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Acceleration of ethanol and acetaldehyde oxidation by D-glycerate in rats

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Abstract

The aim of the present study was to investigate whether D-glycerate (glycerate) could accelerate ethanol and acetaldehyde (AcH) oxidation in vivo in rats by circumventing the rate-limiting step, that is, the reoxidation of the reduced form of nicotinamide adenine dinucleotide. Male rats belonging to the ANA (Alko, nonalcohol) and AA (Alko, alcohol) rat lines were challenged with 1.2 g ethanol per kilogram with or without glycerate administration (0.1-1.0 g/kg). Blood ethanol, blood AcH, and liver free glycerol concentrations were determined during ethanol intoxication. Glycerate treatment, regardless of the dose, accelerated ethanol elimination by approximately 25% ($P < .001$) in the ANA animals. Glycerate also accelerated the AcH oxidation, but perhaps not as much as the ethanol oxidation, as indicated by a trend toward elevated AcH levels. In the experiments with the AA rats, glycerate treatment elevated hepatic free glycerol levels by about 50% ($P < .05$) during alcohol intoxication. The acceleration of ethanol and AcH oxidation in conjunction with elevated glycerol levels by the treatment with glycerate supports the hypothesis that the aldehyde dehydrogenase–mediated AcH oxidation can be coupled with the reduction of glycerate to D-glyceraldehyde catalyzed by the same enzyme. Such a coupling should increase the availability of the oxidized form of nicotinamide adenine dinucleotide and thus accelerate both ethanol and AcH oxidation. Further studies are needed to investigate how the AcH could be even more efficiently oxidized to reduce the harmful effects of ethanol-derived AcH.

1. Introduction

In a recent review it was concluded that acetaldehyde (AcH) plays a role in many of the harmful effects of ethanol [1]. Thus, it is only natural that research should be focused on how to minimize the toxic effects of this metabolite of alcohol. This could be achieved, for example, by reducing the rate of AcH formation (ie, ethanol oxidation), by increasing the rate of AcH oxidation, or by binding the AcH before it exerts its toxic effects.

Reducing the oxidation rate of alcohols by pyrazole and its derivatives has proved effective in some acute toxic conditions related to highly elevated aldehyde levels [2-4], but has not rendered general use because of the toxicity of these compounds [5].

AcH trapping in vivo has been tried in a number of ways. Compounds including penicillamine, cysteine, and methionine (precursor of cysteine) have been tested with limited success [6-8]. In humans, some success has been demonstrated by trapping the AcH in saliva by a slow-release buccal tablet of cysteine [9].

Theoretically, AcH oxidation by aldehyde dehydrogenase (ALDH) could be increased and, subsequently, AcH levels lowered by activation of the reoxidation of the reduced form of nicotinamide adenine dinucleotide (NADH) to the oxidized form (NAD^+) at this reaction site. This could be achieved by a substrate that would use the same ALDH, catalyzing its reduction directly by using the ALDH-NADH complex formed in the oxidation of AcH to acetate. Such an oxidation/reduction coupling, using the ethanol oxidation to AcH by alcohol dehydrogenase (ADH) and the reversal with propionaldehyde reduced to propanol, has been demonstrated in vitro [10].

D-Glycerate (glycerate), being a product of fructose breakdown, could be the compound capable of coupling the ALDH-catalyzed oxidation with a simultaneous reduction. Such an activated NADH reoxidation could preferentially support the activation of the AcH oxidation and, thus, lower the ethanol-derived AcH levels.

In normal physiology, glycerate is formed from another product of fructose breakdown, D-glyceraldehyde (glycer-

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aldehyde), in an ALDH-mediated, NAD^+-consuming reaction, creating NADH. The aim of the present study was to investigate if this reaction could be reversed by a pharmacologic dose of glycerate, thereby activating the reoxidation of NADH into NAD^+, and whether such activation would increase the ethanol and AcH oxidation rates in vivo.

