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Age Effects on Chronic Tolerance to Ethanol Hypnosis and Hypothermia

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YORK, J. L. AND A. W. K. CHAN. *Age effects on chronic tolerance to ethanol hypnosis and hypothermia.* PHARMACOL BIOCHEM BEHAV **49**(2) 371-376, 1994.—Male Fischer 344 rats of three different ages (young, 4 months; middle, 13 months; and old, 25 months) were tested for their hypnotic and hypothermic response to a 3.5 g/kg dose of ethanol on day 1 and after an 8-day exposure to 4.0 g/kg of ethanol administered via intragastric intubation. All three age groups displayed, to a similar extent, an increased rate of blood ethanol disappearance (metabolic tolerance) on day 10 and day 16, as compared to day 1. Both young and middle-age rats demonstrated tolerance to ethanol hypothermia, but older rats did not. The same test dose of ethanol (3.5 g/kg, IP) administered on day 16, after a 5-day interval with no ethanol, produced a hypnosis response similar to that observed on day 1 (no tolerance), but the response to ethanol hypothermia was still significantly reduced over the day 1 and day 10 value in young and middle-age rats, suggesting a persistence and intensification of tolerance to hypothermia over the 5-day rest interval. However, tolerance to ethanol hypothermia was not observed in old rats. Thus, the effects of age on the development of chronic functional tolerance were complex and depended upon the test measure used.

Age Chronic tolerance Ethanol Functional and metabolic tolerance Hypnosis Hypothermia

ATTENTION is now being focused upon the elderly as a population deserving special attention with regard to understanding the actions and toxicities of alcohol. The intoxicating effects of alcohol appear to increase with age in man and rodents, owing partly to higher blood alcohol levels achieved in the aged, as well as to the increased target tissue sensitivities to alcohol associated with aging (21). These findings have prompted precautions regarding the moderate use of alcohol in the elderly (21). Noticeably lacking at this time is information regarding the influence of age on the capability to develop tolerance to alcohol. A decreased capability of the elderly to develop tolerance to alcohol might be expected, for instance, to operate to discourage the escalation of alcohol intake over time that usually accompanies problem drinking. Preliminary studies have, indeed, documented a decreased capability of old rats to develop rapid tolerance to ethanol hypnosis (3).

Chronic tolerance, on the other hand, may involve different biochemical systems and different rates of protein synthesis than those involved in the development of rapid tolerance. Because protein synthesis is probably involved in the development of chronic tolerance to ethanol (5,18) and because the rate of protein synthesis, in general, undergoes a decline with advancing age (12,20), it seems reasonable to predict that old animals may be less capable than young of developing chronic tolerance to ethanol. In the only other study known to us (14) addressing the influence of age on chronic tolerance to ethanol hypothermia, middle-aged male Wistar rats (14 months) were observed to develop little tolerance to ethanol hypothermia (30–90 min postinjection, 3 g/kg IP test doses) after 8 days of chronic exposure to the 3 g/kg/day regimen. Young rats (3 months), on the other hand, displayed less hypothermia after the 8-day exposure. The influence of metabolic tolerance or differing blood alcohol levels at the time of testing was not clear from this report. In a study of young (8–10 months) and old (24–26 months) male Fischer 344 rats, Mayfield et al. (13) reported tolerance to the same extent in old and young animals in a reaction time task after 5 days of exposure to chronic ethanol.

The reports cited above constitute all we know regarding the influence of age on the development of tolerance to ethanol. Clearly, more studies are needed. In pursuing knowledge in this area, researchers now realize that a demonstration of tolerance may depend on the behavioral or physiological measures utilized (5,15). We report here on the initial findings regarding the development of chronic tolerance in rats of different ages using ethanol hypothermia and hypnosis as measures.

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METHOD

Animals

Male Fischer 344 rats of three different ages (10 young, 3 months; 7 middle-age, 12 months; 8 old, 24 months) were obtained from NIA breeding colonies at Harlan Industries, Indianapolis, IN. The animals were housed individually in polycarbonate cages (21 h × 45 l × 25 w, cm) with beddings of wood shavings. Each rat was provided free access to Teklad rat chow and to a bottle containing tap water. Room temperature and humidity were controlled at 22–24°C and 30–60%, respectively, in rooms with 12 h light, 12 h dark cycles. The animals were allowed 4 weeks to adjust to the new environment before testing.

