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Oxidation of Alcohol in Free-Moving Mice from High and Low Preference Strains[1]

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DUCKETT, S., C. W. SCHNEIDER AND R. A. HARTLINE. *Oxidation of alcohol in free-moving mice from high and low preference strains.* PHARMAC. BIOCHEM. BEHAV. 15(3) 495–499, 1981.—Free-moving mice from the high-alcohol preference C57BL/6J strain and the low-preference DBA/2J strain were slowly fed [2-^{14}C]ethanol intragastrically until anesthesia was achieved. Behavior was monitored in a Plexiglas metabolic chamber while $^{14}CO_2$ was simultaneously trapped to determine the rate of ethanol metabolism. Average time to the loss of the righting reflex in the DBA/2J was 21.9 min and 27.9 min for the C57BL/6J strain ($p<0.005$). Elimination of $^{14}CO_2$ was slightly higher (n.s.) in the DBA/2J strain for the entire monitoring period. Infusion of ethanol via the tail vein yielded identical results indicating that the slower elimination rate in the C57BL/6J strain could not be the result of slower absorption across the gut wall. Infusion via the tail vein with radioactive sodium bicarbonate indicated that the DBA/2J strain has a higher rate of CO_2 expiration (n.s.). Consequently, the higher rate of $^{14}CO_2$ expiration from ethanol oxidation may not reflect a higher rate of metabolism. These results are discussed in terms of the apparent differences between these strains in neural sensitivity to ethanol.

Ethanol Metabolism Free-moving mice Intragastric infusion

DIFFERENTIAL alcohol selection among inbred mouse strains has been well-established [18]. One strain, C57BL, is known for its tendency to consume ethanol over water while another strain, DBA/2, almost totally avoids ethanol. Similar patterns of consumption have been observed in these strains with some C_3 [9, 21, 25] and C_4 alcohols [5]. In addition to their differential selection of alcohols these inbred strains also manifest differences in tolerance. Strains with low alcohol preference sleep twice as long as strains with high preference following an intraperitoneal anesthetic dose of alcohol [9, 11, 12, 16]. Compared with several low-preferring strains, the C57BL displays greater resistance to decrement in nest-building when forced to drink 10% ethanol and in the jaw-jerk reflex when ethanol is rapidly infused intraperitoneally [21,22].

Whether observed differences in tolerance are due to metabolic processes, inherent differences in neural response mechanisms or both is not clear. Strain differences in the in vivo and in vitro conversion of alcohol to acetaldehyde are quite small [2, 11, 12, 17, 20, 21, 24, 29]. Some studies indicate that disappearance of acetaldehyde from the blood is more rapid in the C57BL than the DBA/2 strain [19, 21, 23]. This could account for differential tolerance and preference.

The experimental approach in numerous investigations has been to introduce the drug in single large anesthetic or near-toxic doses via a non-oral route. Because of the route of administration and the quantity administered the significance of previous findings is difficult to evaluate. Furthermore, as previously pointed out [22], results obtained under these laboratory conditions may have little relevance for processes that occur under conditions where a free-moving animal voluntarily consumes alcohol. Since single large injections of alcohol could obscure, exaggerate, or decrease metabolic differences because of shifts in the acid base balance, drastic localized changes in the NAD to NADH ratio, and abnormal respiration and heart rate, we have developed a method of slow infusion of ethanol via the stomach in free-moving animals. This method more closely approximates natural consumption and allows minute by minute monitoring of behavioral changes simultaneously with gradual administration of alcohol. Furthermore, in contrast to single injections, the rate of expired $^{14}CO_2$ is an estimate of the rate of oxidation of a compound by the body if that rate is constant or near constant during continuous infusion of the labeled compound [14]. Since the rate-determining step in ethanol metabolism is its oxidation by alcohol dehydrogenase [10, 15, 28] the rate

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of $^{14}CO_2$ expiration during continuous infusion of [2-^{14}C]ethanol should correlate with the in vivo rate of alcohol dehydrogenase activity.

METHOD

Subjects

Fifty-five male mice from each of two strains (C57BL/6J and DBA/2J) were used to assess the behavioral and metabolic effects of infused ethanol. All mice were obtained from the Jackson Laboratory, Bar Harbor, ME, and were 60–65 days of age upon arrival and 80–85 days old at the beginning of the experiment. The animals, housed in a windowless room with a 68°F temperature and an 8 a.m. to 5 p.m. light period held constant, were maintained on standard Purina mouse chow.

