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# BLOCKADE OF HEPATIC ALDEHYDE DEHYDROGENASE IN MICE ON CHRONIC INGESTION OF 4-BROMOPYRAZOLE AND 4-IODOPYRAZOLE

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**Abstract**—4-Halopyrazoles acutely administered decreased the alcohol dehydrogenase activity of livers of treated mice but exerted little or no effect on the activity of aldehyde dehydrogenase. Ethanol administered to mice pretreated in this manner with 4-bromopyrazole disappeared slowly from blood as expected and gave no accumulation of acetaldehyde. In contrast, 4-bromopyrazole and 4-iodopyrazole, administered chronically via the drinking fluid, diminished the aldehyde dehydrogenase activity of livers of imbibing mice and elevated somewhat the alcohol dehydrogenase activity. In agreement with the blockade of aldehyde dehydrogenase observed *in vivo*, ethanol given to mice continually ingesting 4-bromopyrazole or 4-iodopyrazole resulted in the accumulation of acetaldehyde in blood. Moreover, chronic ingestion of 4-bromopyrazole caused a decrease in the natural ethanol preference of C57BL mice, a finding consistent with aldehyde dehydrogenase inhibition and the production of acetaldehyde from the interaction of 4-bromopyrazole and ethanol.

Pyrazole and its 4-substituted derivatives strongly inhibit hepatic alcohol dehydrogenase *in vitro* [1–5] and effectively block the metabolism of administered ethanol in the intact animal [6–10]. Although pyrazole is a specific enzyme inhibitor *in vitro*, affecting only hepatic and yeast alcohol dehydrogenases [3], a decrease in liver catalase activity has been found in rats after a high dose (4·4 m-moles/kg) of pyrazole [11]. The results of our ethanol metabolism and preference studies reported in the present work indicate that 4-bromopyrazole and 4-iodopyrazole, chronically ingested, effect blockade of hepatic aldehyde dehydrogenase *in vivo*.

## MATERIALS AND METHODS

Swiss albino male mice (19–27 g) were CD Charles River (Massachusetts) animals. C57BL/Cum male mice (20–33 g) were obtained from Cumberland View Farms, Clinton, Tenn. 4-Chloropyrazole and 4-bromopyrazole were synthesized by halogenation of pyrazole in carbon tetrachloride [12]; 4-iodopyrazole was prepared by iodination of pyrazole in aqueous solution [13]. Found: 4-chloropyrazole, m.p. 77·5 to 78·5°; 4-bromopyrazole, m.p. 94 to 96°; 4-iodopyrazole, m.p. 108·5 to 109°. Sources of other compounds were: *n*-butyraldoxime, CalBiochem; cyanamide, Eastman Kodak Co. (yellow label); pyrazole, Matheson, Coleman & Bell.

*Enzyme studies.* Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (AldDH) preparations consisted of supernatant fractions from the centrifugation (1100 *g* for 30 min) of 5% (w/v) (for ADH) and 10% (w/v) (for AldDH) homogenates of mouse liver in 0·25 M sucrose. These extracts were kept ice-cold and assayed on the same day by the usual spectrophotometric determination of initial rates of formation of NADH in a Gilford recording spectrophotometer as described previously [14].† Mice in groups of four or five were pretreated with drug or water (controls) and sacrificed after appropriate times. For pretreatments in which animals were allowed to imbibe drug solutions *ad lib.*, drinking bottles were left on until animals were sacrificed at *ca.* 10:00 a.m. Contemporary control groups were always run for each time period. Each liver extract was assayed in duplicate.

*Blood levels of ethanol and acetaldehyde.* Blood ethanol concentrations were determined by gas–liquid chromatographic methods described previously, using aliquots of whole blood from the tail vein or gaseous samples of a dorsal bleb formed by injecting 10 ml nitrogen into the subcutaneous tissue [14]. Ethanol data from blood and gas bleb assays were essentially the same. Blood acetaldehyde concentrations were estimated by the nitrogen bleb method. Each run consisted of 2–3 drug groups and a control group (3–4 mice/group). After the pretreatments, a single dose of ethanol (2–3 g/kg, i.p.) was administered. For animals imbibing drug solutions the drinking bottles were removed at 8:30 a.m., and ethanol injections were given from 9:40 to 11:30 a.m. Ethanol and acetaldehyde concentrations were determined periodically for 6 hr after the administration of ethanol. Pretreated mice without ethanol were routinely checked to ascertain absence of acetaldehyde and ethanol.

