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Behavioral and Enzymatic Interactions Between Benzyl Alcohol and Ethanol

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MESSIHA, F. S., A. PASI AND G. MORNIROLI. *Behavioral and enzymatic interactions between benzyl alcohol and ethanol.* PHARMACOL BIOCHEM BEHAV 43(4) 1071-1074, 1992.—Acute IP injection of benzyl alcohol but not benzaldehyde (0.5 g/kg) caused aversion to voluntary drinking of 5% ethanol solution by male rats with preference to ethanol. Benzyl alcohol noncompetitively inhibited hepatic alcohol dehydrogenase of rats maintained for a short term on 5% ethanol compared to control. The results suggest an adverse interaction between benzyl alcohol and ethanol underlying the observed aversion to ethanol.

Alcohol dehydrogenase Benzyl alcohol Ethanol drinking Liver Rat

BOTH benzyl alcohol (BA) and ethanol (ET) are widely used as organic solvents and preservatives in various industrial and pharmaceutical formulations. BA is also found in certain anesthetic, endocrine, and neuromuscular blocking medications (18,20) and thereby may precipitate adverse effects during ET consumption, in particular in the alcoholic subject. Both BA and ET cross placental and blood-brain barriers and thereby cause neonatal and CNS toxicity (1,2,5-8,10,16,21). The foregoing observations suggest that BA and ET can evoke both behavioral and CNS intoxication. This can be assessed by evaluation of the effects of these alcohols and the corresponding aldehyde metabolites on certain behavioral and metabolic aspects of ET because they share the same mechanism for metabolic detoxification. The ET behavioral test selected was ET preference by the rat and the biochemical measurements made were these of rat liver (L) alcohol (L-ADH) and aldehyde dehydrogenase (L-ALDH).

Hepatic ADH and ALDH possess wide substrate specificity that includes high affinity to BA (14) at a high concentration (11). Therefore, benzaldehyde (BALD) may interfere in the metabolism of ET and/or in certain ET-mediated behavioral responses. The present study also evaluated the effect of BALD on ET preference, on rat L-ADH and L-ALDH as a possible underlying mechanism for a proposed hepatic BA-ET interaction.

METHOD

Adult, male Sprague-Dawley rats, purchased from Holtzman Farm Co. (Madison WI), were used. They were 60 days old at the beginning of the experiment. Animals were housed in a laboratory with a 12 L : 12 D cycle throughout the experiment.

In the first experiment, the effect of an acute equal dose of BA or BALD on voluntary drinking of ET by the rat was evaluated. Animals were habituated to drink 5% ET solution, prepared from 95% ET stock solution and diluted with distilled water, before they were offered a free selection between distilled water and ET. Rats with preference to ET consumption over water as the drinking fluid of choice were the subjects used for the present behavioral test as previously described (12). Individually housed rats had access to Purina pellet food and the cages were supplied with two drinking bottles containing water or 5% ET. These were rotated once daily between 10:00-11:00 a.m. after measurements of food and fluid consumptions were made to avoid position preference. Rats were divided at random into two groups for the BA (n = 6) and BALD (n = 4) treatments. Both BA and BALD were diluted with saline and were IP injected, (0.5 g/kg).

In the second set of experiments, the in vivo effect of BA on L-ADH and L-ALDH was studied as a function of ET intake. Experimentally naive, male rats, of comparable age to those used in the first experiment, were divided into two groups of eight animals each. They had access to Purina food and received distilled water or 5% ET solution ad lib for 12 consecutive days. They were then divided into two subgroups of equal number. They were injected with saline (control) or BA, (0.5 g/kg, IP). Animals were sacrificed by decapitation 1 h after treatment and livers were dissected, blotted with filter paper, and weighed. Individual livers were suspended in

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ice-cold 0.1 M KCl, pH 6.8, buffer and were homogenized by a Waring blender to prepare 10% homogenates (w/v), which were differentially centrifuged to obtain the mitochondrial (MT) and cytoplasmic (CT) subcellular components (15). These were aliquoted for the protein determination by the biuret assay and the determinations of L-ADH (4), L-CT-ALDH (3), L-MT-ALDH isoenzymes with the apparent high (H) and low (L) K_m (19), and the L-ADH kinetics (9).

The results of the behavioral experiments were expressed as means ± SEM for daily food and fluid consumptions (g/24 h). The enzymatic activity was calculated as specific activity, nM/min/mg protein, measured at 25°C. The results were analyzed for statistical significance by Student's *t*-test.

