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Ethanol Potentiates Oxygen Uptake and Toxicity Due to Menadione Bisulfite in Perfused Rat Liver

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SUMMARY

Menadione bisulfite is a hepatotoxicant that damages periportal regions of the lobule in perfused liver in an oxygen-dependent manner. The effect of ethanol on menadione bisulfite toxicity was examined in perfused rat liver. Addition of menadione bisulfite (3 mM) alone to the perfusate increased oxygen uptake by 20–30 μmol/g/hr. Lactate dehydrogenase was released into the effluent after 60 min of perfusion and reached values around 100 units/g/hr. Under these conditions, trypan blue was taken up exclusively in periportal regions of the liver lobule; 44% of periportal cells were stained. In the presence of ethanol, maximal increases in oxygen uptake due to menadione bisulfite were much larger (about 90 μmol/g/hr), and lactate dehydrogenase release occurred earlier and reached higher maximal values (330 units/g/hr). Trypan blue staining was also more extensive; 90% of periportal cells were stained. The effect of ethanol on menadione bisulfite-induced oxygen uptake required metabolism via alcohol dehydrogenase (ADH), because ethanol increased oxygen uptake due to menadione bisulfite from 44 to 81 μmol/g/hr in deermice with ADH but had no effect in deermice lacking ADH. Other agents that increase NADH (xylitol and 2-ethyl-1-hexanol) also potentiated the stimulation of oxygen uptake due to menadione bisulfite, suggesting that ethanol was working by increasing the NADH redox state. Cyanide abolished the increase in oxygen uptake due to menadione bisulfite, both in the absence and in the presence of ethanol, supporting the hypothesis that the effect of ethanol on menadione bisulfite-mediated oxygen uptake involves the mitochondrial respiratory chain. Further, the stimulation of oxygen uptake by menadione bisulfite in isolated mitochondria was enhanced when matrix NADH was increased by addition of β-hydroxybutyrate. These data indicate that ethanol potentiates oxygen uptake and toxicity due to menadione bisulfite most likely by generation of NADH for redox cycling of this model quinone.

In an effort to understand mechanisms by which chemicals that undergo redox cycling exert toxicity, model quinones such as menadione (2-methyl-1,4-naphthoquinone; vitamin K_3) have been studied extensively. Menadione stimulates oxygen uptake in isolated hepatocytes (1) and perfused liver (2–4) due to redox cycling and/or transfer of electrons to the mitochondrial respiratory chain. This is followed by depletion of glutathione, loss of protein sulfhydryls (5, 6), accumulation of intracellular calcium (6, 7), and release of LDH (3, 4). In perfused liver, cell death due to menadione bisulfite is confined nearly exclusively to oxygen-rich periportal regions of the lobule during perfusion in the anterograde direction and to oxygen-rich pericentral areas when the direction of perfusion is reversed, indicating that oxygen is involved in the mechanism of menadione bisulfite-induced toxicity (4). Further support for this conclusion is the observation that reduction of inflow oxygen tension during anterograde perfusion prevented toxicity.

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The pathways of metabolism of menadione have been studied in isolated cells (5). Two-electron reduction of menadione to a hydroquinone is catalyzed by NAD(P)H:quinone reductase (DT-diaphorase; EC 1.6.99.2). This is considered to be a detoxification pathway (5, 8–10), because the hydroquinone is relatively stable and is conjugated to less reactive metabolites (11). One-electron reduction of menadione is catalyzed by NADH:ubiquinone oxidoreductase in the mitochondria and by NADPH-dependent cytochrome P-450 reductase in the endoplasmic reticulum (12). The resultant semiquinone reacts readily with oxygen to produce superoxide anion, regenerating the parent quinone and thereby completing a redox cycle. Superoxide anion produced can be dismutated to hydrogen peroxide, which reacts with other superoxide anions in the presence of iron to produce highly toxic hydroxyl radicals that can cause cell damage. Indeed, oxygen radical scavengers and desferrioxamine, an iron chelator, decrease toxicity due to menadione (13) and menadione bisulfite (4).