*Ethanol preference.* C57BL/Cum mice, individually housed and maintained on a 12-hr light–dark cycle at 72–75° F room temperature, were given free access to Purina Lab Chow and a choice of two drinking cylinders, one containing a 12% (v/v) ethanol solution

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† A possible complication in these enzyme assays of crude supernatants is NADH oxidase activity. However, this potential interference is not expected to affect the findings reported in our present or previous study [14], since (1) virtually no disappearance of NADH occurred when added to either enzyme assay system (without ethanol or propanal substrate); and (2) the hepatic ADH or AldDH activity of drug-treated mice has been expressed throughout as a percentage of the corresponding enzyme activity of contemporary control mice.

Table 1. Aldehyde dehydrogenase (AldDH) and alcohol dehydrogenase (ADH) activities of livers of treated mice*

| Time (hr) | % Control activity 4-Chloropyrazole AldDH | 4-Chloropyrazole ADH | 4-Bromopyrazole AldDH | 4-Bromopyrazole ADH | 4-Iodopyrazole AldDH | 4-Iodopyrazole ADH | Cyanamide AldDH | Cyanamide ADH |
|---|---|---|---|---|---|---|---|---|
| 1 | 84 ± 9 | 40 ± 5† | 87 ± 10 | 25 ± 11† | 82 ± 8‡ | 14 ± 8† | 5 ± 2† | 96 ± 2 |
| 3 | 113 ± 14 | 49 ± 9† | 93 ± 13 | 20 ± 4† | 79 ± 10‡ | 30 ± 4† | 8 ± 4† | 95 ± 10 |
| 6 | 101 ± 8 | 57 ± 5† | 78 ± 15‡ | 26 ± 6† | 81 ± 9 | 53 ± 7† | 14 ± 4† | 87 ± 9 |
| 17 | 113 ± 26 | 164 ± 20† | 82 ± 23 | 156 ± 7† | 126 ± 21 | 155 ± 20§ | 48 ± 9† | 96 ± 3 |

* Entries represent mean relative enzyme activity ± S.D.; ($n$ = 4) for Swiss albino mice. Dose of all drugs administered was 1·5 m-moles/kg, i.p. Complete AldDH system (3·0 ml): 0·316 mM propanal, 0·316 mM $NAD^+$, 10 mM sodium pyrophosphate (pH 10), 0·1 ml of a 10% (w/v) liver homogenate in 0·25 M sucrose, 0·01 mM 4-iodopyrazole (to suppress ADH activity; 4-iodopyrazole had no effect on bovine liver AldDH at $10^{-5}$ M); mean activity ± S.D.: 1·0 ± 0·2 μmole/g/min ($n$ = 12). Complete ADH system (3·0 ml): 16 mM ethanol, 1·6 mM $NAD^+$, 33 mM glycine, pH 11 buffer, 0·1 ml of 5% (w/v) liver extract in 0·25 M sucrose; mean activity ± S.D.: 5·5 ± 1·2 μmoles/g/min ($n$ = 12).

† $P < 0.001$ compared to controls.

‡ $P < 0.05$ compared to controls.

§ $P < 0.01$ compared to controls.

in distilled water and the other only distilled water [14]. The position of the cylinders was alternated randomly every 2–5 days. To ascertain the effect of 4-bromopyrazole on ethanol preference, the compound was dissolved in both the aqueous ethanol and the water drinking fluids (6·8 μmoles/ml). In this manner, mice received 4-bromopyrazole throughout the treatment period, regardless of which drinking cylinder they selected. The amount of fluid consumed from each cylinder was read to the nearest 0·5 ml each morning. The preference ratio is defined as the quotient obtained on dividing the volume of ethanol solution consumed by the volume of total fluids consumed.

