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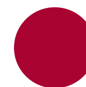

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Ethanol Inhibits Acetate Metabolism in Rat Hepatocytes

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Abstract: The metabolism of acetate at concentrations of 1, 2.5, 5 and 10 mM.was investigated in freshly isolated hepatocytes from 48 hr fasted, female rats in the absence and presence of 10 mM ethanol. The maximal capacity for acetate metabolism was 0.85 µmol/(10^8 cells · min). Ethanol caused a 20% decrease in the apparent V_{max} for acetate metabolism and an increase in the apparent K_m for acetate from 3.0 to 4.6 mM. At physiological concentration of acetate (~1 mM) and in the absence of an inhibitory effect of ethanol, the capacity for acetate metabolism was 15–20% of the rate of acetate formation from ethanol and the inhibitory effcet of cthanol further reduced it to 10–15%. The results thus explain the well-known but hitherto not understood fact that only a small fraction of acetate produced in the liver during ethanol oxidation is further metabolized by the liver, while the majority is exported for oxidation in other tissues. Finally, a new method for calculation of liver acetate uptake in the presence of ethanol is presented.

The metabolism of alcohol in the liver has been studied extensively for almost a century and in fact, at some point in the sixties and early seventies, the unravelling of intermediary metabolism had alcohol oxidation as one of its central themes. Consequently, most aspects of alcohol metabolism is well understood with rather few open questions. One of these are, however, why the liver takes up only a small fraction of the acetate produced during ethanol metabolism, in spite of the fact that there is significant activity of the enzyme acetyl-CoA synthetase (Forsander *et al.* 1960; Lundquist *et al.* 1962; Williamson *et al.* 1969; Fellenius & Kiessling 1973). It should be noted, however, that there are considerable disagreement about the capacity of this enzyme, and values between 0.2 to 3 units per g liver wet weight has been reported, corresponding to 20 to 90% of the rate of acetate production by ethanol oxidation (Aas 1971; Barth *et al.* 1971; Fellenius *et al.* 1973; Knowles *et al.* 1974). Direct inhibition of acetate metabolism during ethanol oxidation has never been demonstrated.

The present work was undertaken to elucidate these questions. We find that acetate metabolism is in fact inhibited by ethanol, and that the capacity of hepatocytes for acetate metabolism is low compared to the rate of ethanol oxidation.

Materials and Methods

Cell preparations. Hepatocytes were isolated from female Wistar rats (230 g) fasted for 48 hr by collagenase perfusion (Quistorff *et al.* 1990). The cells were suspended to a final concentration of 5–7* 10^6 cells/ml in Krebs-Henseleit buffer with 10 mM lactate, 2 mM pyruvate and 1% bovine serum albumin.

Incubations. 1.9 ml of the cell suspension was incubated with shaking (80 strokes/min.) in 25 ml flasks with filter papers in centerwells. The gas phase was 95% O_2/5% CO_2. After 15 min. preincubation at 37° the incubations were started at t=0 by addition of 100 µl acetate and ethanol mixture. The final concentrations in the incubations were 1, 2.5, 5 and 10 mM [2–^{14}C]NaAcetate (12,000 dpm/µmol) and when present 10 mM ethanol. This concentration of ethanol ensured saturation of alcohol dehydrogenase. Incubations were performed in duplicate and with parallel incubations without cells.

The incubations were stopped after 30 min. by addition of perchloric acid to a final concentration of 0.7 M. 125 µl of 4 N NaOH was added to the filter papers and the flasks were allowed to stand for 1 hr at 37° with shaking to collect $^{14}CO_2$. The filter papers were transferred to tubes with 2 ml of water. The day after the incubations the filter papers were removed and the radioactivity of 1 ml of the water was determined. The acidified cell suspensions were centrifuged and the pellets were used for determination of ^{14}C in fatty acids. The supernatants were used for determination of ^{14}C in non-volatile, water soluble products and, after neutralization with KOH and triethanolamine for metabolite determinations.

Metabolite assays. To measure the ^{14}C incorporation in fatty acids the perchloric acid precipitate was washed 3 times in 2 N perchloric acid and twice in water, dissolved in NaOH, and the radioactivity determined. To measure the ^{14}C incorporation in tricarboxylic acid cycle intermediates, ketone bodies, and other non-volatile components, remaining [2-^{14}C]acetate was removed by freeze-drying of the perchloric acid extracts. The amount of [2-^{14}C]acetate was calculated as the total radioactivity in the perchloric acid extracts minus the radioactivity of the freeze-dried perchloric acid extracts.

