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STIMULATORY EFFECT OF ETHANOL ON WEAK ORGANIC ACID UPTAKE IN RAT RENAL TUBULES

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Abstract—Ethanol at relatively low concentrations (10–40 mM) significantly stimulated the uphill uptake of a weak organic acid, fluorescein, in the superficial proximal tubules of rat renal cortex slices, but it did not affect the rate of glucose production from lactate or pyruvate in rat renal cortex fragment suspension. In a low Na^+ medium, ethanol failed to stimulate fluorescein uptake, although under the conditions employed in the present study, the baseline weak organic acid uptake was not dependent on external Na^+. The stimulation of fluorescein uptake by ethanol (20 mM) was abolished by an inhibitor of alcohol dehydrogenase (EC 1.1.1.1), pyrazole (1 mM), or an inhibitor of aldehyde dehydrogenase (EC 1.2.1.3), cyanamide (0.3 mM), suggesting that oxidation of ethanol mediated its effect on the uptake. Among gluconeogenesis inhibitors tested, only D-malate (2 mM) abolished the stimulatory effect of ethanol, while the rest either did not affect (quinolinate) or even slightly augmented (α-cyano-4-hydroxycinnamate and phenylpyruvate) it. The effect of ethanol was markedly increased by an inhibitor of the tricarboxylic acid cycle, fluoroacetate. It was concluded that the stimulation by ethanol of weak organic acid uptake in rat renal tubules was mediated by the production of acetate.

Key words: fluorescein uptake; ethanol; acetate; inhibitors of gluconeogenesis (rat kidney)

Ethanol can alter cellular functions either due to its metabolism or by affecting directly certain plasma membrane constituents. In the liver, where ethanol is mainly metabolized, its conversion into acetaldehyde and acetate gives rise to a decrease in the cytosolic and mitochondrial $NAD^+/NADH$ ratio (for review see Ref. 1). As a consequence, hepatic gluconeogenesis (GNG†) from reduced glucose precursors, such as lactate, is inhibited [2], while that from pyruvate is either not affected [2] or is even stimulated [3] by ethanol. Direct effects of ethanol on hepatocytes, which are associated with its interference with the interactions between G-proteins and receptor or effector proteins in the plasma membrane, are usually observed at rather higher ethanol concentrations (review, Ref. 4). In such a case, the responsiveness of hepatic GNG to hormones may be impaired [5].

Transport processes are inhibited by ethanol at high concentrations (review, Ref. 1). Thus, ethanol (50–100 mM) decreases K^+ uptake in rat hepatocytes [6], which agrees with both the inhibition of hepatic Na,K-ATPase [7] and decline in the intracellular ATP content of rat liver [5]. At the same time, ethanol at relatively low (saturation at 30 mM) concentrations stimulated the uptake of a weak organic anion, 5-methyltetrahydrofolate (5-MTHF), in rat hepatocytes, with the effect being blocked by an inhibitor of alcohol dehydrogenase, pyrazole [8].

Although alcohol dehydrogenase was found in the rat renal cortex [2, 9, 10], any data revealing an influence of ethanol oxidation on renal cellular functions were absent from the literature. Inhibition of K^+ uptake in the rabbit renal cortex [11] and activity of Na,K-ATPase from the rat renal cortex [12] were observed only at high ethanol concentrations.

Recently, we have shown that the uptake of a weak organic acid, fluorescein, in rat renal proximal tubules (RPTs) was modulated by certain inhibitors of GNG, suggesting a regulatory role for the cytoplasmic redox potential in the uptake [13]. Since substrate activation of alcohol dehydrogenase could modulate the redox potential (review, Ref. 1), it was of interest to study whether ethanol oxidation would affect the fluorescein uptake in rat RPTs. The obtained data revealed the stimulatory effect of ethanol (10–40 mM) on the uptake, which was likely to be mediated by the production of acetate.