MEASURES OF RESPONSIVENESS TO ETHANOL

The blood alcohol concentration (BAC) at recovery of the righting reflex served as the index of sensitivity to ethanol hypnosis. The righting reflex was recorded as lost (LRR) when, after IP injection with ethanol, the rat was unable to right itself onto all four feet when placed upon its back. Testing for this effect began at 120 s after injection and was repeated every 20 s until a positive response was obtained. Failure to lose the righting reflex within 10 min was unusual, but resulted in exclusion of the animal from the study. Recovery of righting reflex (RRR; min) was recorded when the animal was observed to recover from the effects of ethanol by righting itself on all fours in its cage. The rat was then required to right itself again within 10 s (two successive trials) when placed upon its back by the experimenter. BAC at RRR represents the amount of alcohol (blood and brain concentration) that the animal was barely capable of overcoming with regard to recovery of the ability to right itself. Higher BACs at RRR indicate that the animal was capable of overcoming a larger challenge of circulating ethanol. Conversely, relatively lower BACs at RRR indicate a relatively greater sensitivity to ethanol; that is, less ethanol was needed to suppress the righting reflex (21).

The lowering of the body temperature (hypothermia) was the other measure of ethanol impairment. Rectal temperatures were assessed by means of a Yellow Springs Instrument Co. model 45TA digital thermometer fitted with a No. 423 small animal probe. Hypothermic effects of ethanol (10% w/v in saline IP) were determined at 0, 6, 7, and 8 h after injection. To obtain body temperatures, the rat was gently grasped at the base of the tail with thumb and forefinger and the lubricated (saline) probe was gently inserted 6 cm into the rectum. Approximately 40 s were allowed for the reading to stabilize. The change in body temperature (ΔT) at the 6, 7, and 8 h readings, compared to the predrug value, was used as the measure of drug effect.

Determination of Blood Ethanol Concentrations

Tail-tip blood samples (20 μl) were taken at RRR and at 6, 7, and 8 h postinjection, immediately after temperatures were taken. The samples were deproteinized by treatment with trichloroacetic acid and then subjected to enzymatic assay using kits supplied by the Sigma Chemical Company (product 332-BT). The amount of reduced NAD (NADH) was determined spectrophotometrically at 340 nm, using a Beckman model 25 spectrophotometer. The concentration of ethanol in the sample was extrapolated from ethanol standard curves. The use of BAC as an index of target tissue ethanol concentration is based upon the finding that the concentration of ethanol in brain tissues closely parallels the concentration of ethanol in the blood after the absorptive phase (4,11) and that the ratio of brain to serum concentration of ethanol remains relatively constant (approximately 90% across age groups) (19).

Statistical Analysis

Data were analyzed for statistical significance by means of two- or three-way analysis of variance or covariance, with repeated measures (ANOVA, Number Cruncher Statistical Package, version 5.0). Post hoc analyses (*t*-tests) were applied when appropriate.

Assessment of Chronic Tolerance

The responsiveness to the hypothermic and hypnotic actions of ethanol (3.5 g/kg IP, 10% w/v in saline) was assessed 1 day before (day 1) and 1 day after (day 10) an 8-day exposure to daily intragastric intubations of 4.0 g/kg doses of ethanol (20% w/v in saline). A 1-week chronic regimen of ethanol (4.0 g/kg) has been demonstrated to produce substantial tolerance to the hypothermic effects of that agent (8). A third test dose (3.5 g/kg) was administered on day 16, after a 5-day respite from ethanol exposure, to assess the persistence of tolerance. All testing and drug administrations took place in the same home environment at approximately the same time of day.