## RESULTS

*Enzyme activities after acute treatment.* Hepatic ADH activity of mice decreased markedly for several hr after treatment with the 4-halopyrazoles, whereas AldDH activity was only slightly reduced or unchanged by these treatments (Table 1). These findings are consistent with the effects *in vitro* of the pyrazoles: potent inhibition of ADH ($IC_{50}$ values of $3 \times 10^{-5}$ M pyrazole and $2 \times 10^{-6}$ M 4-bromopyrazole or 4-iodopyrazole for C57BL mouse liver ADH) and lack of inhibition of AldDH (94 per cent of control activity at $10^{-3}$ M pyrazole for C57BL mouse liver AldDH). The acute effects of 4-halopyrazoles are opposite to those of cyanamide, which lowered hepatic AldDH activity *in vivo* without altering ADH activity (Table 1). The decrease in ADH activity elicited by the pyrazoles did not persist; all three compounds caused an apparent elevation of ADH activity 17 hr after treatment.

*Enzyme activities after chronic treatment.* Chronic imbibing of a solution of 4-bromopyrazole or 4-iodopyrazole (11·5 μmoles/ml) induced a lowering of hepatic AldDH activity (Table 2). As shown in the next section, this blockade of AldDH *in vivo* was accompanied by an accumulation of acetaldehyde in blood after the administration of ethanol. Continual exposure to the pyrazoles in this manner elicited an elevation of ADH activity (Table 2), although the rate of disappearance of ethanol from blood after its administration was slightly retarded (next section).

*Effect on ethanol metabolism* in vivo. The rate of ethanol disappearance from blood in mice after an ethanol dose was substantially decreased by acute intraperitoneal or oral pretreatment with 4-bromopyrazole (Fig. 1A). The pyrazoles administered in the drinking fluid for 6 days prior to the ethanol also slowed the dis-

Table 2. Aldehyde dehydrogenase (AldDH) and alcohol dehydrogenase (ADH) activities of livers of mice ingesting drugs via drinking fluid*

| Drinking fluid (11·5 μmoles/ml) | Drinking period (days) | % Control activity AldDH | % Control activity ADH |
|---|---|---|---|
| 4-Bromopyrazole | 11 | 71 ± 10† | 112 ± 7‡ |
| | 18 | 72 ± 8† | 108 ± 19 |
| | 41 | 67 ± 4† | 151 ± 8§ |
| 4-Iodopyrazole | 41 | 52 ± 5§ | 144 ± 9§ |

* Entries represent mean relative enzyme activity ± S.D.; ($n$ = 4–5) for C57BL/Cum mice. Assay systems are given in Table 1. The average daily fluid intake and dose of 4-bromopyrazole for the 11- and 18-day experiments are similar to those in Fig. 1B. The average daily fluid intake and dose of 4-bromopyrazole and 4-iodopyrazole for the 41-day experiment were similar to those in Fig. 1D.

† $P < 0.01$ compared to controls.

‡ $P < 0.05$ compared to controls.

§ $P < 0.001$ compared to controls.

