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CHARACTERISTICS OF ACETALDEHYDE METABOLISM IN ISOLATED DOG, RAT AND GUINEA-PIG KIDNEY TUBULES

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Abstract—The metabolism of acetaldehyde was studied in isolated dog, rat and guinea-pig kidney-cortex tubules. In contrast with previous observations of Cederbaum and Rubin in rat kidney mitochondria (*Archs Biochem. Biophys.* **179**, 46–66 1977) acetaldehyde was found to be metabolized by the tubules at high rates and in a dose-dependent manner at concentrations up to 5–10 mM. At high acetaldehyde concentrations (1–10 mM) acetaldehyde removal was accompanied by a high rate of acetate accumulation which explained most of the acetaldehyde metabolized in dog and guinea-pig but not in rat kidney tubules. These species differences in acetaldehyde metabolism can be explained by the differences in activities of aldehyde dehydrogenase (EC 1.2.1.3) and acetyl-CoA synthetase (EC 6.2.1.1), the enzymes involved in renal acetaldehyde metabolism which were measured in the renal cortex of the three species. The acetaldehyde carbon removed and not accounted for by acetate accumulation was completely oxidized to CO_2 as demonstrated by the measurement of [U-^{14}C]-acetaldehyde conversion into $^{14}CO_2$. At "physiological" acetaldehyde concentrations (0.1 and 0.2 mM) acetaldehyde utilization was also concentration-dependent but no acetate accumulation was observed.

Acetaldehyde, which forms a complex with amino-oxyacetate [1], a well-known inhibitor of aspartate aminotransferase [2], has been shown to reverse the effects of this inhibitor on gluconeogenesis and ureogenesis in isolated rat hepatocytes [3]. During the course of a study in which we used acetaldehyde to reverse the inhibition of lactate gluconeogenesis by amino-oxyacetate in isolated dog kidney-cortex tubules, we observed that acetaldehyde itself greatly inhibited the metabolism of lactate (unpublished results). This observation led us to examine whether acetaldehyde could be metabolized by the tubules. The data obtained clearly establish that acetaldehyde, the first oxidation product of ethanol metabolism in the liver [4], is metabolized at high rates by tubules isolated from dog, rat and guinea-pig kidney cortex. They also provide precise information on the fate of acetaldehyde carbon in this preparation. In addition, activities of the enzymes involved in the renal metabolism of acetaldehyde are reported in the three species studied.

MATERIALS AND METHODS

Animals. Kidneys were from adult mongrel dogs (15–20 kg) of either sex, male guinea-pigs (400–600 g) of the Dunkin–Hartley albino strain and male Wistar rats (200–300 g), obtained from Iffa-Credo, Saint Germain-sur-l'Arbresle, France. The animals were fed on a standard diet (U.A.R., Villemoisson-sur-Orge, France).

Preparation of kidney-cortex tubules and incubation procedures. After the dogs were anesthetized with i.v. sodium penthiobarbital (25 mg/kg body wt) and rats and guinea-pigs killed by a blow on the head and cervical dislocation, the kidneys were excised and placed in ice-cold isotonic saline. Kidney-cortex tubules were prepared as described previously [5].

Incubations were carried out at 37° in a shaking water bath, in 25 ml hermetically stoppered Erlenmeyer flasks in an atmosphere of O_2/CO_2 (19:1). In all experiments the tubular fragments obtained were incubated with various concentrations of acetaldehyde in 4 ml of Krebs–Henseleit medium [6]. In all experiments each experimental condition was carried out in duplicate. Incubations were terminated by adding $HClO_4$ (2%, v/v, final concn.) to each flask. In each experiment, zero-time flasks were prepared with acetaldehyde by adding $HClO_4$ before the tubules. At the end of each experiment the flasks were cooled at 4° for 30 min to avoid evaporation of acetaldehyde on removal of the stoppers and, after removal of the denatured protein by centrifugation (4000 *g* for 10 min at 0°, 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 described by Baverel and Lund [7]; the medium was then treated as described above.

Analytical methods. Acetaldehyde was determined by the method of Bernt and Bergmeyer [8], and ethanol by the method of Bernt and Gutmann [9]. 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 previously described [5].

Measurement of enzyme activities. Pieces of dog, rat and guinea-pig renal cortex were rapidly excised,

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rinsed in an ice-cold buffer (pH 7.2) containing 0.25 M saccharose, 5 mM Tris–HCl and 0.5 mM EDTA, weighed and homogenized in 9 vol. of the same buffer containing sodium deoxycholate (30 mg/g wet wt of cortical tissue) in a Potter-Elvehjem glass homogenizer with a motor-driven Teflon pestle. The homogenates were then filtered through a double layer of cheese cloth to remove connective tissue as described by Tottmar *et al.* [10] for aldehyde dehydrogenase (EC 1.2.1.3) activity measurement, or centrifuged at 38,000 *g* for 15 min at 2° for acetyl-CoA synthetase (EC 6.2.1.1) and alcohol dehydrogenase (EC 1.1.1.1) activity measurements.

