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Influence of Age on the Development of Rapid Tolerance to Ethanol

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CHAN, A. W. K. AND J. L. YORK. *Influence of age on the development of rapid tolerance to ethanol.* PHARMACOL BIOCHEM BEHAV **47**(3) 567–573, 1994. – Fischer-344 rats of three different ages (4, 13, and 25 months) were tested to determine the extent and duration of rapid tolerance to ethanol-induced hypothermia and hypnosis. There were no significant differences among groups with regard to maximal ethanol hypothermia (3.0–3.5 g/kg ethanol), nor did any of the groups display a significant change (rapid tolerance) in the maximal hypothermic response when tested with a second identical challenge 48 h later. Rapid tolerance to ethanol hypnosis was observed across groups at 48 h, utilizing two different dosing schemes. No tolerance was observed if 14 days were allowed to elapse between the initial and the test challenge. Young rats were observed to develop a greater degree of rapid tolerance than did middle-aged or old rats, using hypnosis as a measure.

Age Alcohol Tolerance Hypothermia Hypnosis

THE physiological basis of tolerance development has been intensely studied, with hypotheses emerging regarding the putative roles of neurotransmitters, endorphins, membrane changes, and classical conditioning (2,11,13,22–25). The available evidence supports the prediction that the capability to develop or maintain tolerance will change with advancing age. For instance, if physiological changes underlying the development of tolerance involve an active process such as protein synthesis [e.g., (13,30)], then the decreased activity in enzyme systems known to occur with aging (19,34) may result in a decreased ability of aging animals to develop or maintain the adjustments underlying tolerance, such as those cited above.

In harmony with this hypothesis, major biochemical changes of mouse and rat brain membranes with aging have been reported, such as increases in total protein and cholesterol content and a significant increase of the cholesterol/phospholipid molar ratio (4,6). Other investigators (9,29,32) have reported age-related changes in brain neurotransmitters, such as serotonin or norepinephrine, changes that may influence capabilities for tolerance development. Well-documented age-related changes in the integrity of those neurotransmitter systems (1,3,7) may be expected to alter the development or maintenance of tolerance with advancing age.

Although first reported in mice by Crabbe and coworkers (8), the phenomenon of rapid tolerance has, until recently, received relatively little attention in experimental studies, perhaps owing to the belief that chronic tolerance plays a greater role in problems surrounding drug abuse and dependence. More attention is now being focused upon the understanding of rapid tolerance and its relationship or contribution to chronic tolerance (14). Rapid tolerance to ethanol-induced motor impairment has been demonstrated to be augmented if practice was provided during the initial intoxication episode (5), much as with chronic tolerance. The development of rapid tolerance to motor impairment may be blocked by prior administration of a protein synthesis inhibitor (31) or an NMDA receptor antagonist (15), also characteristics of chronic tolerance. Thus, rapid tolerance may serve as a model for chronic tolerance, or at least serves as an index of the ability to develop rapid, homeostatic neurological adjustments.

Previous studies have documented rapid tolerance to the hypothermia and motor impairment produced by ethanol in rats (5,15,16). The only study examining rapid tolerance to ethanol hypnosis in rats (17) used a 24-h drug challenge spacing and did not detect rapid tolerance. We report here initial investigations into the influence of age on the magnitude and duration of rapid tolerance to ethanol in rats using a 48-h drug challenge spacing.

METHOD

Animals

Virgin female F344 rats were obtained from NIA breeding colonies (Harlan Industries) and were housed individually in

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clear polycarbonate cages (21 h × 45 l × 25 w, cm) with beddings of wood shavings. Each animal was provided free access to Teklad rat chow and a bottle containing tap water. Room temperature was controlled at 22–24°C and relative humidity between 30–60% in a room with a 12 L : 12 D cycle. Three age groups representative of the life span for the rat were compared: young (4 months), middle-aged (13 months), and old (25 months). Animals were obtained from the supplier a minimum of 2 weeks before the tests were performed.