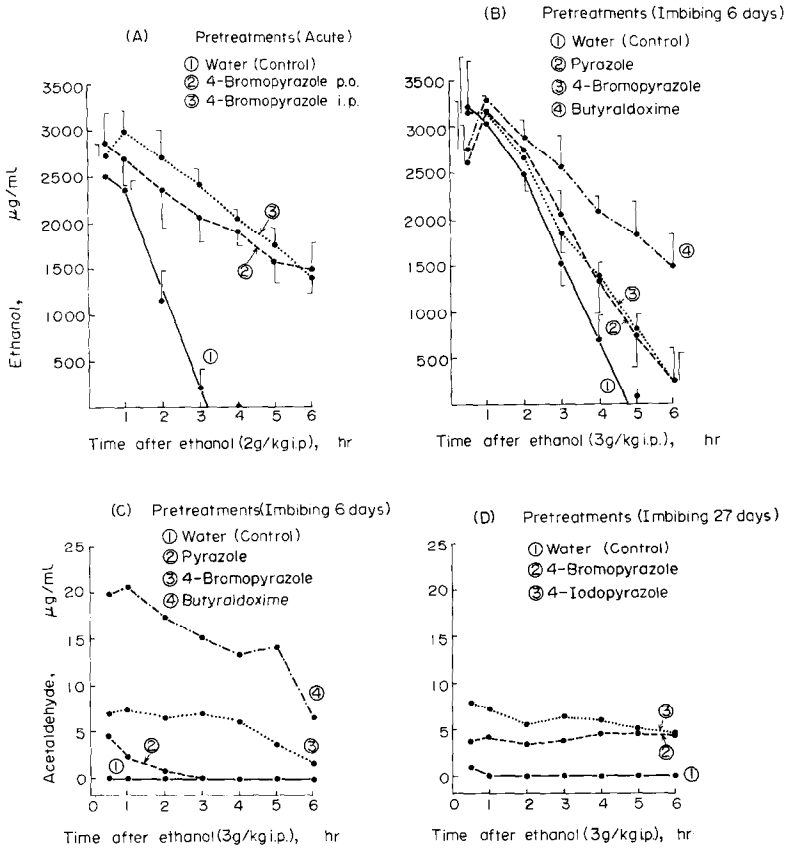


Fig. 1. Top (A and B). Ethanol concentrations in blood of C57BL/Cum mice pretreated with water or drugs as a function of time after an ethanol dose. Each point is mean concentration ($n = 3$) with vertical lines extending 1 S.D. from mean values. Ethanol blood levels were determined by gas chromatography using (A) a subcutaneous nitrogen bleb or (B) whole blood. (A) One m-mole/kg of 4-bromopyrazole was administered i.p. or p.o. 0·5 hr before the ethanol. No accumulation of acetaldehyde in blood from the ethanol was noted in these 4-bromopyrazole mice. (B) Mice imbibed drug solutions (11·5 μmoles/ml) *ad lib.* for 6 days before the ethanol. Mice maintained body weight during this period (20 g). Average fluid intakes (ml/mouse/day) were: water, 4·4; pyrazole, 2·6; 4-bromopyrazole, 3·7; butyraldoxime, 3·4; and average doses (m-moles/kg/day) were: pyrazole, 1·5; 4-bromopyrazole, 2·1; butyraldoxime, 2·0. Approximate rates of ethanol disappearance (g/kg/hr) were: control, 0·63; pyrazole and 4-bromopyrazole, 0·47; butyraldoxime, 0·33. Bottom (C and D). Acetaldehyde concentrations in blood of C57BL/Cum mice pretreated with water or drugs as a function of time after an ethanol dose. Each point is mean concentration (C, $n = 3$; D, $n = 4$). Acetaldehyde levels were determined by the subcutaneous nitrogen bleb method. (C) B and C are parts of the same experiment (see B for doses). (D) Mice imbibed drug solutions (11·5 μmoles/ml) *ad lib.* for 27 days. Mice maintained body weight during this period (33 g). Average fluid intakes (ml/mouse/day) were: water, 5·0; 4-bromopyrazole, 4·4; 4-iodopyrazole, 4·0; and average doses (m-moles/kg/day) were: 4-bromopyrazole, 1·6; 4-iodopyrazole, 1·4.

appearance of the latter from blood (Fig. 1B), although hepatic ADH activity, in contrast to the reductions seen after acute treatments, was elevated (Table 2). No accumulation of acetaldehyde from the administered ethanol occurred after either acute pretreatment with 4-bromopyrazole (not shown). However, allowing the mice to ingest a 4-bromopyrazole solution *ad lib.* for 6 days before the ethanol elicited a sustained accumulation of acetaldehyde (Fig. 1C). Pyrazole similarly ingested caused a transient accumulation of acetaldehyde which was not detectable 3 hr after the ethanol (Fig. 1C). The blockade of liver AldDH was maintained during longer periods of imbibing solutions of 4-halopyrazoles (Fig. 1D and Table 2). The effects of the pyrazoles on blood ethanol disappearance (Fig. 1B) and acetaldehyde accumulation (Fig. 1C) were less than those of butyraldoxime, an ADH inhibitor which also induces AldDH blockade *in vivo* [14].