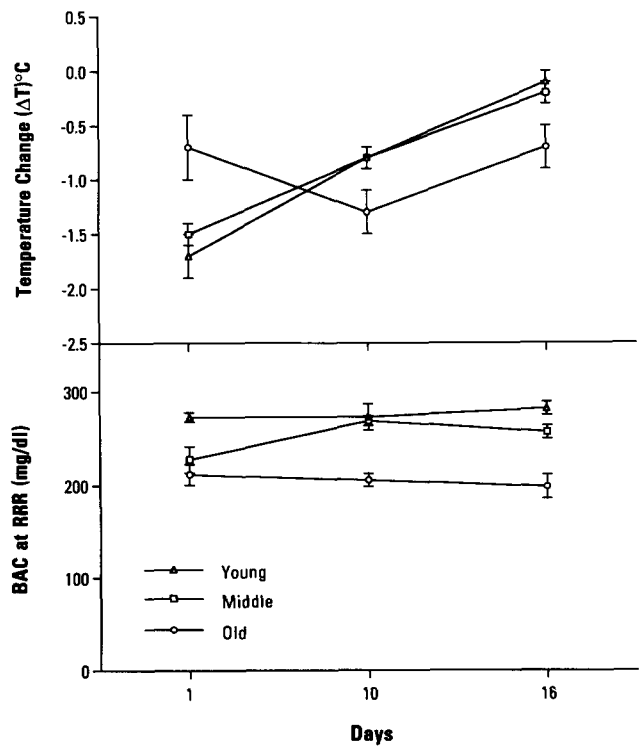

FIG. 1. Effect of chronic ethanol (4.0 g/kg, days 2–9) on the hypothermic and hypnotic response to a 3.5 g/kg challenge on days 1, 10, and 16. The temperature readings refer to the difference (ΔT) between preinjection and hour 6 readings. Blood alcohol determinations were made immediately after the body temperature was taken. Also illustrated are BAC at RRR, which occurred before the 6-h mark. Vertical bars indicate standard error of the mean. n = 9 young, 6 middle, 7 old. Note that owing to the formation of blood clots, the number of animals included in the data of Figs. 1 and 2 is one less for each age group than for Fig. 3.

TABLE 1

SLEEP TIME PARAMETERS IN RATS OF DIFFERENT AGES (MEAN ± SEM)

	Day 1			Day 10			Day 16		
	Y	M	O	Y	M	O	Y	M	O
Weight (gm)	307 (5)	435 (5)	410 (12)	285 (10)	387 (3)	362 (12)	294 (4)	400 (4)	376 (11)
LRR (Sec)	6.3 (14)	3.8 (.5)	3.3 (0.4)	3.9 (.6)	3.6 (.3)	3.6 (.3)	3.5 (.3)	4.1 (.8)	3.4 (.3)
Sleep Time (min)	229 (22)	271 (30)	279 (33)	101 (15)	183 (22)	235 (18)	138 (11)	224 (20)	268 (25)
BAC at RRR (mg/dl)	273 (5)	228 (14)	212 (11)	273 (14)	269 (5)	206 (7)	282 (7)	257 (7)	199 (13)
Baseline Temp	37.4 (.1)	37.3 (.1)	36.6 (.1)	37.6 (.1)	37.5 (.1)	37.7 (.1)	37.2 (.1)	36.8 (.1)	36.6 (.2)
Temp at RRR (°C)	36.4 (.1)	36.4 (.2)	35.6 (.2)	36.3 (.2)	36.2 (.2)	36.3 (.2)	36.0 (.1)	36.1 (.2)	35.8 (.4)
BAC at 6 hr (mg/dl)	217 (9)	202 (13)	167 (17)	172 (6)	202 (8)	158 (10)	165 (3)	191 (9)	152 (12)
Temp at 6 hr (°C)	35.7 (.2)	35.8 (.2)	35.9 (.2)	36.9 (.1)	36.6 (.1)	36.4 (.2)	37.0 (.1)	36.6 (.1)	35.9 (.2)

Rats were injected with 3.5 g/kg test doses on days 1, 10, and 16 and received a chronic regimen of 4.0 g/kg intragastrically on days 2–9. Days 11–15 were drug free. In addition to sleep time parameters, BAC and body temperatures at the 6 h test are also indicated. These are the same animals described in Fig. 1. Baseline temperatures were obtained immediately prior to the injection of ethanol.

On day 7, blood alcohol levels produced by the intragastric intubation procedure were determined a hourly intervals to assess the relative challenges experienced by the different age groups.

RESULTS

The data in Fig. 1 illustrate the difference in response to ethanol hypnosis and hypothermia before and after the 8-day exposure to chronic ethanol. A 2-way ANOVA (age × day) performed on hypnosis data (BAC at RRR) revealed a significant age effect across days 1 and 10 [lower BAC at RRR in old rats, $F(2, 38) = 17.9$, $p < 0.01$], a finding that has been quite common in aging studies (21). However, the values on day 1 did not differ significantly from those on day 10 [no main effect of day; $F(1, 47) = 1.47$, $p < 0.2$], indicating no tolerance to the hypnotic effects of ethanol. The response of the middle-age group on day 10 was in the direction of tolerance, but the age group × day interaction term was not significant, $F(2, 38) = 2.33$, $p < 0.1$. There was no significant change when BAC at RRR was again assessed on day 16. The time to loss of the righting reflex (LRR, Table 1) was longer in young rats on day 1, but there were no significant differences among ages on day 10 or 16.