Ethanol Dosages

Previous studies on the F344 (36) and other rat strains (35) have consistently reported age-related differences in the volume of distribution (body water compartment) for ethanol. Advancing age is usually accompanied by a decrease in the ratio of total body water to total body weight (35). Therefore, the traditional method of determining ethanol dosages on the basis of the total body weight (grams of drug per kilogram body weight) consistently produces higher blood alcohol concentrations in old animals, and, essentially, overdoses older animals. To improve upon dosing methodology, we have made reasoned adjustments in the amounts of drug given to different age groups, such that roughly equivalent peak blood alcohol concentrations can be expected in all age groups. The adjustments were based upon recent pharmacokinetic studies on F344 rats (36). Based on those observations, the dosages for old and middle-aged rats were reduced to 86% and 93%, respectively, of the value given to young animals because these adjustments were observed to produce roughly peak BACs in the different age groups. Thus, in the hypnosis (sleep time) study, young rats were given 3.0 g/kg doses, with 2.8 and 2.6 g/kg, respectively, for middle-aged and old animals. In the hypothermia study, young rats were given 3.5 g/kg, with 3.2 and 3.0 for middle-aged and old, respectively. The findings from these studies are identified in the Results section under the heading Adjusted Doses. As comparisons, we also present data obtained from a study on rapid tolerance to ethanol hypnosis in which conventional doses of 3.0 g/kg were administered to all age groups (identified in the Results section under the heading Conventional Doses).

Measures of Ethanol Sensitivity

The predetermined end point selected for study as the first measure of tolerance was recovery of the righting reflex (RRR). It should be stressed that the validity of the measures of ethanol sensitivity (BAC at RRR) employed in this study does not depend upon the production of identical blood alcohol disappearance or identical peak blood ethanol levels in all age groups. Instead, a principle of "equal responses" was employed that relies upon the measurement of blood alcohol levels at a predetermined "target" level of behavioral intoxication. The blood ethanol concentration (BAC) at RRR was the measure of tissue 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. 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 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 is barely capable of overcoming with regard to recovery of the ability to right itself. Higher BACs at RRR indicate that the animal is 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 is needed to suppress the righting reflex.

The lowering of 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 (IP 10% w/v in saline) were determined at 0, 4, 5, and 6 h after injection in conjunction with the hypnosis study or at 0.5-h intervals in the maximal hypothermia study. 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.

Determination of Blood Ethanol Concentrations

Tail-tip blood samples (20 μl) were taken at RRR and at 4, 5, and 6 h postinjection in the hypnosis study, and at 7 h postinjection in the maximal hypothermia study. 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 (10,12,18) and that the ratio of brain to serum concentration of ethanol remains relatively constant (approximately 90% across age groups) (33).

Statistical Analyses

Data were analyzed for statistical significance by means of one-way or two-way analysis of variance (ANOVA) (Number Cruncher Statistical Package, version 5.0). One-way analyses utilized the three age groups as categorical variables with alcohol effect (e.g., BAC at RRR or maximal temperature depression) as the dependent variable. Two-way analyses (age group by day), utilizing a repeated measures design, were used to statistically evaluate the development of tolerance. Blood alcohol level was used as a covariate in the analysis of alcohol effects on body temperature after recovery of the righting reflex.

Assessment of Rapid Tolerance

Rapid tolerance is typically revealed when the response to a drug is less on the second occasion of administration than it was on the first. Often a 24-h interval between challenges is allowed, as in the study by Le and Kiianmaa (17), who observed no rapid tolerance to ethanol hypnosis when 24 h spacings were utilized. A 48-h interval was utilized for this study, owing to the rather large challenge doses of ethanol employed. In two of the studies of rapid tolerance to the hypnotic effects of ethanol (Adjusted Doses-A, and the study utilizing conventional doses of 3.0 g/kg to all age groups), identical challenge

doses were administered at time zero, 48 h later, and again on day 14 to determine the persistence of tolerance. In a third study (Adjusted Doses-B), challenge doses were administered only on day 1 and day 14. Blood ethanol concentrations at recovery of the righting reflex were taken as indices of changes in sensitivity (tolerance) to ethanol.

Tolerance to the maximal hypothermic effect produced by a challenge of ethanol was utilized as a second measure of rapid tolerance. Identical challenge doses were administered at time zero and 48 h later, and changes in rectal temperature were monitored at 20-min intervals. The lowest temperature obtained from each rat was utilized as the measure of maximal hypothermia.