*Blockade of natural ethanol preference.* When 4-bromopyrazole (1 mg/ml or 6·8 μmoles/ml) was introduced into both water and aqueous ethanol drinking bottles, the mean preference ratio for ethanol of 20 C57BL mice rapidly decreased in 2 days to 0·35 from a stable predrug preference ratio of 0·75 (Fig. 2). A lowered preference ratio (0·4) was maintained for the 16 days of 4-bromopyrazole ingestion (~1 m-mole/kg/day). After removing the 4-bromopyrazole, the mean preference ratio increased in 2 days to ~0·6. Almost normal preference for the ethanol solution was attained in 10 days after withdrawal of the pyrazole.

## DISCUSSION

The inhibition of hepatic ADH by pyrazole and 4-halopyrazoles is competitive with ethanol and involves formation of an inactive ADH–pyrazole–$NAD^+$ ter-

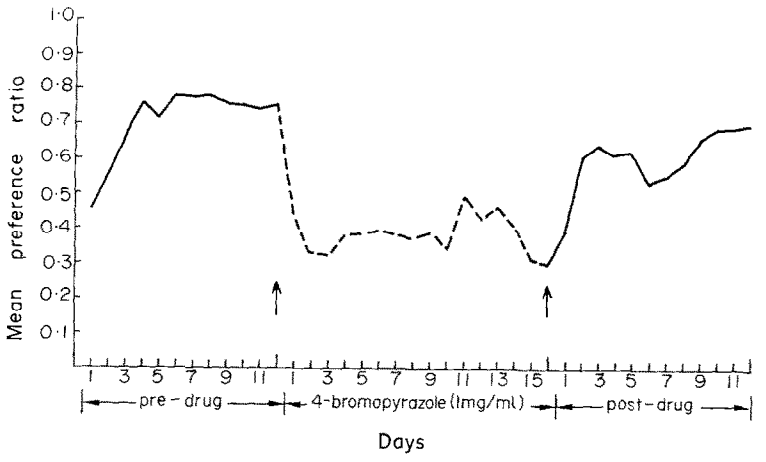


Fig. 2. Mean preference ratio of C57BL/Cum mice for a 12% ethanol solution vs water as a function of time. In the predrug period, the mice ($n = 20$) attained a stable mean preference ratio for 12% ethanol of 0·75. The preference ratio decreased in 2 days to 0·35 and remained at ~0·4 for the 16-day period in which 4-bromopyrazole was dissolved in both water and 12% ethanol drinking fluids at a concentration of 1 mg/ml (6·8 μmoles/ml) (----). After removing 4-bromopyrazole, the preference ratio increased in 2 days to ~0·6 and attained near normal values in 10 days. Preference ratio = volume of ethanol solution consumed/(volume of ethanol solution + water consumed).

nary complex [1, 2]. Aldehyde dehydrogenase (mouse liver, present study; rat liver [15]) and other zinc-containing enzymes [3] are not inhibited *in vitro*. The present observations that 4-halopyrazoles, administered acutely, decrease ADH activity but hardly affect AldDH activity are consistent with their properties *in vitro*. It is noteworthy that Deitrich *et al.* [15] reported no depression of hepatic AldDH activity of rats after a high dose (5·29 m-moles/kg, i.p.) of pyrazole. In respect to AldDH, the pyrazoles are different from cyanamide or butyraldoxime, which, although not active *in vitro*, will strongly lower AldDH activity in mice after a single treatment.