The results of a two-way ANOVA (age group × day) revealed that sleep times on day 10 and on day 16 were significantly shorter than day 1 values in all age groups, $F(2, 34) = 10.30$, $p < 0.001$, perhaps reflecting, in part, metabolic tolerance. With data collapsed across all 3 days (main effect of age), the sleep times of the three age groups were significantly different from each other, $F(2, 34) = 12.89$, $p < 0.001$, with old rats sleeping longest and young shortest. The treatments (days) did not affect the age groups differently with regard to sleep time (no significant age × day interaction).

The maximum hypothermic response was observed at 6 h, and this response was used as the measure of tolerance to ethanol hypothermia. However, the age differences in hypothermia at hours 7 and 8 parallelled those at hour 6, but were lesser in magnitude (Table 2). Tolerance to the hypothermic effect of ethanol was displayed by young and middle-age rats (Fig. 1), but not in old rats. The depression in body temperature (ΔT) produced by ethanol on day 10 was approximately one-half the size of the temperature depression produced by the first challenge on day 1. In old rats, the hypothermia was actually greater on day 10 than on day 1 (ANOVA for repeated measures on ΔT values, day 1 vs. day 10, significant age × day interaction term, $F(2, 37) = 8.78$, $p < 0.001$. Post hoc analyses (*t*-tests, day 1 vs. day 10) revealed that the response for each age group was significantly different ($p < 0.05$) on day 1 and day 10. The tolerance to hypothermia in middle and young rats was even more pronounced on day 16, but little change was seen in ΔT values for old rats compared to their day 1 response.

Because BACs were different on day 1 and day 10 for all three age groups at the 6-h temperature reading (Table 1), an additional analysis of covariance, in which BACs were used as covariates for the ΔT values, was used to correct for these differences, and to permit inferences regarding the develop-

TABLE 2

CHANGE IN RECTAL TEMPERATURE (−ΔT°C) BEFORE AND AFTER TOLERANCE

	Day 1			Day 10			Day 16		
	6 h	7 h	8 h	6 h	7 h	8 h	6 h	7 h	8 h
Young	1.7(.2)	1.4(.3)	0.9(.3)	0.8(.1)	0.8(.2)	0.2(.2)	0.1(0.1)	+0.1(0.1)	+0.3(0.1)
Middle	1.5(.1)	1.0(.2)	0.8(.2)	0.8(.1)	0.6(.1)	0.2(.1)	0.2(0.1)	+0.2(0.2)	+0.3(0.1)
Old	0.7(.3)	0.4(.3)	0.5(.6)	1.3(.2)	1.0(.2)	0.8(.1)	0.7(0.2)	0.1(0.1)	+0.2(0.2)

Values represent changes in body temperature from preinjection value (time 0) produced by 3.5 g/kg IP ethanol tests. All rats received 4.0 g/kg PO daily on days 2-9. All values are negative except those preceded by a + sign. Values in parentheses are standard errors. The number of animals studied is the same as in Fig. 1.