Reduction of menadione in cytosol, microsomes, or mitochondria requires reduced pyridine nucleotides [NADH or

ABBREVIATIONS: LDH, lactate dehydrogenase; ADH, alcohol dehydrogenase (EC 1.1.1.1).

NAD(P)H] as electron donors. Oxidation of ethanol by ADH generates NADH in the cytosol, and the subsequent metabolism of acetaldehyde produces NADH in the mitochondria. Thus, ethanol could theoretically enhance toxicity due to menadione by providing reducing equivalents for redox cycling. The purpose of these studies was to test this possibility, using a water-soluble salt of menadione, menadione bisulfite, in perfused liver. A preliminary account of this work has appeared elsewhere (14).

Materials and Methods

Animals and liver perfusion. Female Sprague-Dawley rats (Zivic Miller, Allison Park, PA), weighing 80–150 g, were used in this study. In addition, deermice that were genetically deficient in ADH (ADH^-) or ADH^+ controls were obtained from a breeding colony, maintained at the University of North Carolina at Chapel Hill, that was established from breeding stock kindly provided by Dr. M. R. Felder of the University of South Carolina. Where indicated, rats and deermice were fasted for 24 hr before use. Livers were perfused in a nonrecirculating system (15) with Krebs-Henseleit bicarbonate buffer (pH 7.4) that was saturated with 95% O_2/5% CO_2 and maintained at 37°. Oxygen concentration in the effluent perfusate was monitored continuously with a Clark-type platinum electrode, and rates of oxygen uptake were calculated from influent minus effluent oxygen concentration differences, the flow rate, and liver wet weight.

Analytical measurements and histological procedures. LDH in the effluent perfusate was determined by standard enzymatic procedures (16), and rates of release were calculated from the effluent concentration, flow rate, and liver wet weight. To detect toxicity in different regions of the liver lobule, trypan blue (0.2 mM) was infused into livers for 10 min at the end of each experiment, followed by fixation of the liver with 1% paraformaldehyde in Krebs-Henseleit buffer. Livers were processed for light microscopy and sections were stained with eosin only. This allows easy identification of trypan blue in the nuclei of irreversibly damaged cells (17). Using light microscopy, five periportal and five pericentral regions were chosen randomly, and the percentage of cells stained with trypan blue in an area 10-cells-wide radiating from the center of each region was identified (18).

Fluorescence of pyridine nucleotides. Fluorescence of NADH was measured from the surface of the perfused liver using a large-tipped (2-mm) light guide (19) and electronics described in detail elsewhere (20). The liver was illuminated at 366 nm and fluorescence at 450 nm was recorded as namp of photomultiplier current.

Preparation of microsomes and mitochondria. Microsomes (21) and mitochondria (22) were prepared by standard techniques of differential centrifugation. All incubations were carried out at room temperature. Microsomes (about 1 mg of protein/ml) were incubated in 2 ml of Krebs-Henseleit buffer (pH 7.4) in the presence of a NADPH-generating system consisting of 0.4 mM $NADP^+$, 30 mM isocitrate, and 0.2 units/ml isocitrate dehydrogenase (Sigma). Mitochondria (about 1 mg of protein/ml) were incubated in 2 ml of 20 mM Tris-HCl buffer (pH 7.2) containing 50 mM sucrose, 100 mM KCl, and 5 mM metaphosphoric acid. Malate (5 mM) and glutamate (5 mM) were added together as substrate. Microsomal or mitochondrial protein was determined by the biuret reaction (23). Oxygen concentration was monitored with a Clark-type platinum electrode, and rates of oxygen uptake were determined from tangents drawn to the oxygen tension versus time curve.