RESULTS

Adjusted Dosages

Rapid tolerance to ethanol hypnosis. The data in Table 1A, in which ethanol challenges were administered at 1, 3, and 14 days, indicated that there were no significant differences among age groups with regard to the latency of onset of hypnosis (LRR). Sleep times were shortest in young rats on each day of testing. Sleep times indicate that the magnitude of disruption in homeostasis produced by ethanol was greater in middle-aged and old rats. This functional disruption is considered to be an important factor in stimulating adjustments (tolerance) to drug effects (21,28) and verifies that the functioning of middle-aged and old rats was indeed challenged as much as that of young rats by the doses of ethanol utilized here.

The results of the hypnosis study are depicted in Table 1 and Fig. 1. A repeated measures two-way ANOVA (age group × day 1, day 3) performed on the BAC at RRR data of Table 1A and Fig. 1A revealed a significant overall effect of age group across days, $F(2, 17) = 10.44$, $p < 0.001$, with old rats waking up at significantly lower BACs. The effect of day of testing (day 1 vs. day 3) was also found to be significant with data collapsed across groups, $F(1, 17) = 56.47$, $p < 0.001$, indicating tolerance had developed across groups (higher BAC at RRR on day 3). The significant ANOVA interaction term, $F(2, 17) = 3.74$, $p < 0.045$, indicated a greater degree of tolerance in young rats than in the other two age groups. Thus, the degree of tolerance was greater in young rats (140% vs. 118% for middle-aged and 123% for old, day 3/day 1 × 100). The tolerance appeared to be maintained up to the 14-day test in young and middle-aged rats.

To obtain further information regarding the time course of decay of rapid tolerance, a second batch of rats was tested 14 days after their first exposure to the same ethanol challenges utilized above with no intervening challenge on day 3 (Table 1B, Fig. 1B). None of the age groups exhibited tolerance on day 14. In fact, all groups exhibited slightly greater sensitivities to ethanol at that time. Sleep times once again indicated that the magnitude of functional disruption produced by the challenges was greater in middle-aged and old rats, and would, therefore, be expected to act as an even greater stimulus to the development of tolerance in these groups.

A three-way ANOVA (age group × day × hour) performed on the blood alcohol values at 4, 5, and 6 h (Fig. 1A) revealed significantly lower BACs in old rats across days and hours [main effect of group, $F(2, 153) = 54.8$, $p < 0.001$]. The main effect of day of test was also significant, $F(2, 153) = 14.0$, $p < 0.004$, with lower mean BAC (data collapsed across groups) produced on day 14 (107.9 ± 3.7) than on day 1 (130.3 ± 3.7) or day 3 (133.7 ± 3.7). This might be taken to suggest metabolic tolerance. However, there was no significant three-way interaction (group × day × hour), which would be expected if the rate of blood alcohol disappearance (steeper slope of hourly data) became greater on day 14. As was true for first batch studies (Fig. 1A), blood alcohol concentrations for old rats in batch 2 (Fig. 1B) were also slightly lower than those for middle-aged and young rats at 4, 5m and 6 h postinjection. There were no significant differences between the slopes of the blood alcohol disappearance curves on day 1 And Day 14. Thus, we conclude that no metabolic tolerance developed.

Absence of rapid tolerance to ethanol hypothermia and maximal ethanol hypothermia. Although the hypnosis study was not expected to yield definitive information regarding ethanol hypothermia, absence of rapid tolerance to ethanol hypothermia is indicated by the temperature readings at 4, 5, and 6 h postinjection in the hypnosis study. The interpretation of these data in terms of tolerance development is complex, owing to the differences among age groups in the baseline values (temp. at time zero) and in the blood alcohol concentrations at the times temperatures were taken. To take into consideration these variables, a three-way analysis of covariance (ANCOVA) (age group × day × hour) was performed, using the change in temperature from baseline (ΔT, °C) as the measure of alcohol hypothermia and correcting for blood alcohol levels by using the BAC at each hourly value as a covariate. Using this approach on Study A data, a significant effect of group was revealed, $F(2, 128) = 11.0$, $p < 0.001$. Examination of the group means collapsed across days and hours revealed a significantly greater hypothermia in old rats ($\Delta T = 1.01$°C vs. 0.52 in middle and 0.11 in young). No other main effects or interaction effects were significant. Thus, no evidence of tolerance or group differences in tolerance were discernable from this approach. We wish to point out that peak hypothermic effects, which usually occur 2–3 h after doses of this size, were not measured in these hypnosis studies because many rats had not recovered the righting reflex by that time.

