AGE-DIFFERENCES IN BRAIN SENSITIVITY AND TOLERANCE TO ETHANOL IN MICE

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Abstract

The present study was designed to evaluate the effect of an acute injection of ethanol on the onset and the duration of the loss of righting response in mice of three different ages, 6, 12, and 24 months old. Measurements were made of the brain ethanol concentrations at the time the righting response was lost as an index of initial central nervous system sensitivity to ethanol. Brain ethanol levels were also measured at the time the righting response was regained. By comparing the difference between these levels and the levels at the time the response was lost, the ability to develop acute tolerance was determined. The rate at which ethanol appeared in the brain, as well and as the drug's accumulation in and disappearance from the blood, was also measured to determine drug uptake and metabolism in the three age groups. The results indicate that old mice: are more sensitive to the central nervous system effects of ethanol, have a slower rate of ethanol uptake into both blood and brain, and metabolize ethanol more slowly than 6 and 12 month old mice. Twelve month old mice differ from 6 month old mice only in the rate at which ethanol is metabolized. All three groups of mice were equally capable of developing acute tolerance to ethanol.

Introduction

Several recent reports have indicated that aged organisms differ in their behavioral and physiological responses to ethanol as compared to young or adult members of the same species. Age-induced changes in the response to an acute dose of ethanol may be due to a number of factors including alterations in: the uptake and distribution of the drug; the rate at which the drug is metabolized; and in regards to behavioral responses, either differences in central nervous system (CNS) sensitivity, or the ability of the CNS to adapt to the presence of the compound i.e., the development of acute tolerance (1). Changes in blood flow (2), and membrane permeability (3) have been reported to occur during aging that could result in differences in the uptake and distribution of ethanol in animals during different stages of development. There have also been reports of a decrease in the rate of ethanol metabolism during aging (4). Concomitantly, there has been shown to be a decrease in enzyme activity in the liver, the primary site of ethanol metabolism, in old as compared to young rats (5). There have been several reports of an increase in the behavioral response to ethanol during aging (6-8). However, these behavioral studies have measured the response to ethanol at a time subsequent to the injection of the drug which corresponds to the descending phase of the brain ethanol concentration curve, therefore making it impossible to differentiate between alterations in initial CNS sensitivity and changes in the ability to develop acute tolerance to ethanol. The present study is designed to determine age differences in CNS sensitivity and the ability to develop acute tolerance to ethanol as well as the involvement of these two aspects of CNS function in the observed alterations of the behavioral response to ethanol which occur during aging. In addition, we also examined the relative contribution that changes in uptake, distribution, and metabolism of the drug may have in the age-induced differences in the response to ethanol.

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Results and Discussion

The analysis of the effect of ethanol on the righting response in mice of the 3 age groups was performed by multiple t-test following significant ANOVA. Ethanol uptake and clearance rates were calculated from regression lines fitted to the data. The slopes of the regression lines were compared by t-test with the aid of a PDP-10 computer program.

There was no significant difference in the time, after the injection of ethanol, at which any of the 3 groups of mice lost the righting response ($P > 0.05$) (Table 1). However, the determination of brain ethanol levels at the time the righting response was lost (Table 1) revealed that the old mice lost the righting reflex at significantly ($P < 0.05$) lower brain ethanol levels than were present in either adult or young mice at the time they lost the response. These results indicate that the old mice had a greater CNS sensitivity than either of the other 2 groups of mice. Since there

TABLE I THE EFFECT OF AN INJECTION OF ETHANOL ON THE LOSS OF RIGHTING RESPONSE (LRR) AND REGAINING OF THE RIGHTING RESPONSE (RRR) IN MICE OF DIFFERENT AGES

	TIME (MINUTES)		BRAIN ETHANOL (mg/g)	
Age Group	LRR	RRR	LRR	RRR
6 Months	2.10 ± 0.35[a](18)[b]	30.14 ± 8.89(9)	2.09 - 0.31(6)	2.63 ± 0.45(6)
12 Months	1.86 ± 0.36(17)	68.16 ± 24.40(9)	2.09 ± 0.31(6)	2.64 ± 0.57(6)
24 Months	2.01 ± 0.34(17)	42.62 ± 18.17(9)	1.47 ± 0.17(6)	2.61 ± 0.52(6)

[a]Standard deviation.

[b]Number of subjects.