Acetate (Holz & Bergmeyer 1970), ethanol (Lundquist 1959), 3-hydroxybutyrate (Williamson & Mellanby 1970), and lactate (Hohorst 1970), were measured using enzymatic assays on the neutralized perchloric acid supernatants. Pyruvate and acetoacetate were measured in a combined enzymatic assay in 0.2 M TRIS, pH7.6 (Mellanby & Williamson 1970).

Calculations. At high concentrations of acetate, and especially in the presence of ethanol, the chemically measured uptake of acetate becomes unreliable due to the small, relative change in the concentration of acetate. Acetate metabolism was therefore determined as incorporation of [2-^{14}C]acetate into products. Acetate uptake in the absence of ethanol as determined by measurements of the concen-

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trations before and after the incubations was 85–116% of the [2-^{14}C]acetate incorporation into products.

When ethanol is metabolized in the cells, the acetate pool is diluted by acetate produced from ethanol, and the specific radioactivity of acetate falls exponentially during the incubation period. Furthermore, the acetate concentration rises, causing an increase in the rate of acetate metabolism at lower-than-saturating acetate concentrations.

In order to calculate the mean specific radioactivity of acetate during the incubation, an iterative procedure was worked out as described below:

The mean of the specific radioactivity at the beginning and at the end of the incubations were used as a first approximation. The rate of acetate metabolism was calculated from this approximated specific radioactivity and the incorporation of radioactivity into products. The rates of acetate metabolism and the corresponding mean acetate concentrations were subsequently used for calculation of the apparent K_m and V_{max} for the metabolism of [2-^{14}C]acetate. The values were then used in a stepwise calculation of the time-course of the acetate concentration and the specific radioactivity of acetate during the 30 min. incubation:

(1) $[\text{acetate}]_{t+\Delta t}=[\text{acetate}]_t+\Delta[\text{ethanol}]_{\Delta t}-\Delta[\text{acetate}]_{\Delta t}$

where square brackets indicate concentrations at time t and t+Δt, respectively. $\Delta[\text{acetate}]_{\Delta t}$ was obtained from the Michaelis-Menten equation using the K_m and V_{max} values obtained above:

(2) $\Delta[\text{acetate}]_{\Delta t}=\Delta t(V_{max}[\text{acetate}]_t/([\text{acetate}]_t+K_m)$

The specific radioactivity of acetate at time t+Δt ($SR_{acetate,t+\Delta t}$) equals total radioactivity of acetate at time t+Δt ($R_{acetate,t+\Delta t}$) divided by $[\text{acetate}]_{t+\Delta t}$ (as obtained from equation (1)):

(3) $SR_{acetate,t+\Delta t}=(R_{acetate,t+\Delta t})/[\text{acetate}]_{t+\Delta t}$

where ($R_{acetate,t+\Delta t}$) was calculated as ($R_{acetate,t}$)−($\Delta R_{acetate,\Delta t}$), in which $\Delta R_{acetate,\Delta t}$ was obtained by multiplication of the specific radioactivity of acetate at time t by the change in acetate concentration in Δt (from equation (2)).

After 3 to 4 iterations using Δt=2 min., a constant mean specific radioactivity of acetate was obtained. This value was then used for calculation of the rate of acetate metabolism.

The calculated specific radioactivity of acetate was about 15% less than the mean value of the specific radioactivity at the start and at the end of the incubations. The best fit was obtained when the rate of ethanol oxidation was 1.4 μmol/(10^8 cells · min.). Changes in V_{max}- and K_m-values had only minor influence on the results of the calculations. The acetate concentrations used in fig. 1 are the means of the experimentally determined concentrations before and after the incubations. The error on this values as compared to that calculated by the iterative procedure was negligable (<1%).

