Characteristics of butanol metabolism in alcohol dehydrogenase-deficient deermice

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Deermice lacking the low- K_m alcohol dehydrogenase eliminated butan-1-ol, a substrate for microsomal oxidation but not for catalase, at 117 $\mu\text{mol}/\text{min}$ per kg body wt. Microsomal fractions and hepatocytes metabolized butan-1-ol also ($V_{\text{max.}} = 6.7 \text{ nmol}/\text{min}$ per nmol of cytochrome *P*-450, $K_m = 0.85 \text{ mM}$; $V_{\text{max.}} = 5.3 \text{ nmol}/\text{min}$ per 10^6 cells, $K_m = 0.71 \text{ mM}$ respectively). These results are consistent with alcohol oxidation by the microsomal system in these deermice.

INTRODUCTION

The existence of alcohol dehydrogenase (ADH)-independent ethanol metabolism *in vivo* has been convincingly demonstrated in a mutant strain of deermouse (*Peromyscus maniculatus*) genetically deficient in low- K_m ADH. ADH-negative deermice eliminated ethanol at rates exceeding 50% of that seen in the wild strain that has normal hepatic ADH (Burnett & Felder, 1980; Shigeta *et al.*, 1984; Glassman *et al.*, 1985; Handler *et al.*, 1986; Takagi *et al.*, 1986). However, considerable controversy still surrounds the question of which enzymic pathway metabolizes ethanol when ADH is absent. Two have been proposed: peroxidation of the ethanol via catalase- H_2O_2 (Handler *et al.*, 1986, 1987) and cytochrome *P*-450-dependent metabolism by a microsomal ethanol-oxidizing system (MEOS) (Lieber & DeCarli, 1970; Shigeta *et al.*, 1984). We have previously addressed this issue by using inhibitors of each pathway (Takagi *et al.*, 1986; Kato *et al.*, 1987, 1988a), and also by using pathway-characteristic isotope effects (Alderman *et al.*, 1987). In the present investigation, metabolism of butan-1-ol, a substrate for microsomal oxidation but not for catalase, has been studied in ADH-negative hepatocytes and *in vivo*. When the kinetic characteristics and rates of butan-1-ol metabolism were compared with those of ethanol in the same systems, it appeared likely that MEOS accounts for most, if not all, ethanol metabolism in ADH-negative deermice.

EXPERIMENTAL

Animals were deermice lacking low- K_m ADH (male and female, 14–19 g), bred in our laboratory from an original stock kindly provided by Dr. Michael Felder. Blood butan-1-ol concentrations *in vivo* were determined at 30 min intervals after intraperitoneal administration of 0.5 g of butan-1-ol/kg body wt. as a 5% (w/v) solution in 0.9% NaCl. Vapour samples (0.5 ml) were withdrawn from a subcutaneously maintained air bubble (Shigeta *et al.*, 1984), followed by head-space g.l.c. analysis. A standard curve was prepared by incubating (at 37 °C) solutions of butan-1-ol in 0.9% NaCl placed in closed

vials with a small vent hole to permit pressure equilibration. After 30 min, 0.5 ml vapour samples were withdrawn, and analysed as described above. The concentrations obtained by this method were validated by taking a blood sample from the supraorbital sinus, and assaying butan-1-ol directly in the blood and also in saline-based butanol standards. Butanol metabolism in hepatocytes was determined from radioactivity in dry matter after incubating the cells with [^{14}C]butanol (American Radiochemicals, St. Louis, MO, U.S.A.). Except for the alcohol substrate, the procedure was identical with that previously described for [^{14}C]ethanol (Kato *et al.*, 1987). The method of Kato *et al.* (1987) was adapted for microsomal oxidation of butan-1-ol by substituting butan-1-ol for ethanol as substrate, and butyraldehyde (Aldrich, Milwaukee, WI, U.S.A.) for acetaldehyde in the standard curve. Catalase haem was measured as described by Oshino *et al.* (1975). Kinetics of butan-1-ol oxidation were determined by non-linear-regression fitting of the substrate and reaction-rate data to the Michaelis–Menten equation, by using the Enzfitter program (Elsevier Science Publishers, Amsterdam, Netherlands) with an IBM PC/AT computer.

RESULTS AND DISCUSSION

At 0.5 h after a dose of 0.5 g of butanol/kg body wt., butan-1-ol elimination proceeded linearly ($y = -4.94x + 9.2$, $r = 0.9997$) for at least 2 h (Fig. 1). After 2 h, the amount of butan-1-ol in the air-bubble-derived gas samples was too small to measure with precision. Doses of butan-1-ol larger than 0.5 g/kg body wt. caused rapid narcosis, frequently followed by death, and were thus not used. The rate of butan-1-ol elimination was 4.94 mmol/h per litre of blood, which corresponds to 117 $\mu\text{mol}/\text{min}$ per kg body wt. This is comparable with previously reported values for ethanol elimination in ADH-negative deermice, i.e. 139 and 269 $\mu\text{mol}/\text{min}$ per kg body wt. (Shigeta *et al.*, 1984) at low (5–10 mM) and high (40–70 mM) ethanol concentrations respectively.

In hepatic microsomes (microsomal fractions), butan-1-ol oxidation proceeded with a $V_{\text{max.}}$ of 6.7 nmol/min

Abbreviations used: ADH, alcohol dehydrogenase; MEOS, microsomal ethanol-oxidizing system.

