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Increased Responsiveness to Ethanol with Advancing Age in Rats

JAMES L. YORK

Research Institute on Alcoholism, New York State Division of Alcoholism and Alcohol Abuse Buffalo, NY 14203

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YORK, J. L. *Increased responsiveness to ethanol with advancing age in rats.* PHARMACOL BICHEM BEHAV 19(4) 687–691, 1983.—Male CD strain rats of three different ages (young—5 months; middle—15 months; old—27 months) were tested for their responsiveness to doses of ethanol sufficient to produce hypothermia or hypnosis. Comparison dosages of ethanol across age groups were based upon the estimated equivalent dilution of the drug into the body water compartments of subjects. In the hypnosis study, there were no statistically significant differences among the groups with regard to the time elapsed until the righting reflex was lost or in total sleep time. However, old animals recovered the righting reflex in the presence of lower blood ethanol concentrations than those observed for young and middle animals, suggesting a greater sensitivity of target tissues to the hypnotic effects of ethanol in old rats. The responsiveness of old rats to the hypothermic effect of ethanol was greater than that of younger rats only when the experiment was conducted at an ambient room temperature of 10°C.

Ethanol Age Rats Body water Hypothermia Hypnosis

PHYSIOLOGICAL changes accompanying the aging process may result in an altered responsiveness of subjects to the pharmacological actions of drugs such as ethanol. Studies in man and in laboratory animals indicate that older subjects are, in general, more responsive to the disruptive effects of ethanol than are younger subjects, particularly when comparisons are made among subjects of different ages by giving doses of ethanol based upon the total body weight (g ethanol/kg body weight) of subjects [1, 6, 16, 17, 18]. When such a procedure is employed, higher peak blood levels of ethanol are often achieved in order subjects, owing to changes in body composition with age [23]. Thus, age-related changes in responsiveness to drugs such as ethanol can, in some instances, be attributed to alterations in drug disposition with age. However, significant age-related differences in the response to ethanol have been reported in cases where no significant differences were observed in blood ethanol concentrations [20].

An important current issue in aging research concerns whether the responsiveness of the critical target tissue mediating the response to ethanol is also altered with aging. One method of obtaining meaningful information on this issue is to measure the responsiveness of subjects (of different ages) to the effects of ethanol when target tissues are presumably exposed to similar concentrations of drug across age groups. Inasmuch as ethanol appears to be distributed almost exclusively into the body water compartments [9,11], an estimate of total body water also serves as an estimate of the volume of distribution for ethanol in rats of different ages. Dosages calculated to produce similar peak concentrations of ethanol in body water compartments (including blood) across age groups allows for the assessment of age-related differences in tissue or organ system sensitivities to the effects of that drug, providing that differences in other pharmacokinetic parameters do not exist among the age groups. Indeed, no significant differences in the hypothermic or ataxic effects of ethanol were observed between young and old rats when ethanol was administered in such a manner as to produce similar blood ethanol disappearance curves in female rats of different ages [1,22]. One goal of the present investigation was to assess age-related responsiveness of rats to the hypnotic effects of ethanol, using body water estimations to determine doses across age groups.

Another goal of the present study was to determine if exposure of animals to cool room temperatures during ethanol intoxication could precipitate age-related differences in responsiveness to ethanol hypothermia. The risk of accidental hypothermia appears to increase with advancing age in man [2,3] and may also be increased by pharmacological agents, including ethanol [10,13]. Although healthy rats of different ages may be capable of maintaining normal body temperatures when the ambient room temperature fluctuates, older animals may display a greater loss in the ability to adapt to temperature extremes when challenged with pharmacological agents [4,15].