Statistical analyses. Results are represented as mean ± standard error. The data were analyzed using Student's *t* test or two-way analysis of variance. When analysis of variance was used, means were compounded using the least significant difference test. The criterion for significance was $p < 0.05$.

Materials. Menadione sodium bisulfite was purchased from Sigma Chemical Company (St. Louis, MO). As in the previous study by Badr *et al.* (4), the sodium bisulfite salt was used for these studies because free menadione is essentially insoluble in Krebs-Henseleit buffer. Because only the unionized fraction of the salt traverses the cell membrane, at physiological pH the concentration of menadione in the cell would be expected to be far less than the concentration of the salt in the perfusate on theoretical grounds. In support of this idea, the stimulation of oxygen uptake in isolated hepatocytes due to menadione sodium bisulfite in the range of 1–3 mM was similar to that caused by 20–50 μM free menadione base.

Results

Increase in oxygen uptake and potentiation of toxicity due to menadione bisulfite by ethanol. Basal rates of oxygen uptake were approximately 120 μmol/g/hr in livers from normal fasted rats (Fig. 1). Addition of menadione sodium bisulfite alone caused an increase in oxygen uptake of about 33 μmol/g/hr within 5 min (Fig. 1; Table 1). This was followed by a decrease in oxygen uptake during continuous perfusion with menadione bisulfite for 60 min, with values declining to 60 μmol/g/hr in about 30 min. The rapid stimulation of oxygen uptake due to menadione bisulfite was much larger, about 97 μmol/g/hr, in the presence of ethanol (Fig. 1; Table 1). The

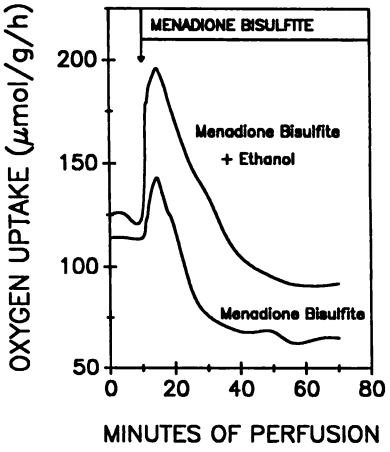

Fig. 1. Effect of ethanol on menadione bisulfite-stimulated oxygen uptake in perfused rat liver. Livers from normal fasted rats were perfused with menadione sodium bisulfite (3 mM) for 60 min in the absence (control) or presence of ethanol (10 mM). Oxygen tension in the effluent perfusate was monitored continuously with a Clark-type oxygen electrode, and oxygen uptake was calculated from the influent minus effluent oxygen concentration differences, the flow rate, and the liver wet weight. Typical experiments.

TABLE 1

Stimulation of oxygen uptake due to menadione bisulfite

Livers from fasted rats were perfused with menadione sodium bisulfite (3 mM) in the absence or presence of ethanol (10 mM), xylitol (2 mM), 2-ethyl-1-hexanol (1 mM), or KCN (2 mM) plus ethanol (10 mM), in experiments similar to those described in the legend to Fig. 1. Oxygen uptake was determined as described in Materials and Methods. Values (means ± standard errors) represent the maximal increase in oxygen uptake (usually observed within 10 min) due to menadione bisulfite ($n = 4$/group except for controls, where $n = 7$).

Addition	Increase in O_2 uptake due to menadione bisulfite
	μmol/g/hr
None	33 ± 6
Ethanol	97 ± 13[a]
Xylitol	86 ± 6[a]
2-Ethylhexanol	59 ± 5[a]
KCN + ethanol	6 ± 1[a]

[a] Significantly different from the value for menadione alone ($p < 0.05$, Student's *t* test).

subsequent decrease in oxygen uptake was also greater in magnitude when ethanol was present. By 40 min of perfusion with menadione bisulfite and ethanol, oxygen uptake decreased from about 190 to 80 μmol/g/hr (Fig. 1). Ethanol alone had no effect on oxygen uptake (Fig. 1), confirming previous results in livers from fasted rats (24).