RESULTS

Figure 1 illustrates the effect of an identical acute dose of BA or BD (0.5 g/kg, IP) on voluntary ingestion of ET by rats with preference to ET as a function of time. Benzyl alcohol (lower panel) caused a gradual voluntary aversion to ET drinking compared to the predrug period. The initial, 24-h, and 48-h reduction of ET drinking by BA was statistically insignificant ($p < 0.1$) and became more apparent thereafter. This amounted to 47% ($p < 0.02$) and 45% ($p < 0.02$) reduction of voluntary ET intake during the 3- and 5-days subsequent to the BA treatment, respectively. This was associated with a marked significant reduction of food but not water consumption beginning 24 h ($p < 0.02$) and lasting for 5 consecutive days after the BA trial ($p < 0.005$–0.05).

Figure 1 (upper panel) also shows that injection of BALD, in equal dose to BA, into a separate group of ET-preferring rats did not alter any of the parameters studied compared to the predrug period.

Table 1 shows the effect of acute administration of BA (0.5 g/kg, IP), on specific activities of rat L-ADH and subcellular L-ALDH isoenzymes as a function of prior exposure to short-term drinking of 5% ET. Rats maintained on 5% ET weighed 478 ± 21 g compared to 463 ± 28 g for animals receiving water as the only drinking fluid. The BA treatment of rats maintained on ET drinking for 12 consecutive days resulted in approximately 37% inhibition of L-ADH from saline control ($p < 0.01$). This decrease was 21% ($p < 0.01$) below that assayed for rats maintained on water and receiving an identical BA treatment. The remaining enzymes studied were not altered from the corresponding saline control by the BA trial with the exception of L-MT-ALDH isoenzymes with the apparent high K_m of water-drinking rats.

Figure 2 shows the kinetics of L-ADH inhibition by BA of rats with prior exposure to ET drinking. This is shown by the reciprocal plots of the velocity of the L-ADH reaction as a function of the ET substrate concentration. The BA-produced inhibition was determined as noncompetitive without a change in the apparent K_m. The V_{max} for the BA treatment decreased to 13.2 from the 19.2 U determined for the saline control.

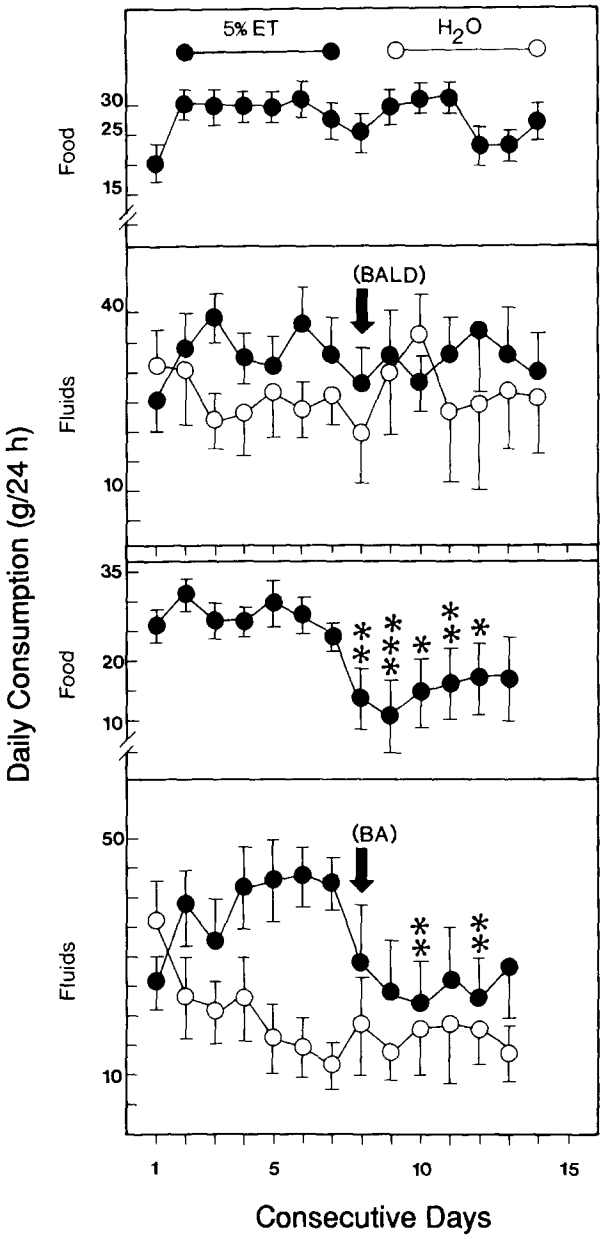

FIG. 1. Effect of an acute dose of benzyl alcohol (BA) or benzaldehyde (BALD) on voluntary intake of ethanol (ET) by the rat. Either BA or BALD (0.5 g/kg) were injected IP. Arrows identify amounts of food and fluid consumption measured 24 h after acute drug injection. The BA and BALD solutions were diluted with saline to the required concentration immediately before injection. Each point represents the mean ± SD of four independent measurements of food and fluid consumptions expressed as g/24 h. (○———○), saline; (●———●), drug treatment. Statistical differences from predrug period: ***$p < 0.005$, **$p < 0.02$, *$p < 0.05$.

