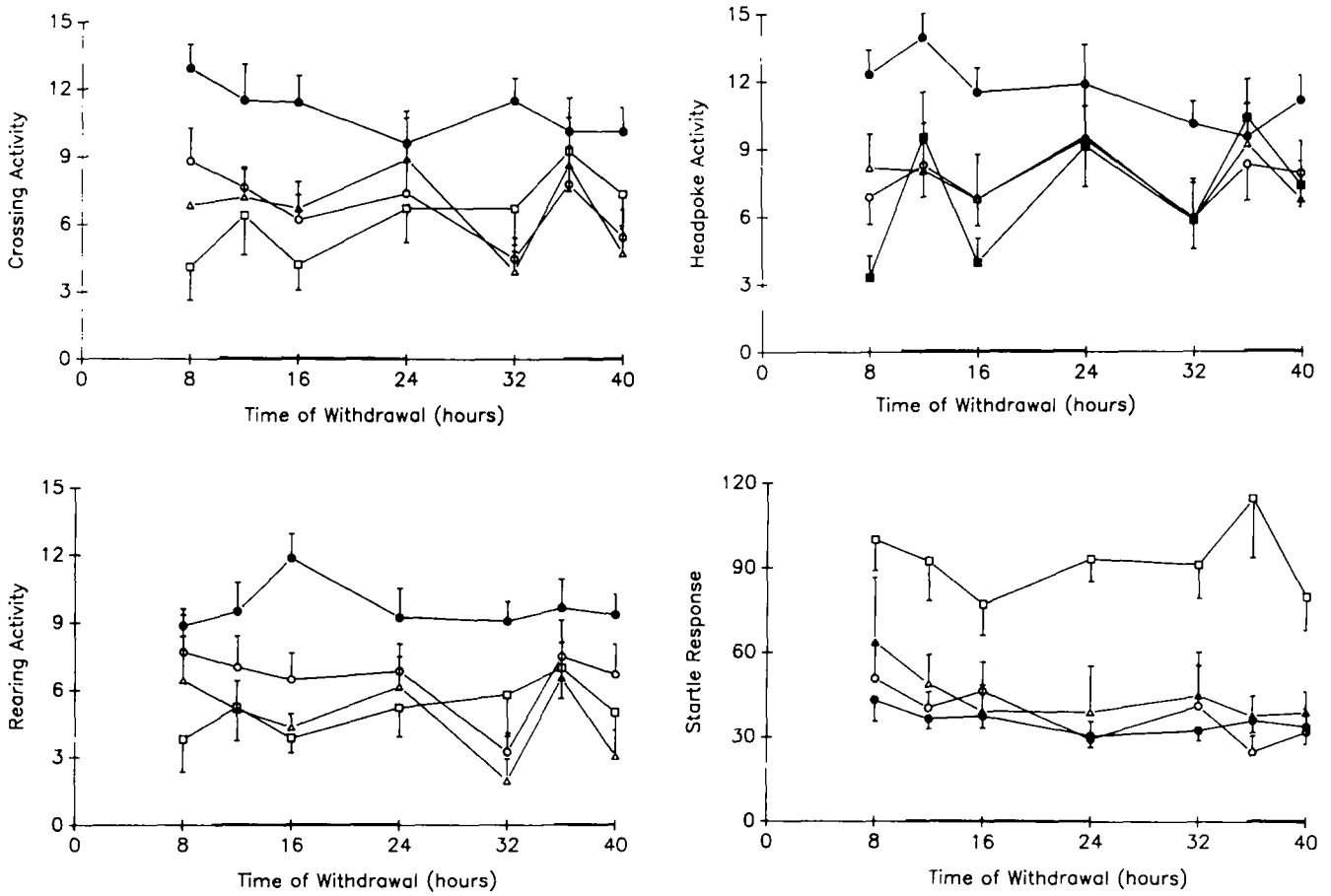

FIG. 5. Open-field activity and startle response of chronically ethanol-treated rats undergoing withdrawal from ethanol. Groups of eight rats were treated daily with 6, 9, or 12 g/kg ethanol or equicaloric dextrin maltose (DM). For details on the chronic treatment, refer to the legend of Fig. 1 and to the Method section. Animals were tested at the indicated times after the last administration of ethanol or DM; 6 g/k ET ○——○; 9 g/kg ET △——△, 12 g/kg ET □——□; Acute ET ◆——◆ and DM ●——●. Results are expressed as the mean ± SEM.

were not altered by the stress of testing (12). The importance of stress-related factors was recently also documented in human subjects (14). Therefore, the 6-g/kg/day group may not have developed chronic tolerance because a) there was no behaviorally augmented tolerance under our conditions (it decayed during the 7-day interval between tests) or b) the in vivo effectiveness of the cumulative testing method, for unknown reasons, differed from that of a bolus test dose, used in all previous studies.