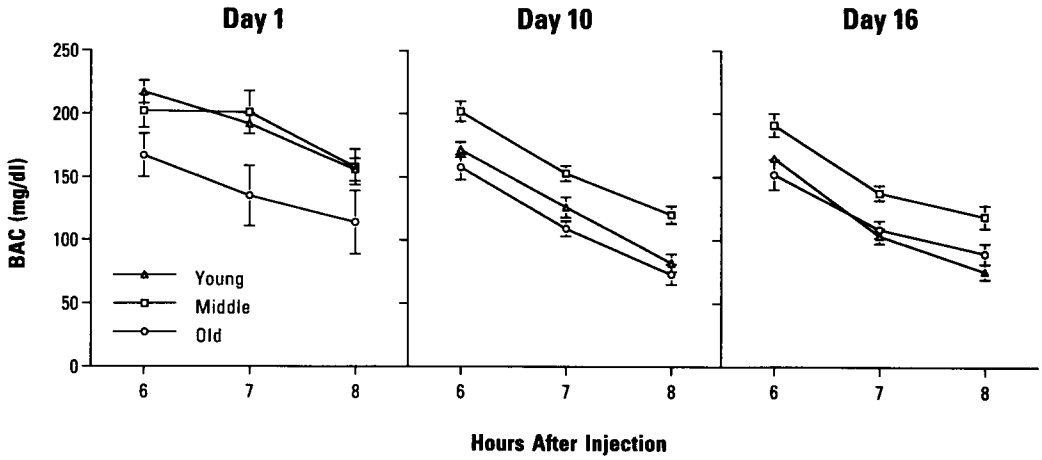

FIG. 2. Metabolic tolerance after chronic intubation with ethanol. Blood alcohol concentrations were determined during the linear descending phase of blood alcohol disappearance, at 6, 7, and 8 h after the injection of the 3.5 g/kg test doses on days 1, 10, and 16. Brackets indicate standard errors of the mean. n = 9 young, 6 middle, 7 old.

ment of functional tolerance. The corrected ΔT values for the 2 days were as follows: day 1: Y = −1.46; M = −1.32; O = −0.88; day 10: Y = −0.87; M = −0.72; O = −1.56. The main effects of age and day were not significant, but a significant interaction of age × day was indicated, $F(2, 36) = 7.94$, $p < 0.001$. Post hoc analyses revealed that young and middle groups were less affected on day 10, whereas the old group suffered an increased hypothermia on day 10 (t-tests, all $p < 0.05$). The statistical analysis of covariance of day 1 vs. day 16 data also yielded a significant main effect of day $F(1, 34) = 22.84$, $p < 0.001$, with less hypothermia on day 16 than on day 1 (tolerance effect). The significant age × day interaction term, $F(2, 34) = 7.51$, $p < 0.002$, was the consequence of the significant tolerance (less hypothermia) in young and middle-aged rats, but no tolerance in old rats. The ΔT values corrected for BAC on day 16 were: Y = −0.30; M = −0.13; O = −0.93.

The collection of blood samples at 6, 7, and 8 h postinjection on each day allowed for a determination of metabolic tolerance (Fig. 2). The rate of disappearance of ethanol from the blood was significantly faster on day 10 than on day 1 [ANOVA with repeated measures, day 1 vs. day 10, significant day × hour interaction, $F(2, 74) = 18.14$, $p < 0.001$]. All age groups displayed this effect to about the same extent (no age × hour interaction). The rate of disappearance of ethanol on day 1 averaged 25 mg/dl per hour vs. 43 mg/dl per h on day 10 (day 10 = 173% of day 1 value). Metabolic tolerance was also present on day 16, after a 5-day drug-free interval, when the rate of disappearance of ethanol (39 mg/dl per hour) was 156% of the day 1 value. Owing to the presence of metabolic tolerance, it was necessary to correct the ΔT values for blood alcohol concentrations present at the time of temperature measurements in the evaluation of functional tolerance to ethanol hypothermia (Fig. 1).

The time course of blood alcohol disappearance after intubation with a 4.0 g/kg dose was determined on day 7 of the study (Fig. 3). These data revealed a similar peak BAC and time course of blood alcohol disappearance in old and middle-aged rats, but lower overall BACs in young rats. The slope of the linear descending phase (hours 4–6) was similar to that observed on day 10 tests (Fig. 2) in all age groups, indicating that the metabolic tolerance had been established by day 7.

DISCUSSION

The finding in all age groups of no chronic tolerance to ethanol hypnosis, but a decreased ability of old rats to develop

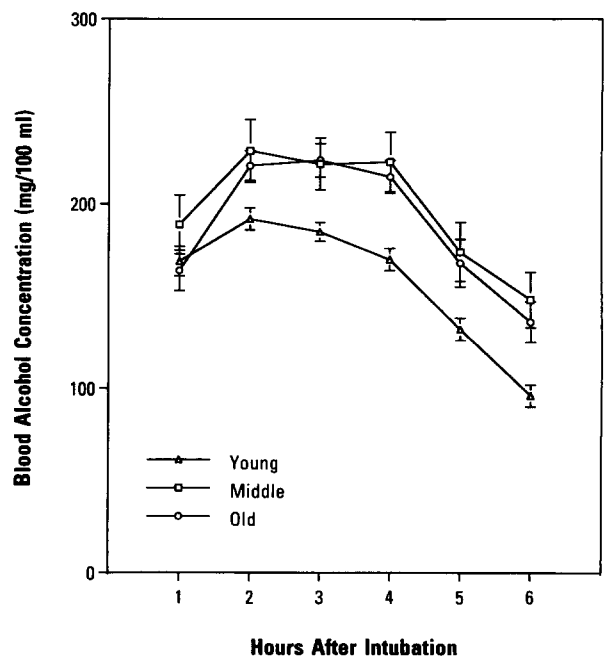

FIG. 3. Blood alcohol disappearance after intragastric intubation on day 6. Rats were intubated with 4.0 g/kg of ethanol at time zero and tail-tip blood samples were obtained at hourly intervals. Brackets indicate standard errors of the mean. n = 10 young, 7 middle-age, 8 old.