2. Materials and methods

2.1. Animals

Male ANA (Alko, nonalcohol) and AA (Alko, alcohol) rats, genetically selected for low and high alcohol drinking preference [11], were used in the present study. These rats are known to display elevated (ANA) or low (AA) AcH levels during ethanol intoxication [12]. The ANA rats belonged to the $F_{84, 85, 87}$ generation and were 2 to 4 months old at experiments 1 and 2. In experiment 3, the AA rats, generation $F_{89\text{-}90}$, were 3 to 5 months old. The animals were given water and a standard laboratory chow (SDS RM1, Witham, Essex, England) ad libitum. They were kept under controlled conditions, with the temperature set at 20°C to 21°C, the humidity at 47.6%, and a 12-hour light-dark cycle (lights on at 6:00 AM). The ANA rats had no previous contact with ethanol before the experimental sessions (experiments 1 and 2). Some of the AA animals had undergone an 18-day voluntary ethanol-drinking period (choice between two bottles containing either tap water or 10% [vol/vol] ethanol solution in water), followed by a 3-day recovery period before the ethanol administration experiments (experiment 3).

2.2. Experiments and procedures

In the experiments, D-glycerate hemicalcium salt monohydrate (Sigma Aldrich Chemical, Milwaukee, WI) was given as 10% (wt/vol) in saline to the animals by intraperitoneal (IP) administration at doses of 0.5 (experiment 1) and 0.1 (experiment 2) g/kg. The ethanol dose was 1.2 g/kg IP, given as 10% (wt/vol) in saline, 1 hour after glycerate administration. In experiment 3, the glycerate was mixed in the diet for the whole experimental period (3 weeks). The animals were divided into 3 groups, which received 2, 10, and 20 g glycerate per kilogram of diet. The average glycerate consumption was 0.1, 0.5, and 1.0 g/kg per day, respectively, as calculated based on weekly food consumption. An additional control group received no glycerate in the diet. Glycerate treatment did not significantly affect ethanol consumption (mean overall ethanol consumption was 4.5 ± 0.3 g/kg per day). No signs of toxicity were observed at the present conditions in any of the experiments.

All experimental sessions started at 8:00 to 9:00 AM. The animals were treated either with ethanol alone or with ethanol together with glycerate. To diminish possible pain during the experiments, all animals were injected subcutaneously with buprenorphine (0.01 mg/kg) a few minutes before the other treatments. Capillary blood samples were collected from the tip of the tail 0.33, 1, and/or 2 hours after ethanol injection. Blood samples were hemolyzed with ice-cold water and AcH and ethanol concentrations were determined by headspace gas chromatography during the same day as described previously [13]. AcH values were corrected for minute (less than 1 μmol/L), artifactual, ethanol-derived AcH formation during the analytical procedures.

Immediately after the last blood sample, the animals were anesthetized with pentobarbital (40 mg/kg IP, 1% [wt/vol] in saline) in experiment 3. Thereafter (5-10 minutes), liver pieces were quickly (within 2-4 seconds) excised and freeze-clamped by means of aluminum clamps precooled in liquid nitrogen. Liver samples were stored at −71°C until glycerol determination.

For the liver free glycerol measurements, the freeze-clamped livers were thoroughly homogenized and diluted 1:6 with mQ water, assuming the liver tissue density of 1 g/mL. The homogenates were incubated in a boiling water bath for 5 minutes and centrifuged at 14 000 rpm for 15 minutes. The resulting supernatants were used for free glycerol measurement with an enzymatic end point commercial assay kit (Boehringer-Mannheim, R-Biopharm, Darmstadt, Germany) according to the manufacturer's protocol except that the assays were modified for use with small volumes of supernatant using a 96-well microplate reader (Labsystems Multiskan RS, Helsinki, Finland). Samples were assayed in duplicate.

All experiments were carried out in accordance with the European Communities Council directive of November 24, 1986 (86/609/EEC). The study was approved by the County Administrative Board of Southern Finland and the ethical committee of the Finnish National Public Health Institute.