MATERIALS AND METHODS

Methods of preparation of rat renal cortex slices and rat renal cortex fragment suspension, as well as those used to determine fluorescein uptake by the superficial RPTs in the slices and the rate of glucose production in the suspension, have been described elsewhere [13]. Briefly, the work was carried out on the kidneys of male Wistar rats weighing 180–250 g. The outermost cortex slices (0.5–0.8 mm) were prepared by hand with the aid of a razor blade and preincubated at 20–22° for 60 min in aerated, substrate-free physiological buffer containing (mmol/L): NaCl 104.7; KCl 15.3; $CaCl_2$ 1.5; $MgSO_4$ 2.5; $NaHCO_3$ 3.6; NaH_2PO_4 3.3; Na_2HPO_4 4.8; pH 7.1–7.3 (20°).

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† Abbreviations: GNG, gluconeogenesis; 5-MTHF, 5-methyltetrahydrofolate; RPT, renal proximal tubule; CHC, α-cyano-4-hydroxycinnamate; T/M, tubule/medium.

After preincubation, the slices were incubated at 20–22° (usually for 20 min) in aerated medium of the same composition supplemented with 0.05 mM fluorescein. In some experiments, an incubation medium with a low Na^+ concentration was used, which contained (mmol/L): NaCl 10; choline chloride 110; $CaCl_2$ 1.5; $MgSO_4$ 2.5; $KHCO_3$ 3.6; KH_2PO_4 3.3; K_2HPO_4 4.8; pH 7.0–7.2 (20°). All the substances tested were added to incubation medium from neutralized aqueous stock solutions. The ethanol-containing incubation media were prepared by adding the appropriate volume of absolute ethanol.

The amount of fluorescein accumulated in the superficial RPTs was determined with the aid of a laboratory-built microfluorimeter with a contact objective lens and glass filters (the primary filter with λ_{max} at 400 nm, the secondary filter with λ_{max} at 540 nm). The filters of the microfluorimeter had wide enough bands of transmission to minimize an influence of intracellular pH changes on the intensity of the fluorescein luminescence. On the surface of each slice, the luminescence intensity in 40 different RPTs was measured. The background luminescence was subtracted. The measurements were repeated on slices from three animals, so that each point represents the mean of 120 individual records. The results were expressed in the form of normalized concentration ratios, T/M (tubule/medium), the T/M value of equilibrium fluorescein accumulation during incubation at 2–4° for 90 min being considered as unity. The uptake data are presented as means ± 2 SE.

The cortex fragment suspension (final content 4–8 mg protein/mL) prepared without treating the tissue with proteases was incubated at 20–22° for 30 min in flasks containing 1 mL of the standard physiological buffer (as above) containing 0.5% bovine serum albumin (fraction V, de-fatted, Sigma Chemical Co., St Louis, MO, U.S.A.) in a shaker at 70 cycles/min. Ethanol, lactate and pyruvate were added to the incubation medium. The incubation was stopped by adding 0.1 mL of ice-cold 30% $HClO_4$. After neutralization and centrifugation, glucose content in clear extracts was determined by the glucose oxidase technique. The pellets were used for measurements of protein content according to a modification of Lowry's method [14].

Chemicals. Fluorescein (disodium salt, uranine, C.I. 45350) was obtained from Koch-Light Laboratories Ltd (Colnbrook, U.K.); pyrazole, cyanamide, quinolinate, α-cyano-4-hydroxycinnamate (CHC) and D(+)-malate from Sigma; monofluoroacetate, pyruvate, lactate and 2-phenylpyruvate from Serva (Heidelberg, F.R.G.). Other reagents were of commercial grade.

RESULTS

When studying the influence of ethanol over a range of concentrations from 10 to 100 mM on the fluorescein uptake in rat RPTs, two media, with standard (Fig. 1A) and low (Fig. 1B) Na^+ content, were used. In controls, sucrose (20, 40 or 100 mM) was introduced into the media. It is seen that in the standard medium, ethanol at the concentrations under test significantly augmented fluorescein uptake. The maximal effect was observed at ethanol concentrations of 10–20 mM. In the low Na^+ medium, ethanol failed to stimulate the uptake, although under the conditions employed in the present study, the baseline weak organic acid uptake did not depend on external Na^+. In further experiments, ethanol at a concentration of 20 mM was administered.