Aldehyde dehydrogenase was assayed spectrophotometrically by measuring the reduction of NAD^+ at 340 nm in a 50 mM sodium pyrophosphate buffer at pH 8.8 as described by Tottmar *et al.* [10]; these assays were performed at 24° to limit acetaldehyde evaporation. Acetyl-CoA synthetase and alcohol dehydrogenase were assayed at 37° as described by Hanson and Ballard [11] and by Krebs *et al.* [12], respectively. The unit of enzyme activity is defined as the amount of enzyme that transforms 1 μmol of substrate/min under the conditions described.

Reagents. Acetaldehyde, which was freshly redistilled prior to use, was supplied by Merck (Darmstadt, F.R.G.); [U-^{14}C] acetaldehyde (7.4 mCi/mmol) was obtained from New England Nuclear (Boston, MA). Enzymes and coenzymes were supplied by Boehringer (Meylan, France). The other chemicals used were of analytical grade.

Calculations. 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 of substance removed, or produced, per g dry wt. of tubule fragments per unit time. They are reported as means ± SEM.

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 radioactivity of the labelled acetaldehyde determined in the zero-time samples for each experiment.

RESULTS AND DISCUSSION

Time-course and concentration dependence of acetaldehyde metabolism

As shown in Fig. 1, acetaldehyde was readily used as substrate by dog, rat and guinea-pig kidney tubules. Acetaldehyde utilization, which was approximately linear with time over a 60 min incubation period, increased with substrate concentration from 1 to 5 mM and then, depending on the species studied, increased, plateaued or decreased at the highest acetaldehyde concentration used (10 mM); this progressive increase in acetaldehyde metabolism with high acetaldehyde concentrations contrasts with the results of Cederbaum and Rubin [13] who found that, in isolated rat kidney mitochondria, acetaldehyde oxidation was saturated at very low concentration (0.08 mM). This suggests that, in rat kidney cortex, the cytosolic isoenzyme of aldehyde dehydrogenase, whose existence has been demonstrated [14, 15], has a much lower affinity for acetaldehyde than the mitochondrial isoenzyme.

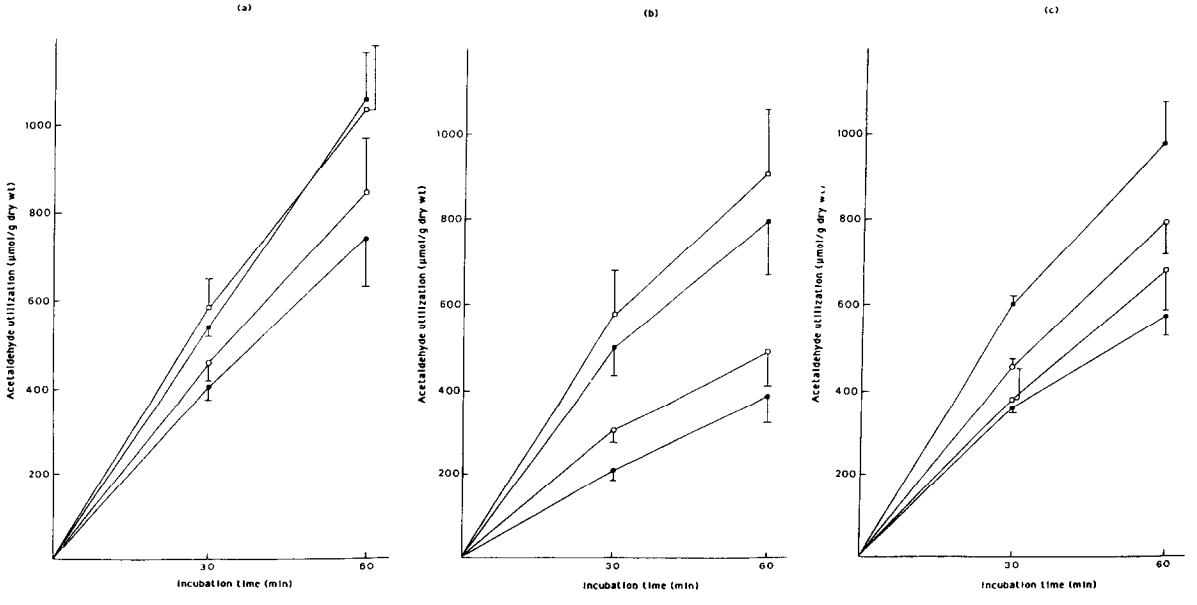

Fig. 1. Time course of acetaldehyde metabolism by dog (a), rat (b) and guinea-pig (c) kidney tubules. Kidney tubules were incubated as described in the Materials and Methods section. Each flask contained 4.0 ± 0.1, 4.6 ± 0.3 and 6.4 ± 0.4 mg dry wt of tubules prepared from renal cortex of dogs, rats and guinea-pigs, respectively. Results are given as means for 4 experiments performed in duplicate for each species; vertical lines represent + or − SEM. ●, 1 mM acetaldehyde; ○, 2 mM acetaldehyde; ■, 5 mM acetaldehyde; □, 10 mM acetaldehyde.