Procedure

A 30% ethanol solution was prepared as follows: 250 μCi of [2-^{14}C]ethanol (ICN) under vacuum in a break-seal ampoule (Type PI) was removed from the ampoule by dilution with 2.5 ml of 95% unlabeled ethanol. The 2.5 ml of radioactive ethanol, further diluted with 10 ml of 95% ethanol, constituted the stock solution which was stored at

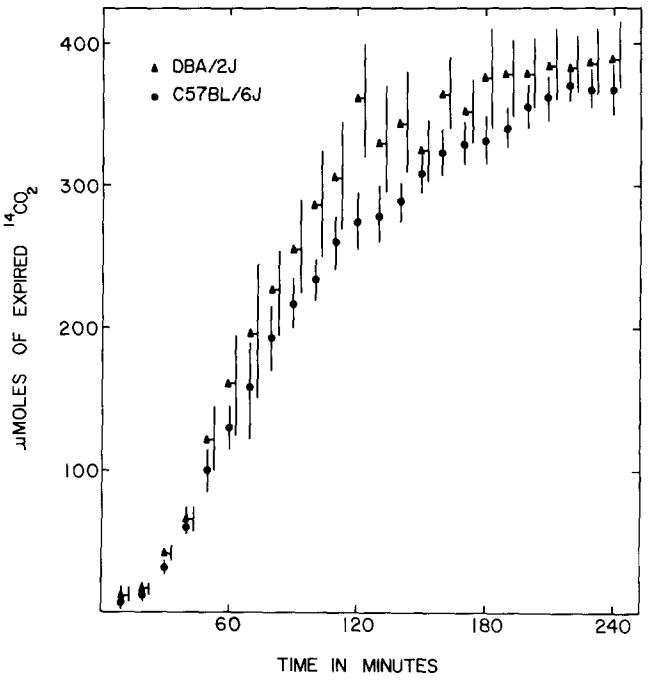

FIG. 1. Expired $^{14}CO_2$ after intragastric infusion of [2-^{14}C]ethanol. Samples taken at 10 min intervals. Vertical lines indicate S.D.