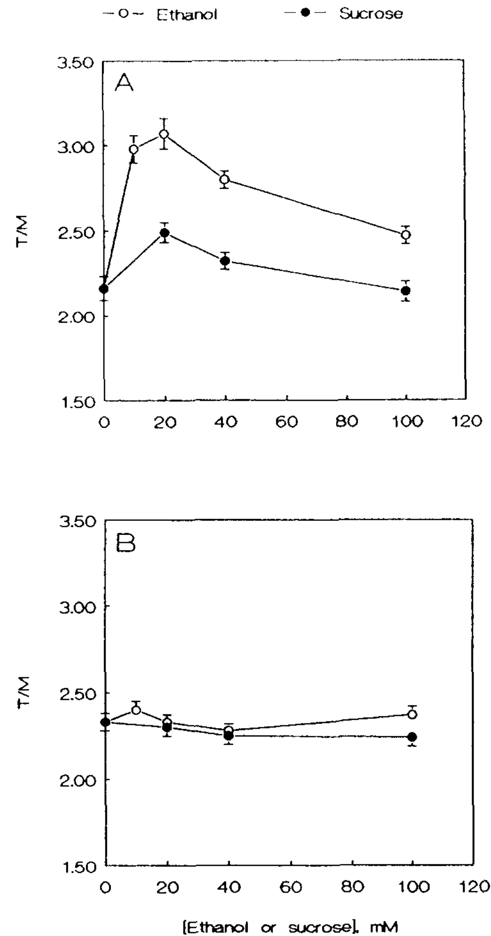

Fig. 1. Influence of ethanol at various concentrations on fluorescein uptake in standard (A) and low Na^+ (B) media. Slices were preincubated in aerated, substrate-free standard physiological solution at 20° for 60 min and then incubated with fluorescein (0.05 mM) at 20° for 20 min. Each point represents the mean of 120 individual records on slices from three rats. The vertical lines show the 95% confidence limits. In controls, equimolar amounts of sucrose were introduced into the bath media.

Time courses of the fluorescein uptake (Fig. 2) revealed that the degree of stimulatory effect of ethanol hardly changed during the incubation period lasting from 10 to 40 min. Stimulation of the fluorescein uptake by ethanol was entirely prevented by addition of inhibitors of alcohol dehydrogenase (pyrazole) or aldehyde dehydrogenase (cyanamide) to the incubation medium (Fig. 3), while neither pyrazole nor cyanamide inhibited *per se* the baseline fluorescein uptake.

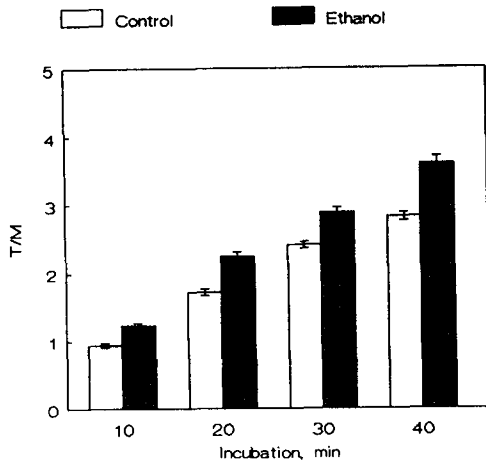

Fig. 2. Time courses of fluorescein uptake with or without ethanol 20 mM). Each bar represents the mean of 120 individual records on slices from three rats. The vertical lines show the 95% confidence limits. Other conditions as in the legend to Fig. 1.

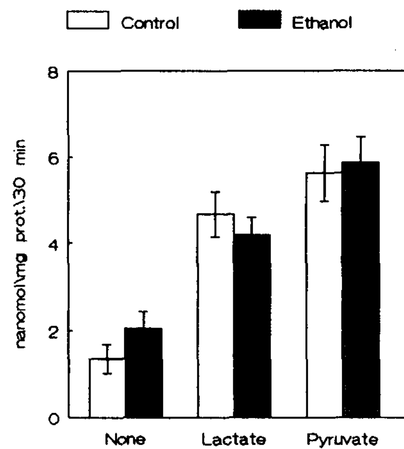

Fig. 4. Rates of glucose production from lactate, pyruvate (both at 5 mM) or endogenous substrates in rat renal cortex fragment suspension incubated with or without ethanol (20 mM). Renal cortex fragment suspension prepared as in Ref. 13 was incubated at 20° for 30 min. Data are presented as means ± SE (N = 9).