METHOD

Subjects

Male CD strain rats (22 each, 3 months and 12 months of age) were obtained from the Carworth Division of Charles River breeding farms, Wilmington, MA. Those two groups comprised the groups hereafter referred to as "young" and "middle." The "old" group (24 months of age) was comprised of retired breeders that had been obtained 12 months earlier from the supplier and had been housed in the animal

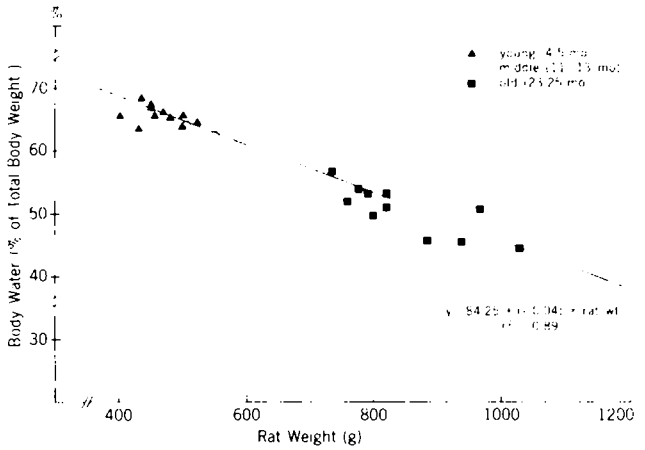

FIG. 1. Body water content of individual rats of different ages. Body water content was determined by lyophilization in male CD strain rats of three different ages (N = 11 each) (see the Method section). Abscissa: the weight of the rat immediately after sacrificing with a lethal dose of sodium pentobarbital. Ordinate: the ratio: body water removed by lyophilization/initial body weight. The linear regression was determined by the "least squares" method.

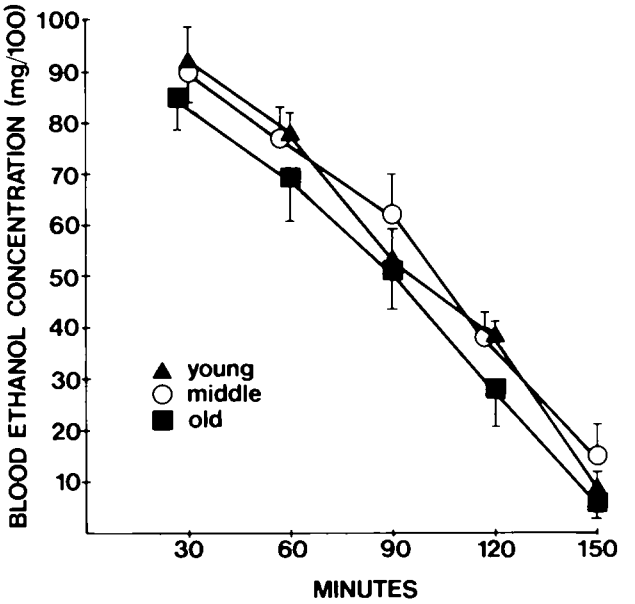

FIG. 2. Blood ethanol disappearance in young, middle, and old rats (N = 10 each). At time zero, all animals were given an intraperitoneal injection of ethanol (10% w/v in saline) in a dose estimated to produce a peak ideal body water concentration of ethanol of 1.53 mg/ml (see the Method section). Tail-tip blood samples were obtained at 30-minutes intervals up to 150 minutes postinjection (abscissa) and prepared for gas chromatographic analysis of blood ethanol concentration (ordinate). Brackets indicate positive or negative aspect of the standard error of the mean.

care facilities of the Research Institute on Alcoholism. All animals were housed individually in polycarbonate cages (23 cm h × 25 cm w × 46 cm l) under a 12-hour light-darkness cycle (21–23°C) and were allowed free access to drinking water and Teklad rat and mouse diet.

Estimations of Body Water Content and Dosages of Ethanol

The procedure used to estimate body water composition was similar to that previously described for studies on female CD strain rats [22]. Eleven subjects, selected at random from each of the three age groups, were sacrificed with injections of sodium pentobarbital, frozen, and then cut into four sections of roughly equivalent weight, consisting of head and neck, shoulders, midsection, and hindquarters. These sections were subjected to lyophilization for 12 days, and serial weighings were made to ensure that the removal of body water was complete. A linear regression performed on the relationship between initial body weight and the contribution of total body water to body weight (%body water) for all age groups yielded a good straight line fit (least squares method) to the data from which estimations of the body water content of live animals could be obtained, using body weight as the predictive variable. Comparison dosages of ethanol for all animals were estimated to produce an "ideal" peak dilution of ethanol in body water of 1.53, 3.06, 4.59, or 6.12 mg ethanol/ml body water. (In an "ideal" system, complete absorbtion and distribution occur rapidly in the absence of elimination.) Since we observed no difference in the rate of elimination of ethanol among age groups in a previous study [22], blood levels of ethanol were expected to be comparable among age groups throughout the time course of drug action. All doses of ethanol were administered intraperitoneally, using 10% w/v ethanol in saline solution.