In contrast to their acute effects, 4-bromopyrazole and 4-iodopyrazole administered chronically to mice via their drinking fluid cause a decrease in hepatic AldDH activity accompanied by somewhat elevated ADH activity. The effects of the continual ingestion of the 4-halopyrazoles on ADH and AldDH activities in livers of treated mice are correlated with those on the disposition of ethanol *in vivo*. Acute doses of the 4-halopyrazoles strongly retard the rate of disappearance of ethanol from blood without any accumulation of acetaldehyde. Chronic ingestion causes a smaller decrease in the ethanol disappearance rate but now elicits accumulation of marked amounts of acetaldehyde. Although 4-bromopyrazole and 4-iodopyrazole resemble butyraldoxime in causing blockade of hepatic AldDH when solutions of the compounds are continuously imbibed by mice, the effects of the pyrazoles are not as pronounced as those of butyraldoxime [14].

Hepatic ADH activity is not depressed and appears to be even enhanced by prolonged drinking of a 4-bromopyrazole solution. However, the rate of disappearance of administered ethanol in such mice is still slightly slower than that in controls. This phenomenon apparently is common to AldDH inhibitors and may be attributed to the reduction of the accumulated acetaldehyde, a reaction catalyzed by ADH [14, 16].* Persistent exposure to the competitive inhibitor, possibly leading to enzyme induction, may explain the enhanced ADH activity observed in the chronic experiments as well as in the acute experiments 17 hr after drug.

Inhibition of hepatic AldDH by the 4-halopyrazoles on chronic ingestion is probably mediated by a metabolite. It is noteworthy that metabolites may also be involved in the blockade of AldDH induced by cyanamide [17] and butyraldoxime [14]. Since acute administration of 4-bromopyrazole is ineffective against AldDH, an accumulation of or prolonged exposure to a metabolite may be involved in its chronic action.

The decrease in mean preference ratio for an ethanol solution elicited by the concomitant ingestion of 4-bromopyrazole is more attenuated than that observed for the anti-alcohol agent butyraldoxime [14]. As in the case of butyraldoxime and other AldDH inhibitors [18], the decreased preference probably results from aversion to the noxious agent, acetaldehyde, derived from the interaction of 4-bromopyrazole + ethanol *in vivo* [19]. The accumulation of acetaldehyde from the latter combination is less than that from the interac-

* The rate of ethanol disappearance in mice chronically drinking a solution of 4-bromopyrazole (Fig. 1B)—less than that in control mice but greater than that in mice receiving an acute dose of this drug (Fig. 1A)—might be interpreted as diminished effects of the competitive inhibitor because of the larger ethanol dose (3 g/kg vs 2 g/kg). This possibility is not considered very likely, because other experiments conducted in our previous study [14] indicated that the characteristic effects of aldoxime inhibitors on ethanol metabolism are essentially the same for ethanol doses of 2, 2·5 or 3 g/kg. Moreover, mice chronically ingesting competitive ADH inhibitors such as butyraldoxime [14] or the pyrazoles (Table 2) appear to have normal or enhanced ADH activity. In these mice, ethanol disappearance should occur at the same rate or slightly faster than in control mice. The actual rate is less (Fig. 1B), probably because of the concomitant AldDH blockade, which elicits acetaldehyde accumulation from the ethanol administered, and the reconversion, $CH_3CHO \rightarrow CH_3CH_2OH$.

tion of butyraldoxime + ethanol. This may account for the quicker recovery to normal ethanol preference after 4-bromopyrazole is withdrawn, in contrast to the protracted (~2 months) low mean preference ratio induced by butyraldoxime [14].

The decrease in aldehyde dehydrogenase activity of livers of treated mice, the accumulation of acetaldehyde in blood from an ethanol dose, and the decrease in the natural ethanol preference of C57BL mice all support the conclusion that the 4-halopyrazoles on chronic ingestion induce blockade of aldehyde dehydrogenase. Regulation of ethanol metabolism in man with the pyrazoles as ADH inhibitors has been proposed with a view toward controlling some of the metabolic derangements induced by ethanol in alcolism [6, 9, 20]. Such studies should consider the possible blockade of hepatic enzymes other than ADH by the pyrazoles under long-term administration.

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