Perfusion with ethanol alone did not cause LDH release.[1] LDH activity was released into the effluent perfusate at about 70 min of perfusion with menadione bisulfite alone (Fig. 2). Maximal values (100 units/g/hr) were reached after about 110 min, and levels declined subsequently to baseline. In the presence of ethanol, LDH release due to menadione bisulfite occurred 10–20 min earlier, peaked after 80 min, and then decreased to basal values over the next 70 min. Maximal LDH activity was nearly twice as high in the presence (296 units/g/hr) as in the absence (164 units/g/hr) of ethanol (Table 2). Consistent with previous reports (3, 4), cell injury due to menadione bisulfite was confined to periportal regions of the liver lobule. No pericentral cells were stained with trypan blue, whereas 44% of periportal cells were damaged irreversibly (Table 2). Ethanol increased the extent of injury due to menadione bisulfite in periportal areas (90% of cells took up trypan blue), but cells in pericentral regions were unaffected. The percentage of cells stained with trypan blue was directly related to stimulation of oxygen uptake due to menadione bisulfite ($r = 0.86$, $p < 0.05$), suggesting an association between toxicity and oxygen uptake.

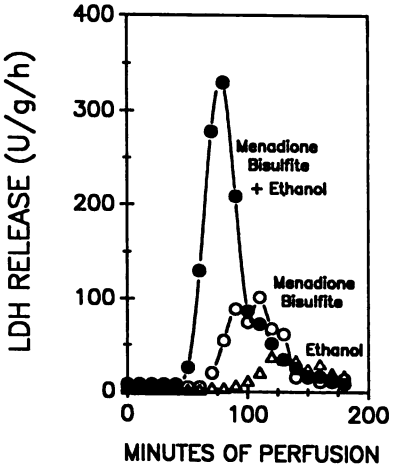

Fig. 2. Potentiation of menadione bisulfite-induced LDH release by ethanol. Livers from normal fasted rats were perfused for 3 hr with ethanol (10 mM) alone (Δ) or with menadione sodium bisulfite (1 mM) in the absence (○) or presence (●) of ethanol (10 mM). The activity of LDH was determined as described in Materials and Methods in perfusate samples taken every 10 min. Typical experiments.

TABLE 2

Toxicity due to menadione bisulfite and ethanol in perfused liver

Livers from fasted rats were perfused with menadione sodium bisulfite (1 mM for percentage of cell staining; 3 mM for LDH release) in the absence or presence of ethanol (10 mM), as described in the legend to Fig. 1. One hour after addition of menadione bisulfite, trypan blue was infused. Livers were fixed and processed for light microscopy, and cell staining was quantified as described in Materials and Methods. Values are means ± standard errors ($n = 4$). No staining was observed in cells in pericentral regions in livers from either group.

Addition	Cell staining in periportal regions	Maximal LDH release
	%	*units/g/hr*
Menadione bisulfite	44 ± 12	164 ± 29
Menadione bisulfite + ethanol	90 ± 6[a]	296 ± 61[a]

[a] Significantly different from the value for menadione bisulfite alone ($p < 0.05$, Student's t test).

[1] R. G. Thurman, unpublished observations.

Role of reducing equivalents in potentiation of menadione bisulfite toxicity by ethanol. Because menadione metabolism requires reducing equivalents, we examined whether ethanol-induced increases in the pyridine nucleotide redox state were responsible for the potentiation of menadione bisulfite toxicity. As expected, ethanol increased pyridine nucleotide fluorescence detected from the liver surface (Fig. 3), due to reduction of NAD^+. Upon infusion of menadione bisulfite, oxygen uptake increased concomitant with a decrease in surface fluorescence, due presumably to utilization of reduced pyridine nucleotides. Both the increase in oxygen uptake and the decrease in surface fluorescence occurred with a half-time of about 3 min. The concentration-response relationship for the increase in pyridine nucleotide fluorescence due to ethanol (Fig. 4A) and the stimulation of oxygen uptake due to menadione bisulfite (Fig. 4B) was also examined. The half-maximal increase in both processes occurred with around 0.25 mM ethanol.