Table 1. Metabolism of various concentrations of acetaldehyde in dog, rat and guinea-pig kidney cortex tubules

Species	Acetaldehyde concentration 1 mM	2 mM	5 mM	10 mM
Dog				
Acetaldehyde removal	−796.4 ± 45.5	−914.7 ± 51.2	−1115.9 ± 46.2	−1133.1 ± 8.1
Acetate accumulation	+490.6 ± 35.7	+564.3 ± 70.5	+708.6 ± 71.9	+768.1 ± 17.9
Rat				
Acetaldehyde removal	−436.7 ± 12.5	−574.4 ± 30.0	−808.6 ± 40.9	−998.5 ± 46.4
Acetate accumulation	+70.9 ± 7.1	+123.0 ± 15.8	+276.9 ± 27.5	+383.6 ± 23.0
Guinea-pig				
Acetaldehyde removal	−646.5 ± 24.6	−782.3 ± 59.8	−1043.2 ± 63.3	−687.5 ± 107.9
Acetate accumulation	+429.7 ± 25.8	+585.8 ± 31.2	+805.7 ± 4.1	+476.8 ± 13.3

Kidney tubules were incubated as described in the Materials and Methods section. Each flask contained 3.9 ± 0.1, 5.4 ± 0.4 and 5.1 ± 0.7 mg dry wt of tubules prepared from the renal cortex of dogs, rats and guinea-pigs, respectively. Results (μmol/g dry wt × hr) for metabolite removal (−) or accumulation (+) are reported as means ± SEM for 4 experiments performed in duplicate for each species.

It should be noted that the high rates of acetaldehyde metabolism in isolated rat kidney tubules observed in the present study are in agreement with the demonstration of Deitrich [14] and of Lamboeuf *et al.* [15] that the rat kidney contains a high aldehyde dehydrogenase activity which is exceeded only by that of the liver; these authors, however, did not use acetaldehyde as substrate but rather indole-3-acetaldehyde [14, 15].

Metabolic fate of acetaldehyde carbon

Table 1 shows that, in all species, the high rates of acetaldehyde removal were accompanied by high rates of acetate accumulation which also increased in parallel with the substrate utilization.

Acetate accumulation indicates that the rate of the aldehyde dehydrogenase reaction, which initiates the metabolism of acetaldehyde, exceeded that of the reaction catalyzed by acetyl-CoA synthetase which converts acetate into acetyl-CoA; thus, acetyl-CoA synthetase is rate-limiting in renal acetaldehyde metabolism.

It should be noted that, at all acetaldehyde concentrations, most of the acetaldehyde removed was recovered as acetate in dog and guinea-pig, but not in rat kidney tubules (Table 1). These differences were presumably due to species differences in the relative rates of acetate synthesis by aldehyde dehydrogenase and of acetate activation by acetyl-CoA synthetase. This is demonstrated by the data of Table 2 showing that the ratio of the activity of aldehyde dehydrogenase over that of acetyl-CoA synthetase is higher in guinea-pig and dog than in rat renal cortex. In agreement with previous findings of other authors [12, 16–19], some alcohol dehydrogenase activity was found in rat renal cortex (Table 2); by contrast, no alcohol dehydrogenase activity could be detected in dog and guinea-pig kidney cortex (Table 2). This is in line with our observation that no ethanol was synthesized from 5 mM acetaldehyde in dog and guinea-pig tubules, whereas very small amounts of ethanol (representing less than 5% of the acetaldehyde removed) were synthesized from 5 mM acetaldehyde in rat kidney tubules (data not shown).

Table 3 shows that there is a relatively good agreement between the conversion of [U-^{14}C] acetaldehyde into $^{14}CO_2$ and the difference between the utilization of acetaldehyde and the accumulation of acetate. This demonstrates that the acetaldehyde removed by dog, rat and guinea-pig tubules which was not accounted for by the acetate accumulation was completely oxidized to CO_2 and H_2O.