Present knowledge of factors that control acute tolerance is limited. Mice inhaling ET for 3-5 h showed a twofold increase in acute tolerance to ET-induced behavioral impairment (1,6). Acute tolerance was related to the dose of ET since acute tolerance was more evident in animals treated daily with the 12-g/kg/dose compared to the 6- or 9-g/kg/day group. Interestingly, acute tolerance as assessed by the impairment index decreased with duration of ET treatment, that is, acute tolerance declined with the development of chronic tolerance. However, because all our tests were carried out with the same cumulative testing dose it is possible that acute tolerance would have been manifested if a larger testing dose had been employed. Our findings confirm an earlier report in rats that acute tolerance disappeared with longer duration of ET treatment (6). This suggests that different mechanisms underlie acute and chronic tolerance to ET.

Determination of tolerance is contingent upon the knowledge of the in vivo concentration of ET at the time of testing. A contributing factor to variations of BEC is the presence of food in the stomach, which retards gastric emptying and the absorption of ET (7,23). Since animals in our study had free access to food and water, it is likely that individual differences in the pattern of food ingestion may have contributed to the observed variability of BEC. Moreover, at high concentrations ET can delay gastric emptying, slowing its own absorption and increasing its intragastric metabolism (5,22). The latter probably contributed to the lower overall peak BEC of the 12-g/kg/day group treated animals.

Another factor to be considered in studies on tolerance to ET is metabolic tolerance. In a study employing an ET-containing liquid diet, metabolic tolerance developed concomitantly with CNS tolerance and accounted for up to 40% of the functional tolerance (26). Although this figure seems somewhat high, metabolic tolerance (e.g., changes in absorption, distribution, metabolism, and excretion) may nevertheless contribute to functional CNS tolerance at high treatment doses, but probably less so at low doses (9). Although meta-

bolic tolerance may also develop to a single exposure to ET (27), its contribution to the expression of acute tolerance is unknown.

Since the early 1980s, it has been apparent that tolerance to ET develops faster in the presence of environmental cues, that is, behavioral and biological components contribute to the expression of tolerance. In contrast to the popular Pavlovian conditioning models, Kesner and Baker (10) suggested the involvement of both associative and self-generated priming factors in the development of tolerance to morphine. According to their view, self-generated priming occurs mostly with high drug doses and short interdrug intervals, while associative priming occurs with low doses and long interdrug intervals. Our results with 12-g/kg/day treatment of ET may have involved self-priming, while the 6-g/kg/day treatment may have been too low to activate the self-priming mechanisms and too high to activate associate mechanisms.

We confirmed our previous findings that the rate of tolerance development differs depending upon the test measure employed. For example, tolerance to the hypothermic effect of ET developed rapidly, while tolerance to startle response hyposensitivity did not develop within the time frame of our study. Furthermore, as we reported previously (13,21), chronic treatment with ET impaired basal performance on the dowel test: Rats receiving the largest daily dose of ET were impaired at the baseline test, prior to administration of the cumulative test dose of ET. This initial impairment in basal motor coordination increased with the duration of treatment with ET. Thus, although the dowel test is simple and readily learned by rats it is physically demanding and is easily disrupted by drugs, stressors, etc. and probably reflects the long-term toxic effects of ET.

The chronic treatment dose of ET also affected the rate of decay of tolerance after withdrawal from ET. With rectal temperature, both chronic and acute tolerances decayed to near normal levels within 3 days after discontinuation of treatment with ET. Thus, the decay of chronic tolerance, except for tolerance to the tail-flick response, is faster than its development under our experimental conditions.

With respect to withdrawal syndrome, the observed general trend supports the group distinctions noted with the tolerance measures. During withdrawal from chronic ET treatment, there was an overall depression of the behaviors exhibited in the modified open-field apparatus. This depression was most evident during the initial 24 h of withdrawal and was minor in the 6-g/kg/day group of animals. Also, only the 12-g/kg/day treatment group showed clear CNS hypersensitivity to an auditory stimulus.

In summary, our studies demonstrated the dose dependence of both acute and chronic tolerance in ET-treated animals using two distinct measures. With chronic treatment, there was dose-related development of chronic tolerance and a decline in acute tolerance with the highest dose of ET. Overall, withdrawal severity was greatest in the group displaying the most initial acute and subsequent chronic tolerance.

ACKNOWLEDGEMENT

The authors offer their sincerest thanks to David Epstein for the statistical data analysis. This research was supported by USPHS Grant AA06236.

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