The observation that half-maximal increases in the fluorescence of pyridine nucleotides (Fig. 4A) and menadione bisulfite-mediated oxygen uptake (Fig. 4B) both occurred with approximately 0.25 mM ethanol, a value close to the K_m of ADH for ethanol (25), suggested that generation of NADH during metabolism of ethanol by ADH was involved in the potentiation of toxicity due to menadione bisulfite. To test this possibility, livers from ADH^+ and ADH^- deermice were perfused with menadione bisulfite in the absence or presence of ethanol. The approach of using a strain deficient in ADH was taken to avoid the use of inhibitors of ADH, which may have nonspecific effects and thereby cloud interpretation. In livers from fasted

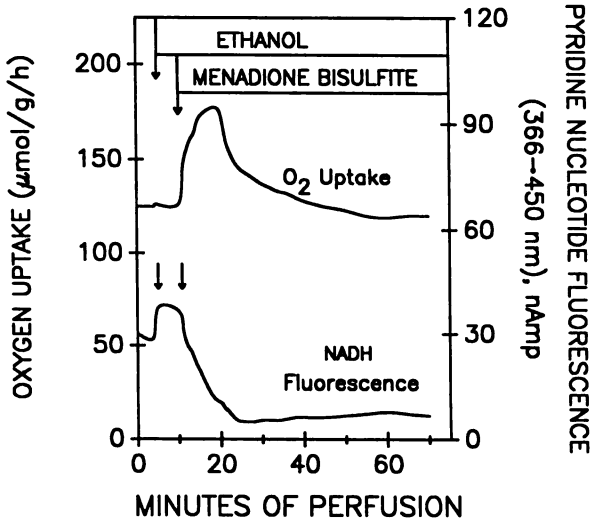

Fig. 3. Oxygen uptake and pyridine nucleotide fluorescence in liver perfused with ethanol and menadione bisulfite. Liver from a normal fasted rat was perfused with menadione sodium bisulfite (3 mM) for 60 min in the presence of ethanol (10 mM). Oxygen tension (*upper trace*) was monitored continuously and oxygen uptake was determined as described in Materials and Methods. Fluorescence (366 → 450 nm) of pyridine nucleotides (*lower trace*) was monitored from the surface of the liver using a large-tipped light guide, as described in Materials and Methods. Typical experiment.

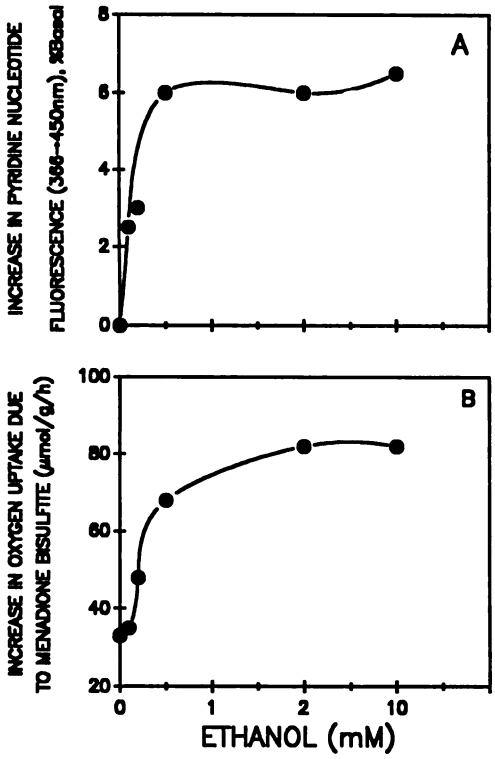

Fig. 4. Effect of ethanol concentration on fluorescence of pyridine nucleotides (A) and stimulation of oxygen uptake by menadione bisulfite (B) in perfused liver. Livers from normal fasted rats were perfused with ethanol and menadione (3 mM) as in Fig. 4. A, Values represent the maximal percentage of increase over basal surface fluorescence due to ethanol before infusion of menadione. B, Points are the maximal increase in oxygen uptake due to infusion of menadione. Each point represents data from one liver.