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Fig. 1. Butan-1-ol elimination *in vivo* in ADH-negative deermice measured from the concentration of vapour in a subcutaneously maintained air bubble (●), and from the concentration in blood (○)

No significant difference was seen between these methods. Values shown are means $\pm$ S.E.M. ($n = 7$).

Fig. 2. Kinetic properties of butan-1-ol oxidation by microsomes from ADH-negative deermice: double-reciprocal plot of reaction rates and substrate concentrations

Microsomal butan-1-ol oxidation was measured by incubating butanol, microsomes and NADPH, followed by g.l.c. assay of butyraldehyde formed. Each plot represents the values from two experiments.

per nmol of cytochrome *P*-450 (Fig. 2), whereas the value found previously for ethanol was 10.5 nmol/min per nmol of cytochrome *P*-450 (Takagi *et al.*, 1986). Similar microsomal rates with butan-1-ol and ethanol have been confirmed by others (Handler *et al.*, 1987).

Fig. 3. Kinetic properties of butan-1-ol metabolism by hepatocytes from ADH-negative deermice: double-reciprocal plot of reaction rates and substrate concentrations

Butanol oxidation by hepatocytes was assessed from the radioactivity obtained in dry matter after incubation of cells with different concentrations of [14 C]butanol. Each plotted value represents the mean of at least five experiments. The mean values ($\pm$ S.E.M.) for reaction velocity (nmol/min per 10^6 cells) at each substrate concentration were as follows: 3.1 ± 0.6 at 1 mM-butanol, 3.9 ± 0.4 at 2 mM, 4.3 ± 0.4 at 5 mM, 5.0 ± 0.8 at 10 mM, 5.5 ± 1.0 at 30 mM, and 5.1 ± 0.9 at 50 mM.

However, the K_m for butan-1-ol was 0.85 mM (Fig. 2), compared with reported values for ethanol of 13 mM (Shigeta *et al.*, 1984) to 15 mM (Takagi *et al.*, 1986) in deermice. This indicates a greater affinity for butan-1-ol, and is consistent with the observed elimination *in vivo*, which was linear down to 2 mM (Fig. 1). Microsomal oxidation has also been found to exhibit a higher affinity for butan-1-ol than for ethanol in rat microsomes (K_m 5 and 10 mM respectively; Teschke *et al.*, 1974) and in cytochrome *P*-450LM3a purified from ethanol-treated rabbits (K_m of 4.4 and 19 mM respectively; Morgan *et al.*, 1982), though not to the degree seen in deermice.

Since exhalation may contribute to butanol disappearance *in vivo*, we have also measured butanol oxidation by hepatocytes in which expiration does not play a role and the product formed rather than butanol lost is measured. Hepatocytes derived from ADH-negative deermice also readily oxidized butan-1-ol (Fig. 3), with kinetic properties ($V_{max} = 5.3$ nmol/min per 10^6 cells, $K_m = 0.71$ mM) very similar to those displayed by microsomal oxidation. Moreover, the maximal rate of hepatocyte butanol metabolism closely resembled that at a concentration (50 mM) of ethanol which saturated MEOS (6.4 ± 0.7 nmol/min per 10^6 cells).

One previous report (Handler *et al.*, 1987) failed to observe comparable butan-1-ol and ethanol uptake in an ADH-negative perfused-liver system. However, those measurements were based on butanol disappearance from the perfusate, in which the total amount corresponded to a concentration decrease of less than 5% for ethanol and 1% for butan-1-ol. Calculations based on such small changes are bound to be unreliable, especially since a 5–10% correction for evaporation had to be made in that system (Handler *et al.*, 1987). The same study reported H_2O_2 -generation rates sufficient to

support peroxidation of the observed ethanol taken up. This H_2O_2 production was calculated by using two parameters found to be uniquely elevated for deermice. The parameters are: a_2 (the methanol concentration needed to degrade half the catalase- H_2O_2 complex present) and the liver catalase haem content. Each was reported to be 4-fold higher in ADH-negative deermice than was found previously in the rat by the method of Oshino *et al.* (1975). Although a_2 was not measurable in our hepatocyte system, since the absorption of catalase- H_2O_2 was small and its assay required a protein density higher than we could achieve, the catalase haem content was assayed and found to be 19.8 ± 2.1 nmol/g of liver ($n = 4$) in deermice and 16.5 ± 1.4 nmol/g ($n = 4$) in rats. Although in disagreement with the value of 79 nmol/g of liver reported by Handler *et al.* (1987), these results are essentially the same as those observed originally in rat liver (19.2 ± 0.4 nmol/g) (Oshino *et al.*, 1975).

In conclusion, we have demonstrated that butan-1-ol, a substrate for MEOS (Teschke *et al.*, 1974), but not for catalase (Chance, 1947; Handler *et al.*, 1987), is metabolized at rates comparable with those for ethanol *in vivo*, and in microsomes and hepatocytes of ADH-negative deermice. These results are consistent with those of previous studies using completely independent approaches (Takagi *et al.*, 1986; Kato *et al.*, 1987, 1988a; Alderman *et al.*, 1987). Although a contribution by catalase has not been specifically ruled out, the data presented support the hypothesis that MEOS mediates most, if not all, of ethanol metabolism in ADH-negative deermice.

This study was supported by DHHS grants AA 03508, AA 05934, AA 07275, and the Veterans Administration. Preliminary accounts of this work were presented at the 1988

meetings of the Research Society on Alcoholism (Alderman *et al.*, 1988), and the International Society for Biomedical Research on Alcoholism (Kato *et al.*, 1988b).

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Received 27 July 1988/2 November 1988; accepted 9 November 1988