EFFECT OF ACETALDEHYDE ON ACTIVITY OF SHUTTLES FOR THE TRANSPORT OF REDUCING EQUIVALENTS INTO THE MITOCHONDRIA

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1. Introduction

Oxidation of ethanol in the liver produces acetaldehyde, which appears in the blood during ethanol metabolism [1]. Apparently, acetaldehyde is produced faster than it is metabolized, despite the fact that, in vitro, aldehyde dehydrogenase is far more active than alcohol dehydrogenase [2]. Mitochondria oxidize several aldehydes, including acetaldehyde [3, 4], and contain 80% of the total aldehyde dehydrogenase activity in the liver [5]. Oxidation of ethanol produces NADH in the cytoplasm; reoxidation of this nucleotide may be rate-limiting for ethanol metabolism [6]. In view of the impermeability of the mitochondria toward NADH [7], several shuttles have been postulated for the transport of reducing equivalents into the mitochondria. These include the α-glycerophosphate [8], the malate–aspartate [9, 10] and the fatty acid shuttles [11]. In view of the importance of the mitochondria in the oxidation of reducing equivalents and of acetaldehyde, we studied the effects of acetaldehyde on the activities of these shuttles. We report here that acetaldehyde (0.6–4.5 mM) is a potent inhibitor of the reconstituted malate–aspartate, α-glycerophosphate and fatty acid shuttles for the transport of reducing equivalents into mitochondria.

2. Methods

Rat liver mitochondria were prepared as previously described [12]. Oxygen consumption was assayed at 23°C using a Gilson oxygraph, equipped with a Clark oxygen electrode in a medium consisting of: 0.3 M mannitol; 10 mM Tris–HCl, pH 7.4; 10 mM KPi, pH 7.4; 2.5 mM $MgCl_2$; 10 mM KCl; 10 mM glutamate ± 1.5 mM ADP and mitochondria equivalent to 2–4 mg protein in a final volume of 3.0 ml. The malate–aspartate, α-glycerophosphate and fatty acid shuttles were reconstituted using the medium described above, 5–10 mg mitochondrial protein and the extramitochondrial components of the shuttles [12, 13]. The α-glycerophosphate shuttle was reconstituted by adding 10 mM α-glycerophosphate, 1 mM ATP and 3 units α-glycerophosphate dehydrogenase. The fatty acid shuttle was assembled by adding 2 mM ATP, 0.2 mM coenzyme A and 0.1 mM albumin-bound palmitate. The malate–aspartate shuttle was formed by adding 3 units of malate dehydrogenase, 3 units of glutamic-oxaloacetic transaminase and either 3 mM glutamate plus 3 mM malate, or 5 mM aspartate plus 1.33 mM α-ketoglutarate. The extramitochondrial NADH-generating system consisted of 6 mM ethanol, 0.25 mM NAD^+ and 16 units alcohol dehydrogenase or 6 mM lactate, 0.25 mM NAD^+ and 20 units lactic dehydrogenase. The rate of ethanol or lactate oxidation, as measured by the disappearance of these substrates from the medium, reflects the

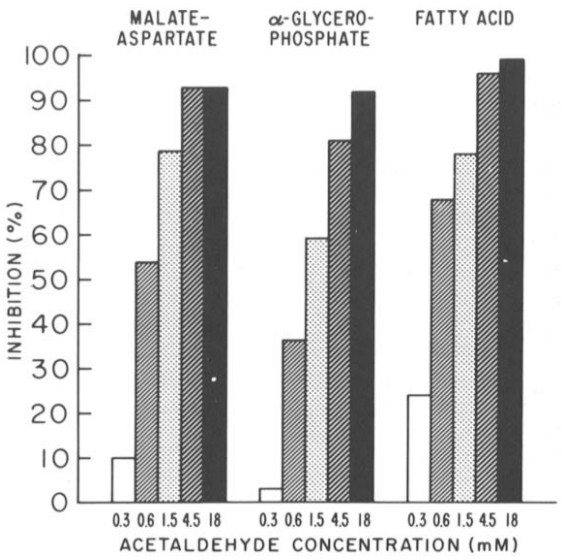

Fig. 1. The effect of acetaldehyde on shuttles for the transport of reducing equivalents into the mitochondria. Ethanol plus alcohol dehydrogenase was used as the extramitochondria NADH-generating system. Control rates of ethanol oxidation (nmoles ethanol/min/mg protein) were: malate–aspartate shuttle 12.60; α-glycerophosphate shuttle, 12.40; fatty acid shuttle, 8.42.

passage of reducing equivalents into the mitochondria. The concentration of ethanol was assayed according to Bonnichsen [14], and that of lactate as described by Hohorst [15]. All values represent the mean of at least three different experiments.

