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Metabolism of acetaldehyde in human and baboon renal cortex

Ethanol synthesis by isolated baboon kidney-cortex tubules

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Acetaldehyde (1–20 mM) was metabolized at high rates and in a dose-dependent manner in isolated human and baboon kidney-cortex tubules. Acetaldehyde removal was accompanied by a large accumulation of acetate in both human and baboon tubules. By contrast, a large synthesis of ethanol was observed only in baboon tubules. Consistent with the latter finding, ethanol was found to be metabolized at significant rates in baboon but not human tubules. In the tubules from both species, a significant fraction of the acetaldehyde removed was also completely oxidized to CO_2 and H_2O. These results suggest that, in both man and baboon, the kidneys participate in the in vivo metabolism of acetaldehyde; they also suggest that, in contrast with the human kidneys, the baboon kidneys contribute to the detoxication of circulating ethanol.

Acetaldehyde; Ethanol; Acetaldehyde metabolism; (Human, Baboon kidney)

1. INTRODUCTION

Acetaldehyde, the first oxidation product of ethanol metabolism in the liver, is a very reactive compound to which many of the toxic effects of ethanol consumption are attributed [1]. Although most of the acetaldehyde formed by liver alcohol dehydrogenase (EC 1.1.1.1) under in vivo conditions is considered to be oxidized instantaneously to acetate by aldehyde dehydrogenase (EC 1.2.1.3), some acetaldehyde escapes from the liver intact and is found in the circulating blood after ethanol administration in man [2,3] and experimental animals [4–6]. Apart from the liver, other organs may contribute significantly to the degradation of circulating acetaldehyde as suggested by the high acetaldehyde concentration found in hepatic venous blood as compared with that in arterial blood [3–6] but their capacity to degrade acetaldehyde has not been well defined.

The above considerations, together with the observation that the human kidney contains some acetaldehyde dehydrogenase activity [7,8], led to the experiments reported here in which the capacity of isolated human and baboon kidney-cortex tubules to metabolize acetaldehyde was determined.

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2. MATERIALS AND METHODS

Fresh normal kidney cortex was obtained from the uninvolved pole of kidneys removed for neoplasm from 18 h fasted patients as well as from normal kidneys removed from 18 h fasted baboons *(Papio papio)* used in heart transplantation experiments. Specimens of cortex were immediately dissected and placed in ice-cold Krebs-Henseleit

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buffer gassed with a mixture of O_2/CO_2 (19:1) until the beginning of the tubule-isolation period (usually within 10 min). Kidney tubules were prepared by collagenase treatment as in [9,10]. Incubations were performed at 37°C in a shaking water bath in 25 ml hermetically stoppered Erlenmeyer flasks in an atmosphere of O_2/CO_2 (19:1). The tubule obtained were incubated for 30 or 60 min with various concentrations of acetaldehyde or with 5 mM ethanol in 4 ml Krebs-Henseleit medium. In all experiments, each experimental condition was carried out in duplicate. Incubations were terminated by adding $HClO_4$ (2%, v/v, final concentration) to each flask. In each experiment, zero-times flasks were prepared with acetaldehyde or ethanol by adding $HClO_4$ before the tubules. At the end of each experiment, the flasks were cooled to 4°C for 30 min to avoid evaporation of acetaldehyde on removal of the stoppers; after removal of the denatured protein by centrifugation ($4000 \times g$ for 10 min at 0°C), the supernatant was neutralized with 20% (w/v) KOH and kept on ice before metabolites were assayed. In experiments where [U-^{14}C]acetaldehyde was used as substrate, incubation, deproteinization, collection and measurement of the $^{14}CO_2$ formed were carried out as in [11]; the medium was then treated as described above.