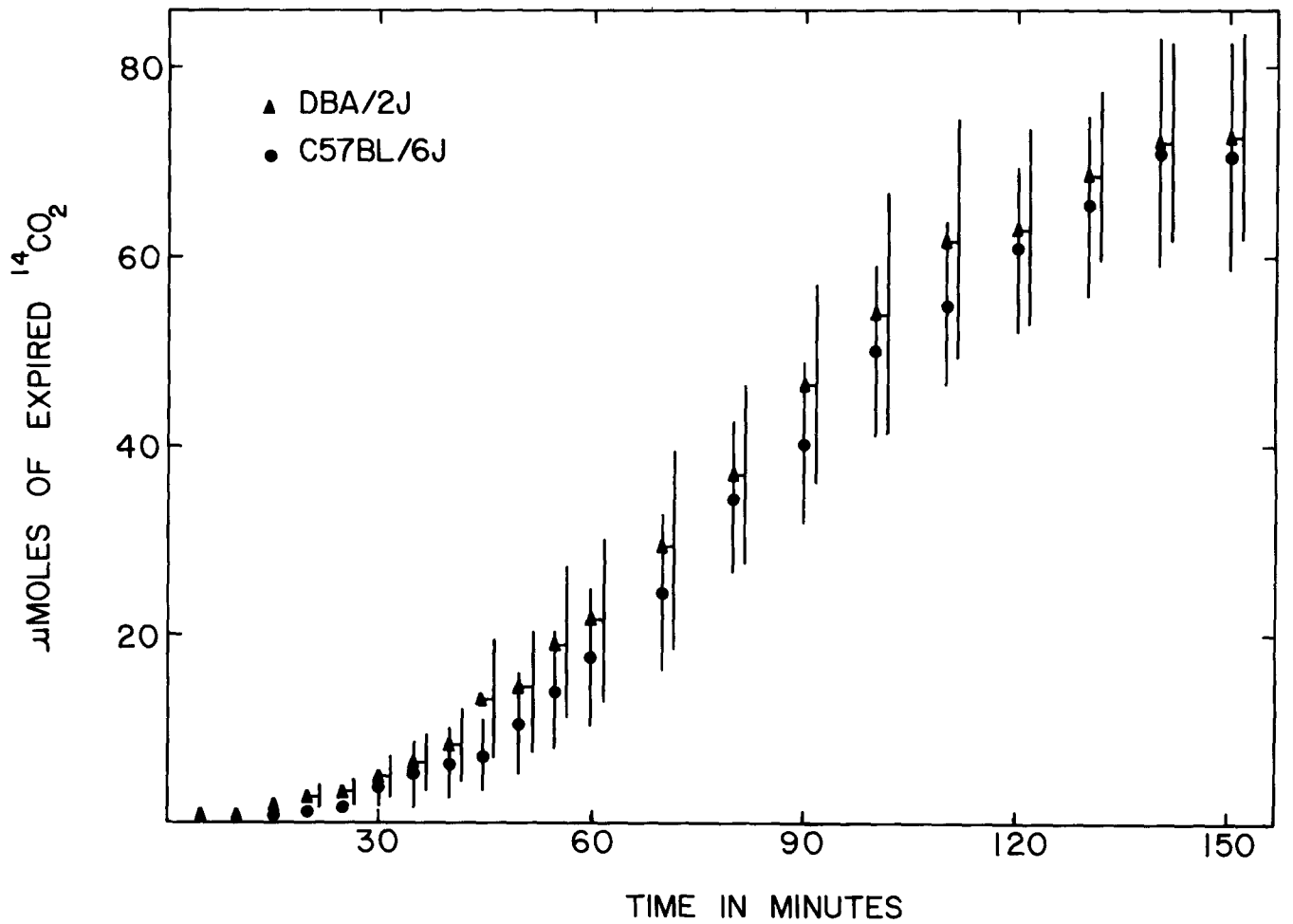

FIG. 2. Expired $^{14}CO_2$ after infusion via the tail vein of [2-^{14}C]ethanol. Vertical lines indicate S.D.

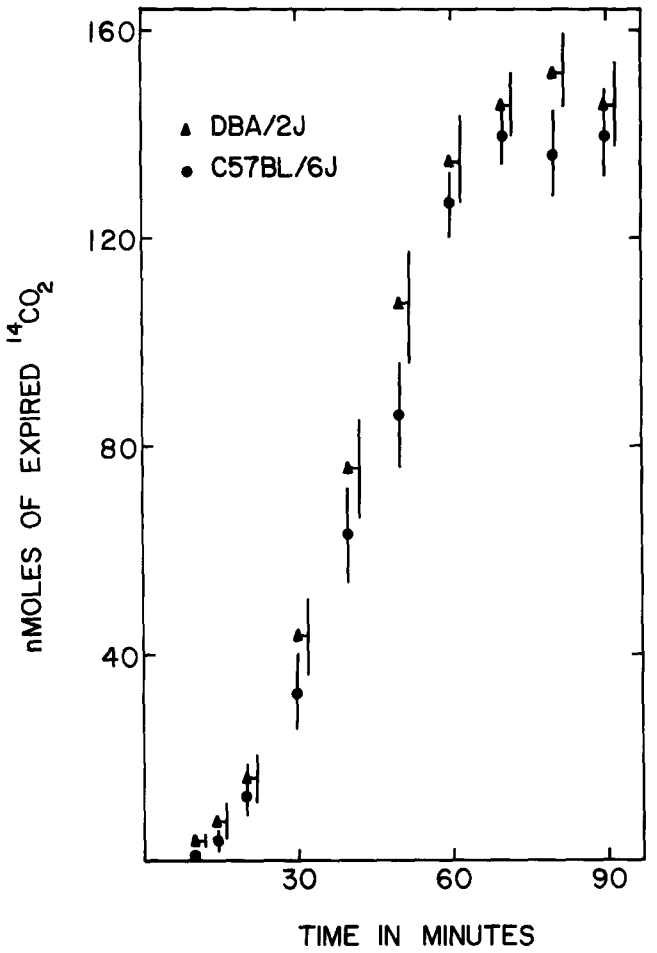

FIG. 3. Expired $^{14}CO_2$ after infusion via the tail vein of radioactive sodium bicarbonate. Vertical lines indicate S.D.

−5°C. For experimental use the stock solution was diluted with distilled water or physiological saline to provide a 30% v/v ethanol solution containing [2-^{14}C]ethanol at the specific activities indicated.