Ethanol Hypothermia

Rectal temperature was determined with the aid of a Yellow Springs Instrument Co. Model 49TA Telethermometer fitted with a small animal thermistor probe (Model No. 423) which was inserted 6 cm into the rectal orifice for approximately 40 seconds to obtain readings. Measurements of ethanol hypothermia were made at ambient room temperatures of 22°C, the usual room temperature in the rat's homeroom, and at 10°C in a large walk-in cold room available in our research facility. Animals were returned to their usual position on the shelving rack after injection and between temperature measurements, as we have observed that body temperature may increase if animal cages are allowed to remain upon the countertops [24]. In the case of cold-room measurements, animals were given a 60-minute acclimitization before injections were made.

Ethanol Hypnosis

Hypnosis (loss of the righting reflex) was induced by an injection of ethanol estimated to produce a peak ideal dilution of ethanol in body water of 6.13 mg/ml, roughly equivalent to a conventional dose of 4.0 g/kg. Times to loss of the righting reflex and to recovery of the righting reflex were recorded. The righting reflex was considered to be intact if, on two successive trials, the animal succeeded in returning to all four feet within 10 seconds after being placed upon its back. Immediately after recovery of the righting reflex, tail-tip blood samples were taken and were prepared for gas chromatographic analysis of blood ethanol concentration according to the procedure outlined by York [22]. Propanol (internal standard, 20 μl, 100 mg/dl) was added to a 20 μl sample of tail-tip blood contained in a 1 ml sample tube. Zinc sulfate (10 μl, 5% solution) and barium hydroxide (10 μl, satu-

TABLE 1

RESPONSE TO ETHANOL HYPNOSIS IN MALE RATS OF DIFFERENT AGES DOSED ON THE BASIS OF ESTIMATED BODY WATER CONTENT

Age	N	Weight (g ± SEM)	Estimated Body Water (ml ± SEM)	LRR (Min ± SEM)	Sleep Time (Min ± SEM)	Blood Ethanol at Waking (mg 100 ml)
Young (5 months)	9	550 ± 19	345 ± 8	3.83 ± 0.16	483 ± 19	286 ± 12
Middle (15 months)	10	611 ± 26	368 ± 9	3.60 ± 0.14	503 ± 21	264 ± 9
Old (27 months)	6	845 ± 51	430 ± 9	4.12 ± 0.34	486 ± 30	215 ± 24*†

Male CD strain rats were injected (IP) with a dose of ethanol estimated to produce an ideal body water concentration of ethanol of 613 mg/100 ml. LRR indicates time to loss of the righting reflex. Sleep time refers to time between the loss and the recovery of the righting reflex. Immediately after recovery of the righting reflex, tail-tip blood samples were taken and analyzed for ethanol concentrations. Note that less ethanol in the blood is required to maintain loss of the righting reflex in old rats.

*$p = 0.025$ as compared to middle; †$p = 0.003$ as compared to young; Students t-tests.

rated solution) were added to precipitate protein. The sample was then vortexed and centrifuged at 3,000 g for 15 minutes. The supernatant was subjected to gas chromatographic analysis by direct injection, in duplicate.