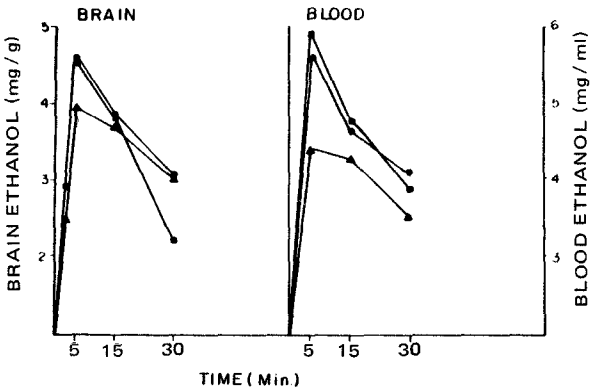

Fig. 1. Old (▲—▲), Adult (●—●), and Young (■—■) mice were injected with ethanol (3g/kg). Brain and blood ethanol levels were determined at various times thereafter. Each data point represents at least 6 determinations.

was no difference in the time after injection when the response was lost, there would appear to be a difference in the rate at which ethanol is taken up into brain in mice of different ages; see below and Fig. 1. The analysis of the duration of the loss of the righting response indicated that the adult mice lost the righting response for a significantly longer period of time than the young mice (P 0.01). However, there were no differences in brain ethanol levels at the time the response was regained (Table 1). All 3 groups of mice regained the righting response at higher brain ethanol levels than were present at the time the response was lost, indicating that there was no impairment in the ability of the CNS to adapt to the presence of ethanol in these mice. The difference in the duration of the loss of the response coupled with the finding that there was no difference in brain ethanol levels at the time the response was regained indicates that there is a difference in the rate at which ethanol is metabolized among these groups of mice.

Both brain and blood ethanol levels were significantly lower in old mice 5 minutes post injection than in the other two groups ($P < 0.05$). At 30 minutes there was no longer any difference between old and adult mice while young mice had lower blood ethanol levels at this time (Fig. 1). The initial lower ethanol levels in the old mice in both brain and blood probably reflect a slower rate of absorption from the peritoneal cavity in these animals than in the other groups. The more rapid decrease in blood ethanol levels during the 30 minutes after the injection of ethanol found in the young mice is probably due to a faster rate of metabolism in this age group compared to the other groups. This premise is supported by the rates of ethanol clearance, between 1 and 4 hours post injection. Young mice were found to clear ethanol at a rate of 0.53 μmoles/min/gram, while adult and old mice were found to clear ethanol at rates of 0.46 and 0.37 μmoles/min/gram respectively. The clearance rate in old mice was significantly lower ($P < 0.05$) than the rate for young mice.

These data indicate that ethanol is taken up in brain to a lesser amount in old mice compared to adult or young mice; however, due to the similar results found in blood this appears to be a peripheral effect. There also appears to be a reduction in the rate at which ethanol is cleared from the blood with increasing age. These findings are in agreement with previous reports of a decrease in ethanol metabolism in aged organisms (8). Neither of these factors could adequately account for the observed changes in the behavioral response to ethanol which occur during aging. The analysis of the effects of ethanol on the righting response indicates that the increase in the response to ethanol in old mice appears to be due to an increase in the CNS sensitivity to the drug rather than a change in the ability to develop acute tolerance to the drug.

Experimental Procedures

Male C57Bl/6 mice in 3 age groups were used in these studies: Young (6 months old), adult (12 months old), and old (24 months old). Mice were housed 5-6 per cage in light (900-1700 hours) and temperature (23 ± 2 °C) controlled rooms and had *ad lib.* access to food (Purina Lab Chow) and water.

Ethanol was injected intraperitoneally at a dose of 3 g/kg, and mice were monitored for the time to the loss of the righting response and the duration of the loss of the righting response (9). Loss of righting response was operationally defined as the inability of a mouse to right itself for at least 30 seconds after being placed in its back. After losing the righting response, mice were immediately decapitated for measurement of brain ethanol levels or were placed on their backs in sleeping troughs (V-shaped plastic troughs) and observed continuously until they regained the righting response. A mouse was judged to have regained the righting response if, upon being repeatedly placed on its back, it was able to right itself 3 times within 30 seconds. Certain groups of mice were decapitated at the time they regained the righting response and brain ethanol levels were determined at this time. To determine ethanol uptake and disappearance, brain and blood samples were obtained at various times after the injection of ethanol in mice not used for the assessment of the effects of ethanol on the righting response.

For the determination of brain ethanol levels, brains were quickly removed, weighed, and homogenized in 2 ml. of ice cold 0.6 N perchloric acid with 25 mM thiourea. Blood samples, 20 μl, were obtained from the tail vein and added to 1ml of 0.6 N perchloric acid with 25 mM thiourea. Brain

and blood ethanol levels were quantitated by gas chromatography as previously described (10).

Acknowledgements:

Supported in part by Campus Research Grant 201-3878-302 to R. F. Ritzmann and Grant AG00871 from the National Institute of Aging to A.D. Springer.

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