3. Results

In the absence of glutamate plus malate (malate–aspartate shuttle), α-glycerophosphate (α-glycerophosphate shuttle) or albumin-bound palmitate (fatty acid shuttle), the rate of ethanol oxidation was 1–2 nmoles per minute per mg protein. This rate increased to 8–13 nmoles upon reconstitution of the shuttles. Acetaldehyde (1–12 mM) had no effect on the permeability of the mitochondria toward NADH, when measured polarographically or spectrophotometrically. However, it was a potent inhibitor of all 3 reconstituted shuttles, when ethanol was used to generate NADH (fig. 1).

Table 1
Effect of acetaldehyde on shuttles reconstituted with lactate–lactic dehydrogenase.

Shuttle	Acetaldehyde concentration (mM)	Activity (nmoles lactate/min/mg protein)	Inhibition (%)
Malate–aspartate	–	9.77	–
	0.6	7.25	26
	1.5	5.60	43
	4.5	3.69	62
	18	3.56	64
α-Glycerophospahte	–	7.27	–
	0.6	4.72	35
	1.5	3.66	50
	4.5	2.62	64
	18	1.79	75
Fatty acid	–	7.04	–
	0.6	5.78	18
	1.5	5.04	28
	4.5	2.36	66

In view of the fact that we assayed the activities of the shuttles by determining the rate of ethanol oxidation, it seemed possible that acetaldehyde was reduced to ethanol, because the equilibrium of the alcohol dehydrogenase reaction favors ethanol formation at neutral pH. Therefore, inhibition of the shuttles by acetaldehyde might be explained as an artefact, with formation of ethanol from acetaldehyde masking the oxidation of ethanol. To examine this possibility, we studied the effect of acetaldehyde on the shuttles using lactate, NAD^+ and lactic dehydrogenase to generate extramitochondrial NADH. The equilibrium of this system (lactate + $NAD^+ \rightarrow$ pyruvate + NADH + $H^+ \cdot K = 2.9 \times 10^{-12}$ moles/ℓ) is similar to that of the ethanol system (ethanol + $NAD^+ \rightarrow$ acetaldehyde + NADH + $H^+ \cdot K = 8 \times 10^{-12}$ moles/ℓ). Therefore, lactate oxidation by the mitochondria is a measure of transport and oxidation of reducing equivalents in the same manner as ethanol oxidation. The rate of lactate oxidation was somewhat lower than that of ethanol. Although acetaldehyde had no effect on the activity of lactic dehydrogenase, it inhibited shuttle activity with that system, though to a somewhat lesser extent than with the ethanol–alcohol dehydrogen-

Table 2
Effect of acetaldehyde on the extramitochondrial oxidation of NADH by α-ketoglutarate plus aspartate.

Acetaldehyde concentration (mM)	Activity (nmoles lactate oxidized/min)	Effect (%)
–	31.85	–
0.6	32.36	+ 2
1.5	27.10	−15
4.5	18.28	−32
18	12.27	−61

ase system (table 1). The difference in the degree of inhibition may be due to reduction of acetaldehyde to ethanol in the presence of alcohol dehydrogenase. Indeed, using lactate and lactic dehydrogenase to produce NADH, the addition of acetaldehyde and alcohol dehydrogenase resulted in the production of ethanol, even in the presence of the reconstituted shuttles. Using 0.6 mM or 1.5 mM acetaldehyde, about 37–39 nmoles ethanol were formed per minute.

In reconstituted shuttle systems, ethanol is oxidized because the products of the reaction (acetaldehyde and NADH) are oxidized in the mitochondria. Competition for NADH may be expected between acetaldehyde (reversal of the alcohol dehydrogenase reaction) and the shuttle systems which transport the reducing equivalents into the mitochondria. To determine whether any of the shuttles could compete with acetaldehyde for NADH more favorably than the others, we measured the rate of ethanol formation from acetaldehyde in the presence of the alcohol dehydrogenase and lactate–lactic dehydrogenase systems. None of the shuttles competed effectively with acetaldehyde (0.6–4.5 mM) and alcohol dehydrogenase (3–72 units) for NADH, although slightly less ethanol was produced from acetaldehyde in the presence of the reconstituted malate–aspartate and fatty acid shuttles than with the α-glycerophosphate shuttle. A 24-fold variation in alcohol dehydrogenase activity did not affect shuttle activity [16].