RESULTS

Body Water Analysis and Ethanol Dosages

The relationship between body weight and body water content for young, middle, and old male rats is illustrated in Fig. 1. Mean body water content (% of total body weight) was found to be 65.60±0.41% for young, 62.85±0.63% for middle, and 50.52±1.18% for old rats, N=11 each. There was virtually no overlap in the body weights for the three groups. The data were nicely described by a straight line (linear regression, least squares method), with the equation: $y = 84.25 + (-0.04) \times$ rat weight (g), and $r^2 = 0.89$ (It should be noted that within any age group, particularly the young and the middle groups, the correlation of body weight to body water is much reduced. Thus, the high correlation across all three age groups would appear to receive a large contribution from body changes that take place over periods of several months, i.e., primarily the deposition of body fat.) Weight differences within the group of old animals are sizeable, and body weight is more highly correlated to body water content.

Owing to the goodness of fit of the data to the straight line, that relationship was used to predict body water content in living animals taken from the same population, using body weight as the predictive variable. Body water content estimated in this manner proved to provide a good estimate of the volume of distribution for ethanol in animals of different ages, as the data in Fig. 2 suggest. There were no statistically significant differences in blood ethanol concentrations among the three groups.

Response to Ethanol Hypothermia

There were no statistically significant differences among the three groups with regard to time to loss of the righting reflex or sleep time (Table 1). Young and middle animals also did not differ with regard to blood ethanol at waking. Old animals, however, recovered the righting reflex in the presence of significantly lower blood ethanol levels than those observed for young and middle animals ($p < 0.01$ ANOVA, followed by post hoc t-tests). Rectal temperatures at 600 minutes postinjection were 36.53±0.27 (young), 36.46±0.20 (middle), and 36.20±0.20 (old) and were not significantly different.

There was an unusual amount of variability in the responsiveness to ethanol hypothermia in this study. When tested at the usual homeroom environmental temperature (22°C), middle-age animals displayed a significant trend in the direction of least responsiveness to ethanol hypothermia, and young animals displayed the greatest response to ethanol hypothermia for the two highest doses of ethanol tested (Fig. 3A). When tested at an environmental temperature of 10°C, an increased responsiveness to ethanol hypothermia was noticed in all groups. The responsiveness of the young and middle groups did not differ from each other for any of the three doses tested. Old animals were significantly more affected by ethanol hypothermia for all three doses of drug tested at the 10°C ambient temperature ($p < 0.01$ ANOVA, repeated measures, Fig. 3B). In contrast to the findings of Finch and co-workers [7], we found no significant influence of exposure to cold alone on the body temperature of any of the three groups of animals studied.

DISCUSSION

Body water analyses performed on male CD strain rats ranging from 4 to 25 months of age have yielded a fairly linear relationship between body weight (which increases with advancing age) and the contribution (percentage) of body water to total body weight. A similar relationship has been previously demonstrated in female CD strain rats 5–26 months of age [22]. Increased adiposity with age would appear to play a predominant role in the expression of the decreased contribution of body water to total body weight as age and body weight advance. In man, in contrast, the percentage contribution of total body water to body weight also decreases with age (approximately a 7% difference between subjects 17–34 and 57–86 years of age), but marked increases