2.3. Statistical analyses

Because of the lack of normal distribution, the ethanol and AcH data were analyzed by the nonparametric Mann-Whitney test for group comparisons. Spearman ρ was used for correlation analyses. Normal distribution was displayed in the glycerol data; here, Student t test was used for group comparisons and Pearson r for correlation analyses. Data are presented as mean ± SEM. In experiment 3, no differences were observed among the 3 different glycerate doses; thus, these groups were combined for further statistical analyses. All statistical analyses were performed by using SPSS 14.0 (SPSS, Chicago, IL).

3. Results

The AcH results from experiments 1 and 2 are listed in Table 1. Compared with ethanol alone, glycerate in conjunction with ethanol elevated the blood AcH levels 107% (lower glycerate dose) and 124% (higher dose) on

Table 1
Effect of D-glycerate on blood AcH levels

Treatment	Blood AcH concentration		
	Time after ethanol administration (h)		
	0.33	1.0	2.0
Experiment 1			
With glycerate (0.5 g/kg)		11.4 ± 2.8 (15)*	6.6 ± 1.9 (15)
Without glycerate		5.1 ± 1.7 (15)	8.1 ± 2.5 (15)
Experiment 2			
With glycerate (0.1 g/kg)	12.2 ± 5.2 (7)	2.9 ± 1.4 (7)	1.7 ± 0.6 (6)
Without glycerate	3.2 ± 1.9 (6)	1.4 ± 0.6 (6)	0.9 ± 0.6 (6)

Acetaldehyde levels are expressed as micromoles per liter (n).
* $P < .1$, with and without glycerate compared.

average 1 hour after the ethanol administration. These elevations were, however, not significant in the separate experiments. Neither was any of the AcH differences at any other time point significant. In experiment 3, although no significant group differences were found in the blood AcH levels, there was a tendency (1 hour after ethanol administration) toward increasing AcH levels at increasing glycerate doses (Spearman $\rho = 0.333$, $P = .1$; from the control mean of 9.1 ± 3.9 μmol/L [n = 6] to the mean from the highest glycerate dose of 12.6 ± 4.2 μmol/L [n = 6]).

The effect of glycerate on blood ethanol levels in experiments 1 and 2 are listed in Table 2. Both doses of glycerate significantly lowered the blood ethanol levels from 1 (20%) to 2 (30%) hours after ethanol administration. Average times for ethanol elimination in experiments 1 and 2 were estimated to be 4.2 and 3.3 hours for the control and glycerate groups, respectively. Thus, glycerate treatment accelerated ethanol oxidation by approximately 25%. In experiment 3, however, no significant group differences between or trends toward differences in blood ethanol concentrations were observed (2 hours after ethanol

Table 2
Effect of D-glycerate on blood ethanol levels

Treatment	Blood ethanol concentration		
	Time after ethanol administration (h)		
	0.33	1.0	2.0
Experiment 1			
With glycerate (0.5 g/kg)		21.8 ± 0.5 (15)***	12.5 ± 0.6 (15)***
Without glycerate		26.8 ± 0.7 (15)	18.0 ± 0.9 (15)
Experiment 2			
With glycerate (0.1 g/kg)	24.1 ± 3.3 (7)	22.4 ± 2.2 (7)*	13.6 ± 1.7 (6)*
Without glycerate	30.6 ± 1.8 (6)	27.3 ± 0.8 (6)	18.8 ± 1.1 (6)

Ethanol levels are expressed as millimoles per liter (n).
* $P < .05$, with and without glycerate compared.
*** $P < .001$, with and without glycerate compared.

Table 3
Effect of D-glycerate on hepatic free glycerol levels during alcohol intoxication

Treatment	Liver free glycerol concentration
Control (no glycerate)	3.06 ± 0.55 (6)
Glycerate (g/kg per day)	
0.1	4.88 ± 1.21 (6)**
0.5	4.23 ± 1.50 (6)
1.0	4.34 ± 1.10 (5)*
Glycerate groups combined	4.50 ± 1.24 (17)**

Glycerol levels are expressed as micromoles per gram wet weight tissue (n).
* $P < .1$ compared with the control group.
** $P \leq .05$ compared with the control group.

administration: control, 20.0 ± 1.0 mmol/L; highest glycerate dose, 19.3 ± 1.5 mmol/L).