Analysis of Group B data in which rats were tested only on day 1 and day 14 also revealed a significant group effect, $F(2, 101) = 5.98$, $p < 0.003$, with less hypothermia again in young rats (mean $\Delta T = 0.33 \pm 0.13$°C vs. 0.85 ± 0.13 in middle-age and 0.91 ± 0.11 in old animals. The group-by-day interaction was significant, $F(2, 101) = 4.37$, $p < 0.01$, indicating young animals showed more hypothermia on day 14, a sensitization-like effect. The extent of hypothermia (ΔT) was similar in all three groups for day 14. The main effect of hour was statistically significant for Group B data.

Rapid tolerance was also assessed by measuring the difference in the maximal hypothermia produced by two identical challenge doses of ethanol separated by 48 h (3.5 g/kg to young and middle-aged rats, 3.0 g/kg to old rats, $N = 9$ in each age group). Baseline (preinjection) temperatures among the age groups did not differ appreciably (Table 2, legend). When absolute temperatures were used as the measure, there were no significant differences among groups (ANOVA) with regard to the peak hypothermia (temp, °C, Table 2) produced by the first challenge with ethanol and the peak hypothermia produced by the second challenge 48 h later. However, when the change from baseline produced by ethanol (ΔT) was used as the measure, all age groups displayed a greater hypothermia with the second ethanol challenge than with the first, an effect in the direction opposite from that of tolerance. This effect may be partially attributable to higher baseline temperatures on day 2. There were no statistically significant differences among groups with regard to the length of time required to

TABLE 1

ETHANOL HYPNOSIS PARAMETERS IN YOUNG, MIDDLE-AGED AND OLD RATS PORTRAYED IN Fig. 1

A. Rapid Tolerance to Ethanol Hypnosis at 48 h

	Young (6)			Middle-Aged (7)			Old (7)		
Day	1	3	14	1	3	14	1	3	14
Weight (g)	199 ± 5	189 ± 4	198 ± 5	242 ± 6	234 ± 6	238 ± 6	286 ± 8	278 ± 8	279 ± 7
LRR (min)	3.0 ± 0.1	2.6 ± 0.2	4.3 ± 0.9	3.0 ± 0.1	2.9 ± 0.1	3.0 ± 0.1	2.9 ± 0.2	3.1 ± 0.2	3.6 ± 0.3
Sleep time (min)	81 ± 11	82 ± 14	59 ± 3	115 ± 9	100 ± 11	100 ± 15	88 ± 6	105 ± 14	104 ± 14
BAC at RRR (mg/dl)	216 ± 18	303 ± 9	272 ± 21	221 ± 9	262 ± 6	257 ± 12	181 ± 9	224 ± 9	193 ± 12

B. Absence of Tolerance With 14-Day Spacing of Challenges

	Young (6)			Middle-aged (6)			Old (8)		
Day	1	3	14	1	3	14	1	3	14
Wt (g)	197 ± 2	No test	193 ± 3	225 ± 5	No test	223 ± 5	296 ± 5	No test	288 ± 5
LRR (min)	2.6 ± 0.1		2.6 ± 0.1	2.8 ± 0.1		3.2 ± 0.2	2.6 ± 0.2		3.4 ± 0.2
Sleep time (min)	122 ± 16		91 ± 11	123 ± 23		101 ± 15	139 ± 10		125 ± 12
BAC at RRR (mg/dl)	245 ± 15		236 ± 9	253 ± 12		230 ± 3	244 ± 8		215 ± 7

C. Rapid Tolerance Using Doses of 3.0 g/kg to All Age Groups

	Young (8)			Middle-aged (8)			Old (9)		
Day	1	3	14	1	3	14	1	3	14
Wt (g)	209 ± 5	221 ± 10	204 ± 4	256 ± 5	254 ± 6	249 ± 5	287 ± 6	263 ± 7	250 ± 7
LRR (min)	2.5 ± 0.1	2.8 ± 0.2	3.5 ± 0.2	3.0 ± 0.1	3.1 ± 0.1	3.2 ± 0.2	2.9 ± 0.3	3.0 ± 0.1	3.3 ± 0.2
Sleep time (min)	105 ± 10	80 ± 13	61 ± 6	170 ± 8	180 ± 12	140 ± 14	218 ± 37	187 ± 12	167 ± 18
BAC at RRR (mg/dl)	231 ± 11	290 ± 10	216 ± 14	249 ± 9	275 ± 17	230 ± 14	214 ± 9	234 ± 12	198 ± 14

Injection schedules and dosing are described in the legend for Fig. 1. After injection, latency to loss of the righting reflex (LRR, min) and recovery of the righting reflex (RRR) were recorded. Sleep time refers to the time elapsing between LRR and RRR. Values shown are ± SEM. The number of animals studied is indicated in parentheses.