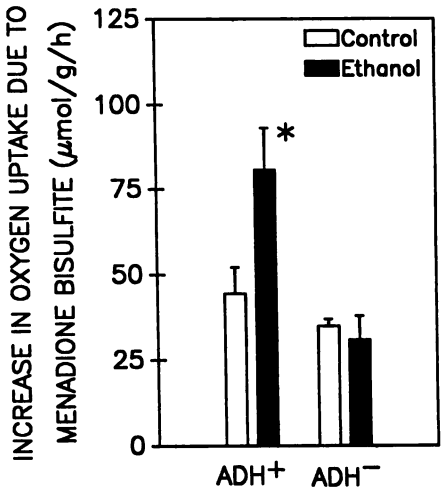

Fig. 5. Oxygen uptake due to menadione bisulfite in livers from ADH$^+$ and ADH$^-$ deermice. Livers from fasted deermice were perfused for 1 hr with menadione sodium bisulfite (1 mM) in the absence or presence of ethanol (10 mM), as described elsewhere (31). Oxygen tension was monitored continuously, and oxygen uptake was determined as described in Materials and Methods. Values represent mean ± standard error of maximal increase in oxygen uptake due to menadione bisulfite (four experiments). *, Significantly different from respective control livers ($p < 0.05$, Student's t test).

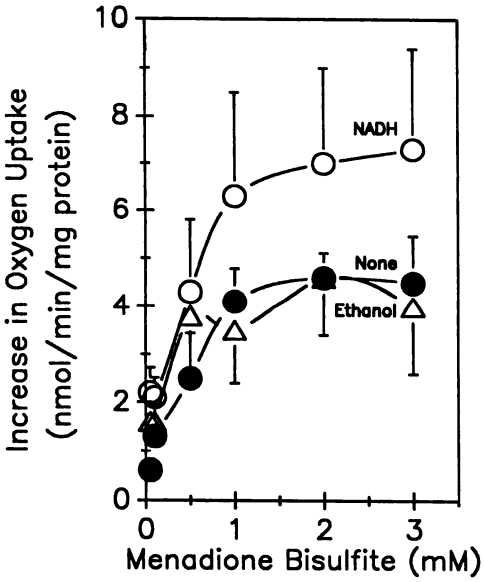

Fig. 6. Effect of ethanol and NADH on menadione bisulfite-stimulated oxygen uptake in microsomes. Microsomes from livers of normal rats were incubated in Krebs-Henseleit buffer at 22° in a 2-ml chamber, in the presence of an NADPH-generating system. Oxygen concentration was monitored continuously with a Clark-type oxygen electrode, and oxygen uptake was calculated from the protein content and tangents drawn to the oxygen concentration versus time curve. Ethanol and NADH were added so that the final concentration was 1 mM. Data represent mean ± standard error of four microsomal preparations. Data were analyzed by analysis of variance, and no differences were observed at $p < 0.05$. ●, No addition; △, ethanol; ○, NADH.

ADH$^+$ deermice, ethanol stimulated oxygen uptake due to menadione bisulfite from 44 to 81 μmol/g/hr (Fig. 5). In contrast, the increase in oxygen uptake due to menadione bisulfite was similar (30 to 35 μmol/g/hr) in the absence and in the presence of ethanol in livers from ADH$^-$ deermice. LDH release was minimal in livers from ADH$^-$ deermice and was not affected by ethanol (data not shown).