Mice from each strain were divided into five groups: 15 were infused with 30% ethanol (distilled water) intragastrically (19 μl/min) while moving in an open field until the righting reflex was lost; 10 were placed individually in a metabolic chamber and were infused intragastrically with 30% [2-^{14}C]ethanol (specific activity 1.1 Ci/mole) at a rate of 19 μl/min for 30 min; 10 animals from each strain were placed in the chamber and infused (0.19 μl/min) through the tail vein with 100 μl of a 30% solution of [2-^{14}C]ethanol (physiological saline; specific activity 5.8 Ci/mole) in order to compare with infusion intragastrically to determine if differences between strains existed in passage through the gut wall; 10 animals from each strain were infused via the tail vein with 0.24 μmoles of Na [^{14}C]HCO_3 (specific activity 60 Ci/mole) in 100 μl of saline to determine if there were strain differences in the rate of CO_2 expiration.

Mice receiving ethanol intragastrically had Silastic tubing surgically implanted approximately 12 hours prior to testing. The animals were lightly anesthetized with methoxyfluorane, and a small incision approximately 2 cm long was made over the midline of the skull. Silastic Medical Grade tubing (0.012 in. i.d.) fitted with a one inch length of 27G stainless steel tubing was threaded between the skin and muscle layers on the right side of the head between the eye and ear from the backmost section of the jaw through the incision at the top of the head. A rubber disc, 1 cm in diameter and 2 mm thick, was punctured in the center in order to pass the tubing through providing a snug attachment when the disc was sewn under the skin. Additional tubing extending from the mouth was then directed down the esophagus and into the stomach. Ten cm tubing extending from the top of the head was attached to a 27G needle in the metabolic chamber wall permitting the feeding of alcohol from an external infusion pump (Sage Model 341). The final step involved surgical attachment of a small rubber muzzle to prevent the animal from biting and puncturing the tubing.

The metabolic chamber and the air flow rate through the chamber have been described elsewhere [4]. The air flowed into the chamber forcing expired $^{14}CO_2$ into two traps in tandem, each containing 150 ml of trapping solution. The trapping solution consisted of 20 ml of monoethanolamine, 161 ml of ethylene glycol monoethylether and 68 ml of toluene. Throughout all experiments 5 ml samples of trapping solution were collected at 10 min intervals in vials containing 5 ml of scintillation fluor. The scintillation fluor contained 5.5 grams PPO 2.5-Diphenyloxazole and 0.1 grams POPOP (1.4-bis [2-(5-Phenyloxazolyl)] Benzene) in 1 liter of toluene. In all experiments 5 ml samples of trapping solution were collected from the first trap every 10 min until the expired $^{14}CO_2$ reached an asymptote. During the last sampling 5 ml was removed from the second trap to test the effectiveness of the first trap. Radioactivity of samples was determined on a Packard model 3320 Liquid Scintillation Spectrophotometer.

RESULTS

Strain differences in expired $^{14}CO_2$ from the oxidation of intragastric infused [2-^{14}C]ethanol are shown in Fig. 1. During the entire sampling time the DBA strain eliminated $^{14}CO_2$ at a higher rate than the C57BL strain. Observation of behavior indicated that the average time to loss of the righting reflex was 21.9±4.6 min for the DBA strain and 27.9±10.1 min for the C57BL strain. A t-test indicated that this difference was significant at $p<0.005$. To determine whether or not the difference in CO_2 evolution could be the result of slower absorption across the gut wall, the strains were infused with ethanol via the tail vein and expired $^{14}CO_2$ measured (Fig. 2). The quantities of expired $^{14}CO_2$ are lower compared to the intragastric infusion because much less ethanol could be used in tail vein infusion. However, the DBA strain still evolved $^{14}CO_2$ at a higher rate than the C57BL strain. It is possible that the different rates of CO_2 expiration could be due to different rates of expiration rather than a result of formation of CO_2 from metabolism. To determine if differences existed between strains in the rates of CO_2 expiration, radioactive sodium bicarbonate was infused via the tail vein and expired $^{14}CO_2$ determined. The results are shown in Fig. 3. The DBA strain was found to have a higher rate of CO_2 expiration. Analysis of all of the data were made using a group by time ANOVA with time as a repeated factor and all differences were found to be nonsignificant (Intragastric ethanol, F=0.0146; Tail vein ethanol, F=2.27; Tail vein Sodium Bicarbonate, F=0.0194).

DISCUSSION

The DBA strain demonstrated a greater behavioral sensitivity to intragastrically infused ethanol than the C57BL strain as indicated by a statistically significant earlier time required to lose the righting reflex. In addition, during the early stages of infusion a marked agitation occurred in the DBA but not the C57BL strain. This type of response has been reported with low doses of other alcohols [5,25], and is a further indication of the difference in neural response to alcohol between these two strains. The greater sensitivity of the DBA strain to alcohol is consistent with the results of other investigations utilizing parameters such as sleep time [9, 11, 12, 16], or jaw-jerk reflex [21] where alcohol was administered intraperitoneally, usually in massive single injections or rapid infusion.