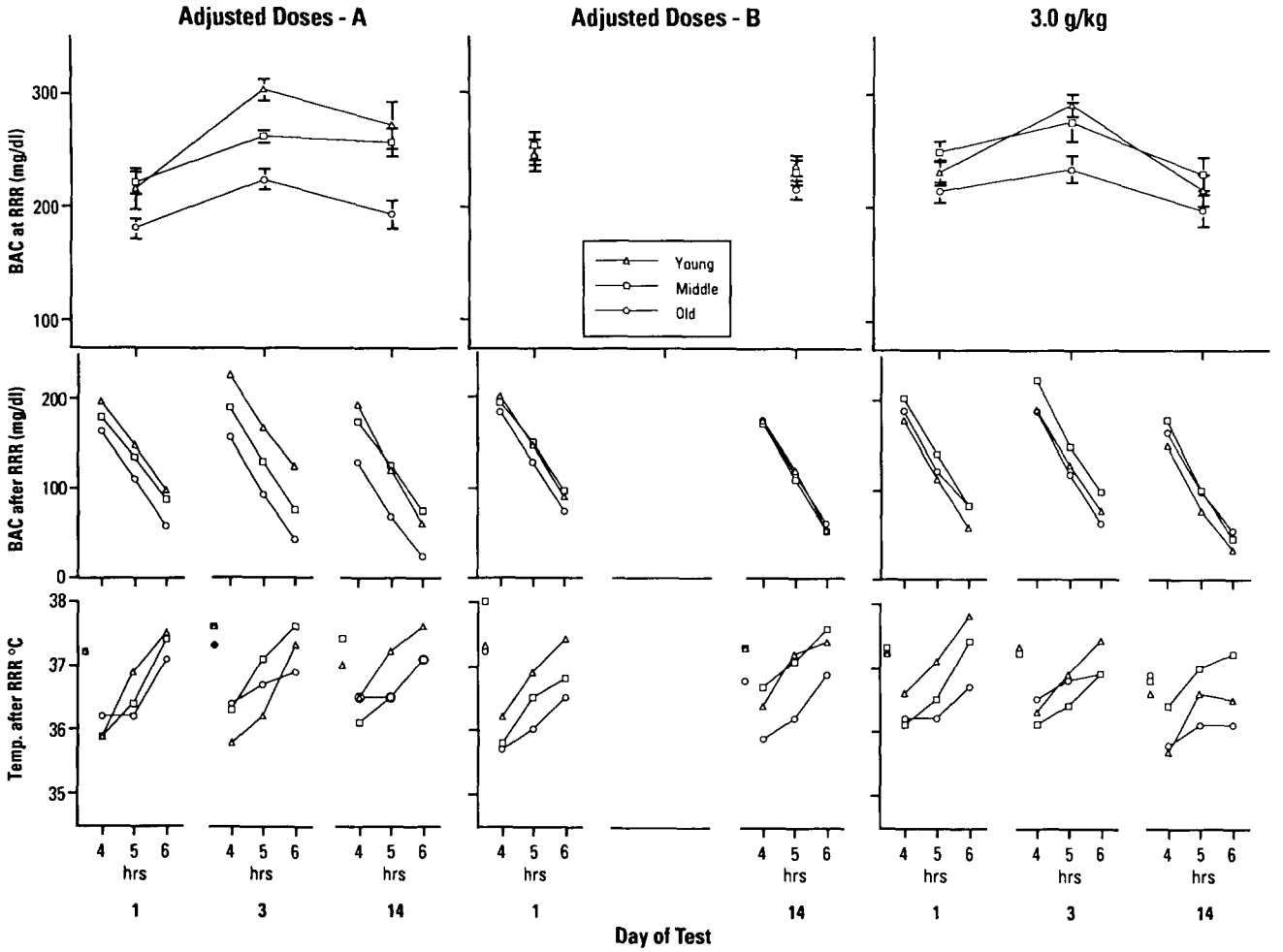

FIG. 1. Rapid tolerance to ethanol hypnosis and accompanying changes in rectal temperatures and blood alcohol disappearance. Rats of three different ages (4, 13, 25 months) were injected at time zero using adjusted doses or a conventional dose approach of 3.0 g/kg to all age groups. Ethanol hypnosis parameters are outlined in Table 1. In Adjusted Doses A, the dose administered on day 1 was given again on day 3 and 14. In B, the doses and procedures were identical to those of A, except that no ethanol was administered on day 3. In the far right sector are data obtained when a 3.0 g/kg dose was given to all age groups on days 1, 3, and 14. Rectal temperature and tail-tip blood samples were obtained after recovery of the righting reflex (4, 5, 6 h). Baseline temperatures obtained immediately before injection are also illustrated to the left of the 4-h values.

reach maximum temperature depression. Tail-tip blood samples obtained in all animals 7 h after injection of the ethanol 2 challenge, as a check on the effectiveness of the dosing scheme in producing equivalent blood alcohol levels, revealed no significant differences among groups (42.2 ± 8.5 mg/dl for young; 48.0 ± 11.0 mg/dl for middle-aged; 31.0 8.7 in old mg/dl).

Conventional Dosages

The extent of rapid tolerance observed using 3.0 g/kg challenges for all age groups (Fig. 1, Table 1) was slightly less than that observed when adjusted doses were employed. The main effect of day of treatment (day 1 vs. day 3) was significant [two-way ANOVA, days of treatment vs. age group, $F(1, 22) = 16.20$, $p < 0.001$]. As with the adjusted doses, this data also yielded a significant interaction [day × age, $F(2, 22) = 4.29$, $p < 0.027$], indicating a larger development of rapid tolerance in young rats. Blood alcohol levels observed after RRR, at 4, 5, and 6 h postinjection, were quite similar in all age groups, and no evidence for metabolic tolerance