Hepatic free glycerol levels in experiment 3 are displayed in Table 3. Glycerate significantly elevated the hepatic free glycerol levels during alcohol intoxication with an average of 47%. No differences were observed among the different glycerate dose groups.

4. Discussion

The ALDH-catalyzed oxidation of AcH is currently supposed to be essentially irreversible. Thus, the functional in vivo importance of a reductive pathway of ALDH-catalyzed reactions has not been suggested before. However, the present results, which display glycerate-mediated increases in ethanol and AcH oxidation and elevated glycerol levels, demonstrate that the ALDH-catalyzed AcH oxidation may be coupled to simultaneous reduction of glycerate to glyceraldehyde at conditions involving a reduced milieu in combination with an excess of glycerate (pathway proposal depicted in Fig. 1).

That glycerate treatment elevated glycerol levels during ethanol intoxication supports the notion of an acceleration of ethanol oxidation caused by increased reoxidation of NADH (the rate-limiting step for ethanol oxidation) derived from the ALDH reaction and/or from the reduction of glyceraldehyde to glycerol at the ADH step. Coupled ethanol oxidation and reduction at the ADH reaction by the use of glyceraldehyde [14] and propionaldehyde [10] has earlier been demonstrated in vitro.

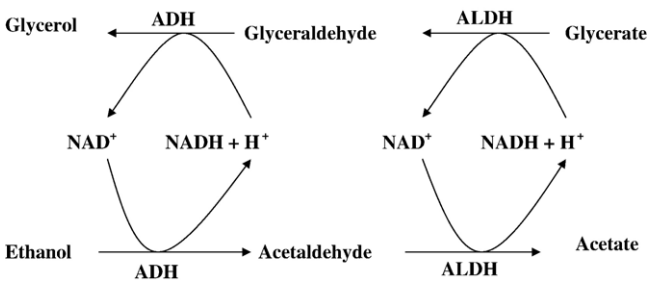

Fig. 1. Proposed mechanism for the effect of D-glycerate on ethanol and AcH metabolism.

The coupling of glyceraldehyde reduction to glycerol with ethanol oxidation to AcH may explain earlier findings, according to which fructose accelerates ethanol oxidation [14]. Thus, depending on the intracellular site of action, glyceraldehyde, the product of fructose breakdown, could directly couple (see Fig.1) with the ADH-mediated ethanol oxidation [15] and/or exert its actions indirectly through an intramitochondrial activation of NADH reoxidation [14]. Theoretically, activated NADH reoxidation could be the consequence of activated consumption of mitochondrial adenosine triphosphate for kinase-mediated phosphorylation reactions in both the fructose and the glycerate cases. This possibility, however, seems unlikely, because such mechanisms would primarily activate the mitochondrial AcH oxidation, which would lead to decreased AcH levels in conjunction with increased ethanol oxidation [16].

In the present study, AcH levels, if anything, were elevated. However, given that ethanol oxidation rate increased about 25% in experiments 1 and 2 and that the efficacy of hepatic acetaldehyde oxidation is more than 95% [17], we may conclude that AcH oxidation rate also increased by more than 20% in those conditions. Altogether, the coupling of the ALDH-catalyzed oxidation/reduction seemed to slightly increase more the ethanol oxidation compared with that of AcH. Earlier, it has been demonstrated that fructose loading elevates AcH levels during ethanol intoxication [18]. These AcH elevations were more significant compared with the present data, perhaps because coupling of the fructose-derived glyceraldehyde can only take place at the ADH site.

In the present conditions, it seems as if the ethanol oxidation would have been more affected by the glycerate treatment compared with the AcH oxidation. This may be explained by the increase in the reoxidation of NADH by the coupled reactions at both the ALDH and the ADH sites and by the ADH reaction gaining more from the recapture of NAD^+ compared with the ALDH reaction. A contributory factor for the slightly less efficient increase of the AcH oxidation could be that the glycerate-derived glyceraldehyde exerts some competitive inhibition on the ALDH-catalyzed Ach oxidation, whereas the glycerol is not a good competitor for the ADH reaction [19]. Further studies are needed to find a more efficient detoxification method of the AcH.

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