The relatively constant rate of $^{14}CO_2$ expiration during intragastric infusion of radioactive ethanol should be a measure of the rate of in vivo alcohol dehydrogenase activity since it represents the rate limiting step in ethanol metabolism [10, 15, 28]. Therefore, these data indicate that the DBA strain expires $^{14}CO_2$ originating from oxidation [2-^{14}C]ethanol at a slightly higher rate than the C57BL strain, but the difference is not significant. Since differences in $^{14}CO_2$ evolution during infusion via the stomach are the same as those during tail vein infusion, absorption across the gut wall plays no role in these slight differences. Furthermore, infusion via the tail vein produces no sign of agitation in the DBA strain. Therefore it is unlikely that the curve is elevated during stomach infusion because of the excessive movement that occurs during the early agitation. Infusion of sodium bicarbonate indicated that the rate of CO_2 expiration was slightly higher in the DBA strain. This could account for some or all of the elevation in CO_2 expiration observed after infusion of alcohol via the tail vein or stomach. However, it is important to note that the difference between strains in CO_2 expiration after infusion with sodium bicarbonate is non-significant just as it is after alcohol infusion. Therefore, the difference between strains probably does not reflect differences in the rate of catabolism of alcohol. In any case, these differences are opposite those expected for the animals because the more sensitive strain shows a slightly higher apparent rate of ethanol metabolism.

In another experiment it has been determined that the expiration of ethanol and acetaldehyde after an oral dose of ethanol are identical in both of these strains [4] indicating that tolerance could not result from higher rates of expiration. It has been confirmed that expired ethanol reflects the relative levels of that compound in the blood [7]. Therefore, the identical expiration of ethanol by the two strains indicates that blood levels throughout the period examined are the same. This applies to acetaldehyde if the reasonable assumption is made that expired acetaldehyde also reflects blood levels.

There are a large number of studies that suggest that high and low alcohol-preferring strains differ in their neural response to ethanol, as well as other substances [5–7, 9, 11–13, 16, 21, 22, 25–27] and that metabolic differences play little if any role. Others [1,3] suggest metabolism may play a larger role in tolerance. It is difficult to know what factors may have contributed to these differences. Perhaps as suggested in the introduction, large doses administered through unnatural routes may yield inconsistent results. Recently, Tabakoff and Ritzmann [26] and Grieve [7] have suggested that a distinction be made between initial neural sensitivity and acute tolerance which may develop rapidly after exposure to ethanol. While Tabakoff and Ritzmann [26] found no difference between the DBA and C57BL strains in loss of the righting reflex after a single injection of ethanol, the DBA slept for a significantly longer time.

It is not clear how long acute tolerance takes to develop, but if indeed this is the case, it must be very rapid since IP infusion (20 × metabolic rate) can produce a significant decrement in the jaw jerk reflex in 3–4 min in the DBA strain while requiring 12–15 min in the C57BL strain. Whether or not the difference we have observed in time to lose the righting reflex during slow intragastric infusion is the result of initial or acute tolerance is not clear. If our results reflect an initial or innate difference in tolerance, then the large IP injection employed by Tabakoff and Ritzmann [26] may have obscured those differences. It may be that the initial tolerance in these strains is small and observable only under those conditions where gradual infusion is employed. Perhaps the issue could be resolved under conditions where the infusion rate is increased until differences in the loss of the righting reflex between strains is obscured.

In this investigation we have attempted to observe the metabolism of alcohol under the most natural conditions achievable in the laboratory, i.e. animals receiving alcohol via the natural route while free-moving. We have monitored the CO_2 evolved from in vivo ethanol oxidation, and the lack of any significant differences between the strains when considered with the significant difference in the time to pass out and the pronounced agitation of the DBA during the early infusion period, makes metabolism as a factor in differences in ethanol tolerance unlikely. It may well be, as previously suggested [21], that there is an inherent difference between these strains in their CNS sensitivity to ethanol either initially or, as Tabakoff and Ritzmann [26] suggest, after some exposure to the drug. Further investigations of possible differences between strains in neural and biochemical changes induced by alcohol should provide insight into the mechanisms underlying alcohol tolerance.

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