was observed. Thus, failure of old animals to develop rapid tolerance to the same extent as young rats cannot be attributed to a smaller challenge (BAC or sleep time, Table 2) in old rats on day 1. As in the study on adjusted doses, old animals were again observed to be more sensitive to ethanol hypnosis, showing lower BAC at RRR when age group performance was collapsed across days 1 and 3, $F(2, 22) = 5.99$, $p < 0.01$.

DISCUSSION

To our knowledge, the present report supplies the first observations regarding the influence of age on the development of rapid tolerance to ethanol. Unlike the study utilizing ethanol hypnosis as a measure, the study using maximal ethanol hypothermia as a measure found no evidence for the development of rapid tolerance in any of the three age groups. Thus, different measures may not respond to the same extent in tolerance studies, as others have suggested (13,27). This

TABLE 2

ABSENCE OF RAPID TOLERANCE TO MAXIMAL ETHANOL HYPOTHERMIA

	Young			Middle-Aged			Old		
	Temp (°C)	ΔT (°C)	Time* (h)	Temp (°C)	ΔT (°C)	Time* (h)	Temp (°C)	ΔT (°C)	Time* (h)
Day 1	35.93 ± 0.11	1.43 ± 0.18	2.00 ± 0.33	35.69 ± 0.11	1.18 ± 0.20	2.50 ± 0.28	35.88 ± 0.21	1.10 ± 0.17	2.20 ± 0.33
Day 3	35.57 ± 0.23	1.87 ± 0.22	1.90 ± 0.11	35.68 ± 0.22	1.47 ± 0.22	2.50 ± 0.55	36.10 ± 0.19	1.32 ± 0.08	3.40 ± 0.44

Values shown are ± SE. N = 9 rats in each group. Preinjection temperatures for day 1 were as follows: 37.23 ± 0.10 for young; 36.88 ± 0.17 for middle-aged, and 36.99 ± 0.09 for old. Preinjection temperatures for day 3 were 37.44 ± 0.20 for young; 37.36 ± 0.17 for middle-aged, and 37.39 ± 0.16 for old. ΔT values refer to differences between baseline and maximal hypothermia.

*Time required to reach maximal hypothermia (Temp, °C) after IP doses of 3.5 g/kg (young and middle-aged) and 3.0 g/kg (old rats) on day 1 (day 3) and again 48 h later (ethanol 2).

possibility emphasizes the importance of using more than one measure of tolerance.