Acetaldehyde was determined by the method of Bernt and Bergmeyer [12], and ethanol according to Bernt and Gutmann [13]. Acetate was measured by the Boehringer acetate assay kit (Meylan, France). The dry weight of the amount of tubules added to the flasks was determined as in [9]. Net substrate utilization and product formation were calculated as the difference between the total contents of the flasks (tissue + medium) at the start (zero-time flasks) and after the period of incubation. The metabolic rates are expressed in μmol substance removed (or produced)/g dry wt tubule fragments per unit time. They are reported as means ± SE. The rates of conversion of [U-^{14}C]acetaldehyde into $^{14}CO_2$ were calculated by dividing the radioactivity in $^{14}CO_2$ by the specific activity of the labelled acetaldehyde determined in the zero-time samples for each experiment.

Acetaldehyde, which was freshly redistilled prior to use, was supplied by Merck (Darmstadt) and absolute ethanol was obtained from Prolabo (Paris). [U-^{14}C]Acetaldehyde (7.4 mCi/mmol) was supplied by New England Nuclear (Boston, MA). Enzymes and coenzymes were supplied by Boehringer (Meylan).

3. RESULTS AND DISCUSSION

3.1. *Characteristics of acetaldehyde metabolism in human and baboon kidney tubules*

As shown in tables 1 and 2, both human and baboon tubules readily metabolized acetaldehyde at all concentrations of acetaldehyde tested. Acetaldehyde utilization, which was approximately linear over a 60 min incubation period, increased with the substrate concentration and reached its maximum at 10 mM acetaldehyde. This observation that intact renal tubules metabolized high concentrations of acetaldehyde at high rates and in a dose-dependent manner with increasing substrate concentrations is in contrast with the results of Cederbaum and Rubin [14] who found that, in rat kidney mitochondria, acetaldehyde oxidation was saturated at a substrate concentration of 0.08 mM. This also suggests that one (or several) of the isoenzymes of aldehyde dehydrogenase, which initiate acetaldehyde metabolism and are found in renal cortex [7,8,15], has a much higher K_m for acetaldehyde than that found in rat kidney mitochondria.

In both human and baboon kidney tubules, a large fraction of the acetaldehyde removed was recovered as acetate, whose accumulation was also approximately linear with time (tables 1,2). This means that, thanks to aldehyde dehydrogenase, acetate was formed at a faster rate than it was capable of being metabolized by acetyl-CoA synthetase (EC 6.2.1.1), an enzyme also known to be present in renal tissue [16,17]; thus acetyl-CoA synthetase appears to be rate-limiting in renal acetaldehyde metabolism in the two species studied here.

In human kidney tubules, ethanol synthesis was also linear with time and dose-dependent but it occurred at very low rates and never represented more than 5.3% of the acetaldehyde utilization (table 1). In contrast, high rates of ethanol synthesis were observed in baboon tubules at all the acetaldehyde concentrations used (table 2). This suggests that the alcohol dehydrogenase found in human kidney [18,19] is not very active when compared with that in baboon kidney. This view is fur-

TABLE 1

Metabolism of various concentrations of acetaldehyde in human kidney-cortex tubules

Parameter measured	Incubation (min)	Acetaldehyde concentration				
		1 mM	2 mM	5 mM	10 mM	20 mM
Acetaldehyde removal	30	−277.0 ± 30.2	−335.2 ± 25.8	−372.3 ± 73.1	−518.4 ± 49.7	−489.3 ± 82.2
	60	−568.3 ± 27.6	−669.1 ± 30.0	−728.0 ± 29.2	−885.3 ± 37.0	−835.5 ± 21.4
Ethanol synthesis	30	+ 2.8 ± 0.3	+ 10.8 ± 3.7	+ 17.6 ± 4.4	+ 22.4 ± 4.1	+ 21.0 ± 2.0
	60	+ 5.7 ± 2.7	+ 20.9 ± 2.6	+ 37.1 ± 7.6	+ 46.5 ± 0.3	+ 36.8 ± 6.5
Acetate accumulation	30	+225.2 ± 34.5	+226.9 ± 32.6	+244.8 ± 29.1	+227.3 ± 29.6	+214.7 ± 43.8
	60	+385.3 ± 36.4	+413.4 ± 33.5	+424.8 ± 23.2	+419.9 ± 20.7	+337.6 ± 27.1