Table 2. Activity of enzymes of acetaldehyde utilization in the cortex of dog, rat and guinea-pig kidney

Species	Enzyme activity: Aldehyde dehydrogenase	Acetyl-CoA synthetase	Alcohol dehydrogenase	Aldehyde dehydrogenase / Acetyl-CoA synthetase
Dog	2.25 ± 0.16 (4)	1.99 ± 0.06 (4)	N.D. (4)	1.13
Rat	1.12 ± 0.07 (4)	1.72 ± 0.15 (3)	0.93 ± 0.16 (3)	0.65
Guinea-pig	5.14 ± 0.26 (4)	2.29 ± 0.21 (3)	N.D. (3)	2.24

Enzyme assays were performed as described in the Materials and Methods section. Values (units/g fresh wt × min) are reported as means ± SEM.

The number of preparations used for the enzyme activity determinations are given in parentheses. N.D. = not detectable.

Table 3. Fate of 5 mM [U-^{14}C]acetaldehyde in dog, rat and guinea-pig kidney tubules

Species	Incubation (min)	Acetaldehyde removed	Acetate found	Acetaldehyde not accounted for	[U-^{14}C]acetaldehyde converted into $^{14}CO_2$
Dog	30	−434.3 ± 17.4	+317.5 ± 34.8	116.8 ± 24.4	+70.5 ± 14.6
	60	−786.6 ± 40.3	+548.6 ± 54.3	238.0 ± 20.0	+195.3 ± 18.6
Rat	30	−283.1 ± 6.6	+121.8 ± 6.4	161.3 ± 8.3	+233.8 ± 26.6
	60	−529.7 ± 7.5	+191.9 ± 3.7	337.8 ± 11.6	+383.0 ± 38.9
Guinea-pig	30	−421.9 ± 29.6	+410.7 ± 25.6	11.2 ± 14.6	+69.0 ± 12.4
	60	−785.7 ± 65.4	+705.2 ± 65.9	80.5 ± 11.0	+132.9 ± 7.3

Kidney tubules were incubated for 30 and 60 min as described in the Materials and Methods section. Each flask contained 13.5 ± 0.3, 12.0 ± 1.1 and 16.1 ± 2.0 mg dry wt of tubules prepared from the renal cortex of dogs, rats and guinea-pigs, respectively. Results (μmol/g dry wt) for metabolite removal (−) or accumulation (+) are reported as means ± SEM for 3 experiments performed in duplicate for each species. The "acetaldehyde not accounted for" was calculated as the acetaldehyde removed minus the acetate found.

Table 4. Time-course of the removal of "physiological" concentrations (0.1 and 0.2 mM) of acetaldehyde in isolated dog, rat and guinea-pig kidney tubules

Species	Incubation (min)	Acetaldehyde removal 0.1 mM acetaldehyde	0.2 mM acetaldehyde
Dog	30	−220.4 ± 9.1	−268.1 ± 13.9
	60	−459.3 ± 23.6	−500.0 ± 20.7
Rat	30	−205.9 ± 25.2	−226.6 ± 26.7
	60	−404.0 ± 39.3	−430.3 ± 60.8
Guinea-pig	30	−256.4 ± 44.9	−268.9 ± 43.1
	60	−475.3 ± 52.1	−535.1 ± 72.1

Kidney tubules were incubated as described in the Materials and Methods section. Each flask contained 0.9 ± 0.3, 0.9 ± 0.1 and 0.9 ± 0.1 mg wt of tubules prepared from the renal cortex of dogs, rats and guinea-pigs, respectively. Results (μmol/g dry wt × hr) for metabolite removal (−) are reported as means ± SEM for 3 experiments performed in duplicate for each species.

Physiological relevance

Although most of the acetaldehyde formed by liver alcohol dehydrogenase under *in vivo* conditions is considered to be oxidized instantaneously to acetate [20], some acetaldehyde escapes intact from the liver and is released into the circulating blood after ethanol administration [21–23]. After ethanol ingestion or injection, blood acetaldehyde concentration in man [24] or rats [22, 25, 26] can reach 0.1 to 0.2 mM. The data of Table 4 show that at these "physiological" concentrations of acetaldehyde, this compound is very actively metabolized by dog, rat and guinea-pig kidney-cortex tubules; under these conditions, no acetate accumulation was observed. Therefore, this study, which is the first to demonstrate that intact renal cortical cells metabolize acetaldehyde at high rates *in vitro*, suggests that, *in vivo*, the kidneys of the three species studied, have a large capacity to remove circulating acetaldehyde. Although the urinary excretion of acetaldehyde was not measured in their study, the observation by Marchner and Tottmar that acetaldehyde concentration in renal venous blood was 50% lower than that in arterial blood of a rat after ethanol administration [22] is in agreement with the proposal that the kidneys play a significant role in the detoxication of circulating acetaldehyde, a very reactive compound to which many of the toxic effects of ethanol consumption are attributed [27].

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