chronic tolerance to ethanol hypothermia, stands in contrast to observations reported earlier on rapid tolerance at 48 h (3), in which none of the age groups displayed rapid tolerance to ethanol hypothermia, but all age groups displayed rapid tolerance to ethanol hypnosis, with young rats displaying a greater capability than middle-age or old rats. Thus, the activity of different compensatory mechanisms appears to underlie the expression of rapid and chronic tolerance. This difference may be linked to the different time course of the two types of tolerance. Thus, aging would appear to have a differential effect upon the type of tolerance (rapid or chronic) and upon the measure (e.g., hypothermia, hypnosis) utilized.

Of particular interest is the observation of the persistence and intensification of tolerance until day 16, after 5 drug-free days in young and middle-aged rats. The response to ethanol on day 16 was, in fact, quite small (-0.1, and -0.2°C, respectively) in the young and middle-aged rats. These findings suggest that the processes underlying the expression of tolerance on day 10 continued to proliferate during the 5-day drug-free interval. Only a few studies have addressed this issue of persistence of tolerance over drug-free intervals. In one of the few studies known to us, persistence of tolerance to motor impairment for 21 days after two exposures, with practice (1.7 g/kg on days 1 and 2), has been reported (2). Persistence, but not intensification, of tolerance to ethanol hypothermia has been reported (16) after a 5-day drug-free interval in male Sprague-Dawley rats (age unspecified) after a 27-day regimen of intragastric (6 g/kg) ethanol. However, caution must be used when comparing different studies because of strain differences in ethanol tolerance. For instance, Le and Kiianmaa (9) reported that AA rats, but not ANAs, exhibited tolerance to ethanol (3.5 g/kg) hypnosis and hypothermia after daily treatment with 5.0 g/kg PO for 24 days.

Traditionally, it has been considered important in tolerance studies that the chronic drug administrations produce similar blood alcohol disappearance curves in different comparison groups to ensure that all animals were challenged to the same extent to develop tolerance. The data in Fig. 3 illustrate that younger rats were exposed to lower blood (and presumably, brain) concentrations of ethanol, a finding that was not unexpected (21). Yet, in spite of the smaller challenge, young rats developed chronic tolerance to a greater extent than older rats. Thus, the blood alcohol levels displayed in Fig. 3 would be expected to bias the experiment in a conservative direction; that is, in a direction opposite from the main conclusion. It could, of course, be argued that the most important challenge was the extent of hypothermia experienced during the chronic daily challenges (17). Although we did not record body temperature during those exposures, there is ample evidence that old rats suffer more profound hypothermia when their BACs are similar to those in younger rats (21).

In addition to the findings on functional tolerance to ethanol hypothermia discussed above, this experiment also provided information regarding the influence of age on the development of metabolic tolerance. All three age groups displayed, to a similar extent, an increase in the rate of blood alcohol disappearance on days 7, 10, and 16. The lower blood alcohol levels at the 6-h target on those days compared to day 1 made it necessary to use BAC as a covariate to the temperature readings in the assessment of tolerance.

Factors that influence the time course of the development and persistence of tolerance are clearly of interest to researchers, as well as clinicians. For instance, Le, Khanna, and Kalant (8), using hypothermia as a measure, have demonstrated that tolerance to 3 g/kg IP challenges with ethanol was nearly maximal after an 8-day exposure to 4.0 g/kg daily intragastric doses. In the same animals, tolerance to motor impairment (inclined plane) developed more slowly and to a lesser extent. The present findings also demonstrate that tolerance to ethanol hypothermia develops to a greater extent than another form of tolerance (ethanol hypnosis) in young and middle-age rats. Future studies should address the reasons why old rats fail to develop chronic tolerance to ethanol hypothermia, with particular attention to the possibility that old rats may display deficits in the NMDA receptor systems or vasopressin systems implicated in the development of chronic tolerance (6,7).

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