The significant main effect of age group when data are collapsed across days in the hypnosis study indicates a greater sensitivity to ethanol in old rats (smaller BACs are required to maintain hypnosis). The association of advancing age with greater tissue sensitivity to ethanol has now been reported in many studies [see (36) for a review of these findings] and would appear to be firmly established. The rapid tolerance data provide additional evidence that older animals suffer the additional disadvantage of possessing lessened capabilities to mobilize adaptive processes (tolerance) that operate to counteract the untoward effects of a repeated exposure to alcohol.

The extent to which age-related differences in acute tolerance may have contributed to the age-related sensitivity differences (BAC at RRR on day 1) we observed cannot be discussed with certainty at this time, although recent observations indicate that the F344 rat does not develop acute tolerance to ethanol hypnosis, using brain ethanol concentrations at LRR and RRR as indices (37). This finding would also cast doubt on the possibility that age-related differences in the proliferation of acute tolerance processes taking place before the second ethanol challenge (day 3) contributed to the rapid tolerance we observed.

The time course of persistence (or decay) of rapid tolerance is now receiving attention in experimental studies. In pioneering observations on mice (8), a persistence of rapid tolerance to ethanol hypothermia of only 24 h was reported. Although rapid tolerance to ethanol hypothermia (2.0 g/kg challenge) in rats was also reported (15), it was followed only for a 24-h period of time. Persistence of rapid tolerance to ethanol-induced motor impairment in rats has been reported for periods up to 48 h in one study (19) and up to 5 days in another (15). The only previous study of rapid tolerance using ethanol hypnosis reported no difference in BAC at RRR at a 24-h test interval in Finnish rats (17). Thus, the present study using a 48-h test interval is the first to report rapid tolerance to the hypnotic effects of ethanol, using BAC at RRR as a measure.

The present study also provides information regarding the time course of rapid tolerance. The tolerance to hypnosis was detectable 48 h after the administration of the tolerance-inducing ethanol challenge, with BAC at RRR averaging (mean across age groups) 131% of the value achieved with the first challenge. When the second challenge was administered 14 days after the first, no tolerance was detectable. A tolerance-enhancing effect of a second "booster" dose was reported by Bitran and Kalant (5), who observed that the tolerance to ethanol motor impairment was extended up to 3 weeks when two challenge doses were administered within 8–24 h in a paradigm that allowed motor "practice" during intoxication.

The findings obtained from the traditional dosing were somewhat similar to those obtained using adjusted doses. That is, all age groups developed rapid tolerance, but the magnitude of the effect in the young was more than double that observed in middle-aged and old rats. Unlike the data obtained with adjusted doses, the data obtained from the 3.0 g/kg challenges did not indicate a persistence of tolerance at the 14-day test in young and middle-age rats. Thus, we cannot conclude from our combined observations that there is a persistence of tolerance to the 14-day point, or that there are age-related differences in the persistence of this particular type of tolerance.

The finding that adjusted doses produced slightly lower BACs in old rats indicated that the adjustments overcompensated for the age-related differences in volume of distribution for ethanol. This was found to be the case also in an earlier study (36). Because of the difficulties in making exact adjustments, we have also presented data using traditional dosing schemes (3.0 g/kg to all age groups) based upon total body weight, with the expectation that, if anything, this approach would bias the experiment in a conservative direction, that is, in the direction of higher BACs in old rats, favoring a greater development of tolerance. We wish to emphasize once again, however, that the validity of the sleep time studies, relying upon BAC at RRR as the key measure, does not require identical blood alcohol disappearance curves in all age groups.

We were unable to detect any rapid tolerance to maximal ethanol hypothermia when ethanol challenges were separated by 48 h. Perhaps rapid tolerance to maximal ethanol hypothermia is more short-lived than rapid tolerance to ethanol hypnosis, and requires a 24-h spacing for its detection, as others have reported (see above). The available evidence suggests that the extent and duration of rapid tolerance are dependent upon a variety of subject characteristics (age, gender, species, and strain), as well as drug dosage and the biological or behavioral system studied.

These findings on age decrements in rapid tolerance reinforce prevailing notions regarding losses with age in plasticity and adaptability of neural tissue. For instance, losses with age in learning and short-term memory capabilities have been well documented. The possibility that similar neural processes underlie tolerance, learning, and memory is now receiving increased attention (13).

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