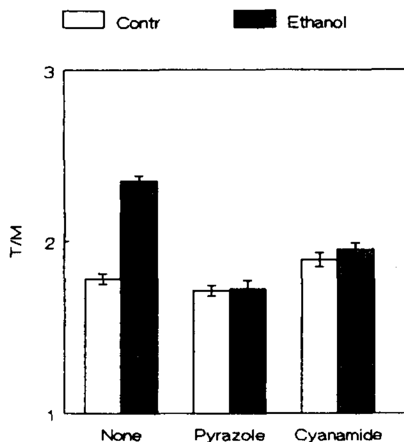

Fig. 3. Influence of pyrazole (1 mM) and cyanamide (0.3 mM) on the effect of ethanol (20 mM) on fluorescein uptake. Conditions as in the legend to Fig. 1.

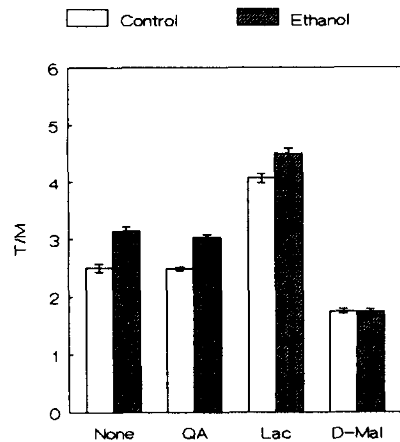

Fig. 5. Influence of quinolinate (QA; 2 mM), lactate (Lac; 5 mM) and D-malate (D-Mal; 2 mM) on the stimulatory effect of ethanol (20 mM) on fluorescein uptake. Conditions as in the legend to Fig. 1.

At the same time, ethanol fails to affect GNG from either lactate or pyruvate in the rat renal cortex fragment suspension (Fig. 4), which is in agreement with the data obtained by Krebs *et al.* [2] on rat renal cortex slices. Gluconeogenesis from endogenous substrates was practically unaffected by ethanol as well.

The stimulatory effect of ethanol on the fluorescein uptake was almost additive to that of lactate (Fig. 5). Quinolinate failed to modify the ethanol effect, while D-malate abolished it. Except for D-malate, none of the tested GNG inhibitors prevented the stimulatory effect of ethanol on the uptake. Moreover, such inhibitors of intramitochondrial metabolism of pyruvate as CHC, phenylpyruvate and, especially, fluoroacetate augmented the effect of ethanol (Fig. 6).

DISCUSSION

Ethanol at relatively low concentrations (up to 40 mM) significantly stimulated the 5-MTHF uptake in rat hepatocytes [8]. This effect was abolished by an inhibitor of alcohol dehydrogenase, pyrazole, suggesting that it was oxidation of ethanol which mediated its effect on the uptake. The stimulation of the 5-MTHF uptake was concluded to be associated with a reductive shift in the cytoplasmic

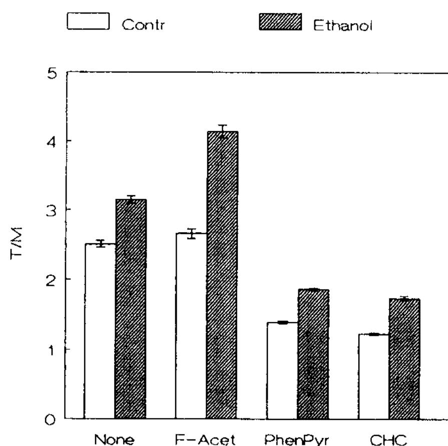

Fig. 6. Influence of fluoroacetate (F-Acet; 1 mM), phenylpyruvate (PhenPyr; 1 mM) and CHC (0.1 mM) on the effect of ethanol (20 mM) on fluorescein uptake. Conditions as in the legend to Fig. 1.