The malate–aspartate shuttle may also be reconstituted by the addition of α-ketoglutarate and aspartate, instead of malate and glutamate. Under these conditions, there is considerable oxidation of ethanol or lactate in the absence of mitochondria, since the generation of oxaloacetate by the transaminase reaction allows oxidation of NADH in the presence of

Table 3
Effect of acetaldehyde on glutamate oxidation.

Acetaldehyde concentration (mM)	Oxygen uptake (natoms oxygen/min/mg protein)		Effect (%)	
	State 4	State 3	State 4	State 3
–	8.5	58.5	–	–
1.0	9.2	44.8	+ 8	−24
3.0	7.8	35.1	− 8	−40
12.0	5.0	14.2	−41	−76

malate dehydrogenase, with the subsequent accumulation of malate and glutamate [16]. Acetaldehyde inhibited this extramitochondrial system (table 2) considerably less than the mitochondrial system reconstituted with glutamate and malate (table 1).

Since the 3 shuttles are linked to NAD^+, and operate most efficiently under state 3 conditions [16], it is possible that acetaldehyde may inhibit the shuttles by interfering with NAD^+-dependent state 3 respiration. Acetaldehyde indeed inhibited coupled respiration with glutamate as the substrate (table 3), at concentrations which did not significantly affect state 4 respiration, or state 3 respiration with succinate or ascorbate as the substrate. Acetaldehyde also inhibited the oxidation of NADH by mitochondria which were disrupted by 3 cycles of freezing–thawing. Furthermore, addition of excess NAD^+ to disrupted mitochondria did not prevent the inhibition of glutamate oxidation by acetaldehyde. Therefore, inhibition of NAD^+-dependent substrate oxidation by acetaldehyde is not due to mere competition between acetaldehyde and the substrate for NAD^+.

4. Discussion

Ethanol consumption in man leads to the presence of acetaldehyde in the blood; after the administration of disulfiram (an inhibitor of aldehyde dehydrogenase [17]) concentrations as high as 1 mM have been reported [1]. Since acetaldehyde is highly soluble, its concentration in the blood reflects, at the least, its distribution in total body water. Hence, the concentration in the liver, where acetaldehyde is generated, may be considerably higher than that in the

blood. We find 0.6–1.5 mM acetaldehyde to be a potent inhibitor of 3 different shuttles for the transport of reducing equivalents into the mitochondria. Owing to the formation of ethanol from acetaldehyde and NADH, the inhibition observed with the ethanol–alcohol dehydrogenase system is greater than that with the lactate–lactic dehydrogenase system. When corrected for ethanol formation, comparable inhibition is observed using either extramitochondrial NADH generating system.

Inhibition by acetaldehyde of NAD^+-dependent state 3 respiration may account, in part, for the decrease in shuttle activity induced by acetaldehyde, especially since the extramitochondrial system which oxidizes NADH is less sensitive to acetaldehyde than the mitochondrial one. However, other factors may also be involved, since the inhibition of the shuttles is greater than the respiratory inhibition induced by a similar concentration of acetaldehyde. Acetaldehyde also inhibits the transport of several anions which participate in the shuttles, e.g., glutamate, phosphate and citrate [18]. Acetaldehyde (0.6–4.5 mM) had no effect on the activities of glutamic–oxalacetic transaminase, malate dehydrogenase or lactic dehydrogenase. Purified α-glycerophosphate dehydrogenase activity was inhibited 2, 14, 23 and 63% by 0.6, 1.5, 4.5 and 18 mM acetaldehyde, respectively. The postulated interaction of acetaldehyde with Co-A [19] might contribute toward inhibition of the fatty acid shuttle. Hence, a combination of effects may explain the sensitivity of the shuttles to acetaldehyde. The acetaldehyde-induced inhibition of shuttles for the transport of reducing equivalents suggests that the presence of acetaldehyde may be a limiting factor in ethanol metabolism.

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