DISCUSSION

The present behavioral evaluation shows that acute injection of BA but not BALD resulted in aversion to voluntary intake of ET. This reflects a BA–ET interaction because neither a compensatory water intake to replenish the reduction of fluid intake nor an adequate food consumption were evident subsequent to the BA trial. The aversion to voluntary intake of ET by BA might have been precipitated by inhibition of L-ADH, the rate-limiting step in ET oxidation. This has been demonstrated in the present study by administration of an identical acute dose of BA to rats maintained for a short term on a similar 5% ET drinking fluid. The inhibition of L-ADH by BA is unlikely due to modification of endogenous L-ADH by consumption of the 5% ET solution because ET-preferring

TABLE 1

EFFECT OF ACUTE DOSE OF BA ON ENDOGENOUS HEPATIC RAT ALCOHOL AND ALD-ALDH ISOENZYMES AS A FUNCTION OF SHORT-TERM INTAKE OF ETHANOL

	Drinking Fluid (12 days)			
	Water		5% Ethanol	
Hepatic Enzyme	Saline	BA	Saline	BA
L-ADH	17.1 ± 4.3	18.0 ± 2.7	22.5 ± 3.6	14.2 ± 1.5*
L-CT-ALDH (H)	13.1 ± 1.2	13.6 ± 3.0	13.6 ± 1.5	13.6 ± 1.5
L-CT-ALDH (L)	9.5 ± 1.8	8.9 ± 1.4	10.1 ± 1.8	9.2 ± 2.2
L-MT-ALDH (H)	30.5 ± 1.5	33.1 ± 1.6	34.1 ± 2.1	33.4 ± 0.5
L-MT-ALDH (L)	7.3 ± 1.7	6.7 ± 1.0	7.8 ± 1.9	6.8 ± 0.4

BA was injected IP (0.5 g/kg) into rats maintained for 12 consecutive days on tapwater or 5% ethanol drinking fluid ad lib. Hepatic alcohol dehydrogenase (L-ADH) and aldehyde dehydrogenase (L-ALDH) were measured. The latter was determined in both cytoplasmic (CT) and mitochondrial (MT) subcellular fractions. The L-ALDH isoenzymes with the apparent high (H) and low (L) K_m were assayed in each subcellular component. Values are means ± SEM of four independent assays of specific activities (nM/min/mg protein).

*$p < 0.01$.

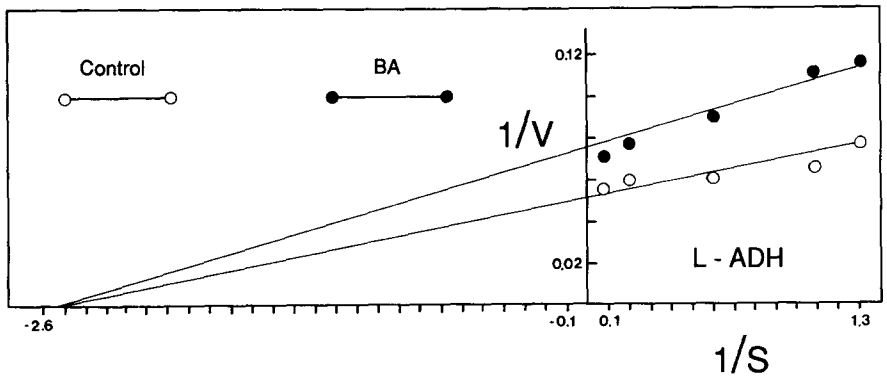

FIG. 2. Kinetics of benzyl alcohol (BA) inhibition of rat liver alcohol dehydrogenase (L-ADH). Rats were maintained on 5% ethanol (ET) solution for 12 consecutive days prior to injection with saline (control) or BA (0.5 g/kg, IP). Reciprocal plots of specific activity (nM/min/mg protein) are presented as a function of substrate (ET) concentration. (○-----○), saline: (●———●), BA.

and -nonpreferring rats of the same strain showed similar L-ADH activity (13).

The observed reduction of voluntary intake of ET by BA may be also due to the development of a metabolic acidosis by BA. This is supported by BA-produced inhibition of lactate dehydrogenase isoenzymes of human infants' cord blood and of rodents' blood serum (17), which will facilitate the build up of lactic acid. However, a systemic toxic reaction to BA and/or ET as a consequence of prolonged exposure to ET and inhibition of L-ADH cannot be excluded.

The inhibition of L-ADH by BA may also augment other adverse effects such as the potentiation of CNS depression of anesthetics containing BA (18) by alcoholics. Moreover, the use of commercial or medical preparations containing BA (18,20) by alcoholics during consumption of ET could render subjects more susceptible to ET intoxication.

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