$NAD^+/NADH$ redox potential due to the substrate activation of alcohol dehydrogenase. Recently, we have assumed that the fluorescein uptake in rat RPTs was dependent on the cytoplasmic redox potential, so that the stimulatory effect of lactate on the uptake was presumably mediated by a reductive shift related to the activation of lactate dehydrogenase (EC 1.1.1.27) [13]. Since the rat renal cortex was known to contain not only aldehyde dehydrogenase [10] but also alcohol dehydrogenase [2, 9, 10], in the present work we investigated whether or not ethanol would affect the fluorescein uptake in rat RPTs.

As it turned out, ethanol stimulated the fluorescein uptake at rather low concentrations (10–40 mM), which along with the preventive action of pyrazole, suggested that the effect of ethanol was conditioned by its oxidation. Acetaldehyde, known to be a potent physiologically active intermediate (review, Ref. 15), could hardly mediate by itself the effect of ethanol, since this effect was blocked by an inhibitor of aldehyde dehydrogenase, cyanamide. Thus, it might well be that the stimulation of fluorescein uptake by ethanol is associated with a reductive shift in the cytoplasmic redox state. If this was so, the effect of ethanol on the uptake would be similar to that of lactate. However, this was not the case. The stimulatory effect of lactate on the fluorescein uptake in rat RPTs was attenuated by CHC, phenylpyruvate or quinolinate, while D-malate augmented the lactate effect [13]. In contrast, the stimulation of the uptake by ethanol was blocked by D-malate, whereas the effect of ethanol was augmented moderately in the presence of CHC or phenylpyruvate, and was not modulated by quinolinate. Besides, the effects of lactate and ethanol on the fluorescein uptake were almost additive. Thus, it is very likely that the alcohol dehydrogenase activity in the rat renal cortex is too low to alter directly the cytoplasmic redox state, which is in agreement with the fact that ethanol fails to modulate renal GNG [2] (present work).

It is noteworthy that the effect of ethanol on the fluorescein uptake in rat RPTs is modified by the GNG inhibitors in the same manner as that of acetate, including augmentation of the acetate effect by fluoroacetate [13] and its dependence on external Na^+ [16]. Thus, one can conclude that it is the production of acetate which mediates the stimulatory effect of ethanol on the weak organic acid uptake in rat RPTs.

Three mechanisms explaining the stimulatory effect of acetate on renal weak organic acid uptake have been considered in the literature (review, Ref. 17). Acetate could (1) serve as a metabolic substrate for enhanced ATP production needed to maintain or increase the Na gradient across the basolateral membrane; (2) be metabolized to α-ketoglutarate or (3) favor formation or accumulation of α-ketoglutarate, increasing the dicarboxylate gradient across the basolateral membrane and thus accelerating the exchange of organic anions, such as fluorescein, with the intracellular dicarboxylates. Since the bulk of acetate produced from acetaldehyde in rat renal cortex mitochondria is converted into acetyl-CoA [10], the first and second mechanisms must involve activation of the tricarboxylic acid cycle. However, the fact that fluoroacetate not only fails to prevent the stimulatory effect of acetate on fluorescein uptake, but even augments it [13], does not support the involvement of the tricarboxylic acid cycle in the development of this effect.

The third mechanism demands further experimental efforts to be elucidated. At present, it is obvious that such a mechanism must comprise both intra- and extramitochondrial events. Thus, the stimulatory effect of acetate on the renal fluorescein uptake is shown [18] to be dependent on the activity of Na,K-ATPase, which in turn determines the metabolic state of the mitochondria (review, Ref. 19). The failure of acetate to stimulate fluorescein uptake in the presence of an inhibitor of cytoplasmic malate dehydrogenase (EC 1.1.1.37), D-malate [13], suggests that the export of malate from the mitochondria and/or its oxidation in the cytosol is required for the development of the stimulatory effect of acetate. Re-distribution of α-ketoglutarate between the mitochondria and the cytosol is obligatorily associated with the operation of some exchange carriers of metabolites in the inner mitochondrial membrane (review, Ref. 20). Also, in this connection, it should be emphasized that acetyl-CoA regulates certain intramitochondrial reactions related to mitochondrial metabolite transport (review, Ref. 21).

In conclusion, it may be suggested that upon administration of ethanol *in vivo* the metabolic state of rat renal cortex tissue can be altered not only by the products of its oxidation in the liver but also as the result of ethanol metabolism in the RPT cells.

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