Experiments were performed to determine whether acetaldehyde, acetate, or NADH, products of ethanol metabolism by ADH, were involved in the mechanism of ethanol-stimulated menadione bisulfite toxicity. Perfusion with acetaldehyde would also produce NADH from metabolism via ADH; therefore, two agents that produce NADH but not acetaldehyde or acetate were examined. In the presence of either xylitol or a metabolite of the plasticizer diethylhexylphthalate, ethylhexanol, oxygen uptake due to menadione bisulfite was 2–3 times greater than with menadione alone (Table 1). In addition, perfusion with acetate (10 mM) alone did not affect menadione bisulfite-stimulated oxygen uptake or toxicity (data not shown). These results suggest that increases in NADH are responsible for potentiation of oxygen uptake and toxicity due to menadione bisulfite by ethanol.

Site of action of ethanol on menadione bisulfite-stimulated oxygen uptake. To determine whether ethanol potentiated redox cycling of menadione bisulfite by cytochrome P-450 reductase, oxygen uptake due to menadione bisulfite (3 mM) was examined in isolated microsomes. Basal rates of oxygen uptake in isolated microsomes were around 3 nmol/min/mg of protein. Menadione bisulfite increased oxygen uptake in isolated microsomes in a concentration-related manner (Fig. 6). The maximal increase in oxygen uptake due to menadione bisulfite was about 4 nmol/g/hr, and half-maximal stimulation was observed at 0.5 mM. This increase was similar to that observed with about 50 μM free menadione (data not

shown). Hoever, ethanol did not affect oxygen uptake due to menadione bisulfite in microsomes (Fig. 6). There was a tendency for greater stimulation of oxygen uptake due to menadione bisulfite by NADH; however, this increase was not statistically significant.

Menadione transfers electrons to the mitochondrial respiratory chain in isolated hepatocytes (1); therefore, the effect of cyanide, an inhibitor of cytochrome oxidase, on the potentiation of menadione bisulfite-stimulated oxygen uptake by ethanol was examined. Basal rates of oxygen uptake during perfusion with KCN were reduced from about 120 to 40 μmol/g/hr. Moreover, perfusion with KCN nearly completely abolished the increase in oxygen uptake due to menadione bisulfite in the presence of ethanol (Table 1). Furthermore, menadione bisulfite (0 to 3 mM) stimulated oxygen uptake in isolated mitochondria (Fig. 7), and β-hydroxybutyrate (10 mM), which elevates the mitochondrial NADH redox state (26), increased menadione bisulfite-induced oxygen uptake by up to 60%. These results indicate that ethanol stimulates oxygen uptake due to menadione bisulfite by increasing the reducing equivalent supply for redox cycling predominantly in the mitochondria.

Discussion

Menadione undergoes redox cycling and also transfers electrons to the mitochondrial respiratory chain (1, 5). It has been shown that reducing equivalent supply is rate limiting for redox cycling in perfused liver for another quinone-containing chemical, doxorubicin (27). Ethanol increased menadione bisulfite-mediated oxygen uptake 3-fold (Fig. 1; Table 1) and toxicity 2-fold (Fig. 2; Table 2); therefore, it is possible that NADH produced during oxidation of ethanol by ADH is involved in the mechanism of this effect.

Three experiments presented in this study support this idea. First, the time course of oxidation of NADH was similar to the time course of the increase in oxygen uptake due to menadione bisulfite (Fig. 3). Second, both the increase in NADH fluorescence and the stimulation of oxygen uptake due to menadione bisulfite occurred half-maximally with about 0.25 mM ethanol (Fig. 4), a value close to the K_m of ADH for ethanol (25). Third, potentiation by ethanol of oxygen uptake due to menadione bisulfite was observed in livers from ADH^+ deermice but not in livers from ADH^- deermice (Fig. 5). Taken together, it is clear that potentiation of menadione bisulfite-induced oxygen uptake requires metabolism of ethanol by ADH. Because the stimulation of oxygen uptake due to menadione bisulfite was directly proportional to the percentage of cells stained with trypan blue (see Results), suggesting that oxygen uptake and toxicity are closely related, it seems reasonable to propose that metabolism of ethanol by ADH is also required for potentiation of menadione bisulfite-induced toxicity by ethanol. The observation that xylitol and ethylhexanol, which generate NADH but not acetaldehyde or acetate, also increased oxygen uptake due to menadione bisulfite (Table 1) provides further support for the hypothesis that NADH is central to the mechanism by which ethanol enhances menadione-induced toxicity.