Kidney tubules were incubated as described in section 2. Each flask contained 4.9 ± 1.0 mg dry wt tubules. Results (in μmol/g dry wt) for metabolite removal (−) or production (+) are reported as means ± SE for 4 experiments performed in duplicate

TABLE 2

Metabolism of various concentrations of acetaldehyde in baboon kidney-cortex tubules

Parameter measured	Incubation (min)	Acetaldehyde concentration				
		1 mM	2 mM	5 mM	10 mM	20 mM
Acetaldehyde removal	30	−481.7 ± 59.5	− 644.1 ± 88.4	− 751.1 ± 187.3	−1177.0 ± 134.7	− 944.9 ± 55.7
	60	−929.3 ±123.4	−1168.7 ± 156.1	−1534.0 ± 219.5	−1723.0 ± 292.5	−1480.6 ± 238.2
Ethanol synthesis	30	+ 83.4 ± 13.0	+ 155.2 ± 25.9	+ 262.7 ± 40.1	+ 231.5 ± 47.5	+ 199.8 ± 42.6
	60	+142.1 ± 17.1	+ 255.9 ± 32.3	+ 501.7 ± 68.3	+ 441.3 ± 86.5	+ 303.5 ± 46.0
Acetate accumulation	30	+259.1 ± 44.5	+ 295.4 ± 31.6	+ 394.4 ± 51.1	+ 412.4 ± 64.1	+ 381.9 ± 61.5
	60	+475.9 ±114.3	+ 571.4 ± 64.9	+ 751.3 ± 102.6	+ 790.1 ± 119.9	+ 598.2 ± 62.3

Kidney tubules were incubated as described in section 2. Each flask contained 2.8 ± 0.2 mg dry wt tubules. Results (in μmol/g dry wt) for metabolite removal (−) or production (+) are reported as means ± SE for 4 experiments performed in duplicate

ther supported by our observation that, with 5 mM ethanol as substrate, appreciable amounts of ethanol were removed by baboon tubules (180 ± 29.7 μmol/g dry wt per h; $n = 4$), whereas negligible amounts were removed by human tubules (10.1 ± 5.8 μmol/g dry wt per h; $n = 4$).

Tables 1 and 2 also show that, in both human and baboon kidney tubules, significant amounts of the acetaldehyde removed were not accounted for by the sum of the acetate accumulated plus the ethanol synthesized, suggesting that some acetaldehyde was further oxidized beyond the stage of acetate. Complete oxidation of acetaldehyde was demonstrated by measuring the conversion of 5 mM [U-^{14}C]acetaldehyde into $^{14}CO_2$ in baboon kidney tubules; in these experiments ($n = 4$), acetaldehyde removal was 1084.9 ± 13.6 μmol/g dry wt per h and its conversion into $^{14}CO_2$ was 191.2 ± 5.1 μmol/g dry wt per h, representing 17.6% of the acetaldehyde removed, a value close to that calculated from the data in table 2. Thus, a significant fraction of the acetaldehyde removed was completely oxidized to CO_2 and H_2O in baboon and presumably also in human tubules.

3.2. *Physiological relevance*

This study is the first to demonstrate the large capacity of the human and baboon renal cortex to metabolize acetaldehyde. Although the concentration of acetaldehyde in arterial blood of humans and baboons after ethanol consumption is much lower (1–20 μM) than the acetaldehyde concentrations used in the present work, our data strongly suggest that the kidneys of these two species can significantly participate in the extrahepatic elimination of acetaldehyde in vivo. In this respect, it should be noted that, after ethanol administration, the rat kidney has been shown to extract 50% of the acetaldehyde present in the renal artery [4]. It should also be stressed that all the isoenzymes of aldehyde dehydrogenase present in renal cortex are not sensitive to the inhibitory effect of disulfiram [7], a compound used to deter alcoholics from drinking ethanol; since the administration of disulfiram leads to an increase in blood acetaldehyde to concentrations of up to 2.3 mM [2], it is conceivable that our observations are of 'physiological' value and that, in disulfiram-treated patients, the kidneys contribute to maintain the blood acetaldehyde concentration at relatively safe levels by converting this very toxic compound into acetate, CO_2 and H_2O.