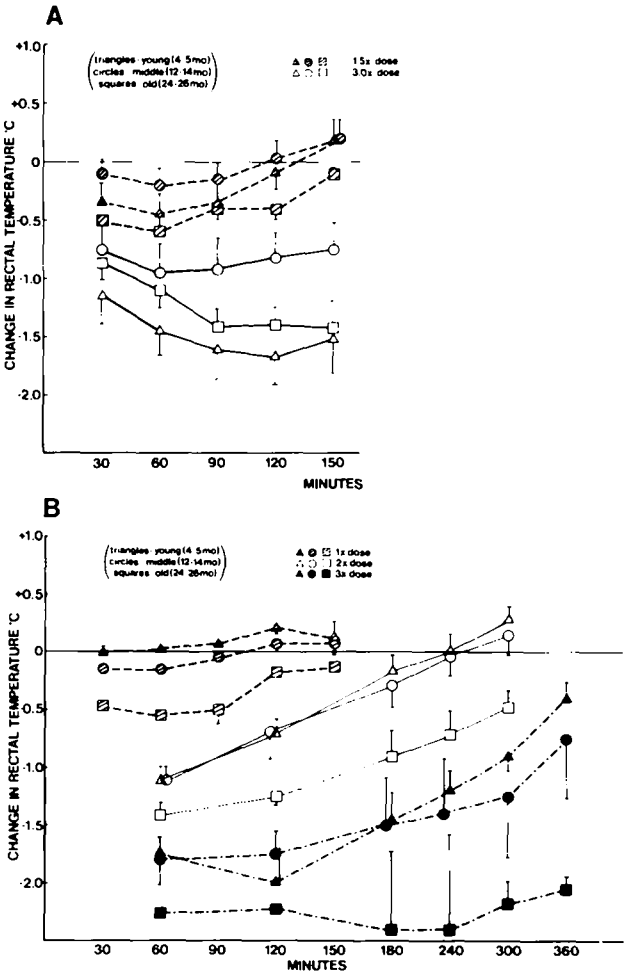

FIG. 3. Ethanol hypothermia in rats of different ages at two different ambient room temperatures (A—22°C; B—10°C). Subjects were injected intraperitoneally at time zero with ethanol (10% w/v in saline) in doses estimated to produce peak body water concentrations of 1.53 (1×), 3.06 (2×), or 4.59 (3×) mg/ml of ethanol. Values along the abscissa indicate minutes elapsed since drug injection. The change in rectal temperature was calculated with reference to the temperature reading obtained immediately prior to injection (time 0). Representative Temperatures at time zero were 37.61±0.09 (young), 37.45±0.10 (middle), and 37.40±0.08 (old) for Fig. 3A and 37.29±0.11 (young), 37.26±0.13 (middle), and 36.80±0.14 (old) for Fig. 3B. Brackets indicate positive or negative aspect of the standard error of the mean. N = 10 young, 9 middle, 6 old.

in body weight with age are not observed [5]. In contrast to the CD strain rat, the Fischer 344 rat, which the National Institute on Aging recommends for aging Studies, does not display marked changes in body weight with age and is considered the preferred rat for many kinds of aging studies [8].

The use of the linear regression (% body water vs. body weight) to predict the volume of distribution for ethanol in live animals resulted in roughly similar blood ethanol disappearance curves in all three age groups. Blood alcohol levels for old animals were slightly, but consistently, lower throughout the entire ethanol-disappearance phase, suggesting that the volume of distribution for ethanol was slightly underestimated in that group. A similar finding in female CD strain rats has been reported [23].

The observation that old animals recovered the righting reflex in the presence of lower blood ethanol levels than those observed for young and middle rats suggests a greater sensitivity of target tissue to the hypnotic effects of ethanol in old rats, or alternatively, a less rapid development of acute tolerance in old rats, or a combination of those influences. Of course, this reasoning derives from the assumption that the concentration of ethanol in important brain tissues closely parallels the concentration of ethanol in the blood after the absorptive phase [11,12]. Lower blood levels of ethanol at waking in old rats suggest that blood ethanol values throughout the entire course of the sleep time study may have been lower in old rats than in young or middle rats, owing either to a systematic error in the estimation of body water content or to unequal clearances of ethanol at high doses. Irregardless of these eventualities, the interpretation from the sleep time studies of greater sensitivity to ethanol in old rats is still reasonable. The suggestion of greater sensitivity to the hypnotic effects of ethanol with age in male CD rats is consistent with an earlier finding in female CD rats [1] and observations in mice [14,19].

Other measures of ethanol impairment have not unanimously demonstrated age-related differences. No differences in the effects of ethanol on rotarod performance [1] or hypothermia [22] have been reported when similar blood alcohol levels are achieved in different age groups at the time of testing. Earlier reports of age-related differences in responsiveness to ethanol have not always controlled for blood ethanol levels [24]. We believe the present study demonstrates that old male rats are more responsive to ethanol hypothermia than young and middle-age rats when tested at a below-normal ambient room temperature. Even through blood concentrations of ethanol or ethanol clearances may not have been perfectly equal in all comparison groups, the same methods for administering doses were employed in the cold-room study (Fig. 3B) as in the normal room temperature observation (Fig. 3A). Yet only the cold-room study revealed a clear tendency for older animals to be more affected by ethanol hypothermia.

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