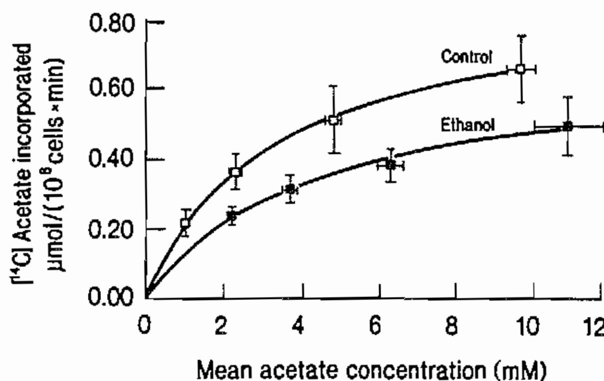

Fig. 1. ^{14}C-Incorporations from [2-^{14}C]acetate into metabolic products in the presence and absence of ethanol. Values are means±S.D. for 5 experiments. (□) without ethanol, (■) with 10 mM ethanol.

Apparent K_m's for acetate and V_{max}'s of [2-^{14}C]acetate metabolism were calculated from Lineweaver-Burk plots by linear regression with proportional weighting.

Statistics. K_m's and V_{max}'s in the presence and absence of ethanol were compared by paired-data t-test. All values given are means±S.D. with the number of animals in parenthesis.

Results and Discussion

Fig. 1 shows the incorporation of ^{14}C from [2-^{14}C]acetate into metabolic products at different concentrations of acetate in the presence and absence of ethanol. The metabolism of [2-^{14}C]acetate appeared to follow Michaëlis-Menten kinetics with an apparent K_m for acetate of 3.02±0.37 mM and an apparent V_{max} of 0.845±0.118 μmol/(10^8 cells · min.) in the absence of ethanol. In the presence of ethanol, K_m for acetate was increased to 4.06±1.34 mM, while there was a statistical significant decrease in V_{max} to 0.662±0.148 μmol/(10^8 cells · min.) ($P<0.05$, n=5). The inhibition of acetate metabolism at 1 mM acetate by ethanol was 36%±7%.

The incorporation of [2-^{14}C]acetate into non-volatile water soluble products and carbon dioxide was inhibited by 40% and 60%, respectively by ethanol at 1 mM acetate. The incorporation of [2-^{14}C]acetate into fatty acids was enhanced two-fold in the presence of ethanol (results not shown).

In accordance with established results (Forsander *et al.* 1965; Williamson *et al.* 1969), the lactate/pyruvate and 3-hydroxybutyrate/acetoacetate ratios were 3.13±0.07 and 1.09±0.40 in the absence of ethanol and increased to 38.0±16.6 and 3.12±0.18 in the presence of ethanol (n=5). There was no dependency on the concentration of acetate, except for a slight increase in the lactate/pyruvate ratio with increasing acetate concentrations in the absence of ethanol (2.69±0.01 and 3.49±0.19 at 1.0 and 9.7 mM acetate, respectively). Total ketone body formation was unchanged (results not shown). The rate of oxidation of ethanol was in the range 0.74−1.14±0.25 μmol/(10^8 cells · min.) (n=4).

A possible explanation for the inhibition of acetate metabolism by ethanol could be the well known fall in tricarboxylic acid cycle flux (Williamson *et al.* 1969; Lindros 1972) during ethanol oxidation combined with an unaltered ketone body synthesis (Grunnet & Kondrup 1983; present study). Since the oxidation of acetyl-CoA in the TCA-cycle has a low apparent V_{max} and in particular a low K_m compared with ketone body synthesis (Ontko & Jackson 1964), a decrease of the tricarboxylic acid cycle flux would result in a lower apparent V_{max} and a higher apparent K_m for acetate uptake, as observed. The possibility that the relatively small oxidation of ethanol-generated acetate in the liver could be caused by metabolic zonation with a predominant location of the cytosolic acetyl CoA synthetase in the upstream part of the sinusoids has been discarded in a recent study (Knudsen *et al.* 1992).

The concentration of acetate in the hepatic vein is about 1 mM during ethanol metabolism (Lundquist *et al.* 1962; Nuutinen *et al.* 1985). Based on the K_m and V_{max} values

above, the capacity for acetate metabolism at this concentration of acetate can be calculated to 0.21 and 0.13 μmol/(10^8 cells · min.) in the absence and presence of ethanol, respectively, which can be compared to a rate of acetate formation from ethanol of 1–1.5 μmol/(10^8 cells · min.). In conclusion, the present results show that the small fractional metabolism of acetate by the liver during ethanol oxidation is due primarily to a low capacity of hepatocytes for acetate metabolism with a minor contribution from inhibition of acetate metabolism by ethanol.

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