This work also shows that the baboon renal cortex is unique when compared with a rat, dog, guinea-pig [20] and human renal cortex in that it is able to synthesize and metabolize ethanol at significant rates. Therefore, our data suggest that the baboon kidneys contribute to the combustion of ethanol to a much greater extent than previously believed in other species where ethanol is considered to be almost exclusively metabolized by the liver [21,22]. In addition, our results indicate that this species difference should be considered when using the baboon as an experimental model for alcohol metabolism in humans.

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REFERENCES

[1] Sorrell, M.F. and Tuma, D.J. (1979) Clin. Sci. 57, 481–489.
[2] James, T.N. and Bear, E.S. (1967) Am. Heart J. 74, 243–255.
[3] DeMaster, E.G., Redfern, B., Weir, E.K., Pierpont, G.L. and Crouse, L.J. (1983) Alcoholism Clin. Exp. Res. 7, 436–442.
[4] Marchner, H. and Tottmar, O. (1976) Acta Pharmacol. Toxicol. 38, 59–71.
[5] Kesäniemi, Y.A. (1974) Biochem. Pharmacol. 23, 1157–1162.
[6] Jauhonen, P., Baraona, E., Mikyakawa, H. and Lieber, C.S. (1982) J. Lab. Clin. Med. 100, 908–916.
[7] Harada, S., Agarwal, D.P. and Goedde, H.W. (1980) Life Sci. 26, 1773–1780.
[8] Goedde, H.W. and Agarwal, D.P. (1981) J. Clin. Chem. Clin. Biochem. 19, 179–189.
[9] Baverel, G., Bonnard, M., d'Armagnac de Castanet, E. and Pellet, M. (1978) Kidney Int. 14, 567–575.
[10] Baverel, G., Bonnard, M. and Pellet, M. (1979) FEBS Lett. 101, 282–286.
[11] Baverel, G. and Lund, P. (1979) Biochem. J. 184, 599–606.
[12] Bernt, E. and Bergmeyer, H.U. (1974) in: Methods of Enzymatic Analysis (Bergmeyer, H.U. ed.) vol. 3, pp. 1506–1509, Academic Press, New York.

[13] Bernt, E. and Gutmann, I. (1974) in: Methods of Enzymatic Analysis (Bergmeyer, H.U. ed.) vol. 3, pp. 1499–1502, Academic Press, New York.
[14] Cederbaum, A.I. and Rubin, E. (1977) Arch. Biochem. Biophys. 179, 46–66.
[15] Deitrich, R.A. (1966) Biochem. Pharmacol. 15, 1911–1922.
[16] Barth, C., Sladek, M. and Decker, K. (1972) Biochim. Biophys. Acta 260, 1–9.
[17] Knowles, S.E., Jarrett, O.G., Filsell, O.H. and Ballard, F.J. (1974) Biochem. J. 142, 401–411.
[18] Schmidt, E. and Schmidt, F.W. (1960) Klin. Wochenschr. 38, 957–962.
[19] Moser, K., Papenberg, J. and von Wartburg, J.P. (1968) Enzym. Biol. Clin. 9, 447–458.
[20] Michoudet, C. and Baverel, G. (1985) in: Kidney Metabolism and Fuction (Dzurik, R. et al. eds) pp. 143–149, Martinus Nijhoff, Dordrecht.
[21] Lundsgaard, E. (1938) CR Lab. Carlsberg 22, 333–337.
[22] Krebs, H.A. and Perkins, J.R. (1970) Biochem. J. 118, 635–644.