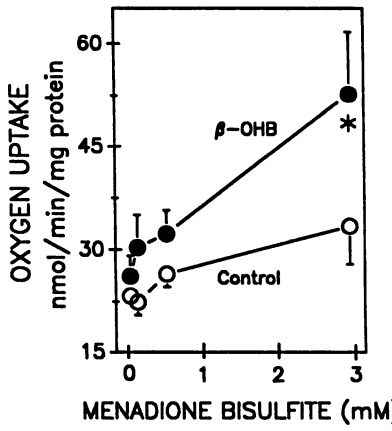

Fig. 7. Effect of β-hydroxybutyrate on menadione bisulfite-stimulated oxygen uptake in isolated mitochondria. Rat liver mitochondria were isolated and incubated as described in Materials and Methods. Oxygen uptake was monitored with a Clark-type oxygen electrode. β-Hydroxybutyrate (*β-OHB*) (10 mM) was added 1 min before menadione sodium bisulfite, which was added at concentrations indicated on the *abscissa*. Values represent mean ± standard error of four mitochondrial preparations. *, Significantly different from control by analysis of variance, $p < 0.05$.

How might generation of NADH during metabolism of ethanol increase toxicity due to menadione bisulfite? One possibility is that NADH may stimulate one-electron reduction of menadione bisulfite at the level of the smooth endoplasmic reticulum. However, neither ethanol nor NADH significantly enhanced menadione bisulfite-induced oxygen uptake in isolated microsomes, rendering this hypothesis unlikely (Fig. 6). This finding is consistent with the observation that menadione stimulated superoxide formation to a greater extent in microsomal fractions when NADPH rather than NADH was the cofactor (5, 28). A second possibility is that NADH could stimulate redox cycling of menadione bisulfite in the mitochondria or reduction of menadione bisulfite with subsequent transfer of electrons to the mitochondrial respiratory chain. Menadione-mediated singlet oxygen production was inhibited by antimycin A (2), and KCN prevented oxygen uptake due to menadione in isolated hepatocytes (1) and perfused liver (4), favoring the latter possibility. Further, KCN abolished menadione bisulfite-mediated oxygen uptake even in the presence of ethanol (Table 1). Stimulation of oxygen uptake due to menadione bisulfite in isolated mitochondria by β-hydroxybutyrate (Fig. 7), which increases NADH in the mitochondrial matrix, also supports this hypothesis.

Thus, it is possible that ethanol potentiates menadione bisulfite-induced oxygen uptake and toxicity by increasing mitochondrial NADH, which enhances reduction of menadione bisulfite to a semiquinone or hydroquinone by NADH:ubiquinone oxidoreductase. This in turn increases the transfer of electrons to the mitochondrial electron transport chain. During perfusion with menadione bisulfite, oxygen uptake is initially elevated due to the increased transfer of electrons in the mitochondrial respiratory chain (Fig. 1). Although it is not clear how increased transfer of electrons may lead to toxicity, it is of note that the subsequent decline in oxygen uptake, indicating either inhibition of respiration or damage to the mitochondria and, therefore, decreased production of ATP, was also greater during perfusion with ethanol. A decrease in ATP during perfusion with menadione has been reported (7), and in these studies a decline in oxygen uptake and ATP production precedes cell injury, assessed as release of LDH (Fig. 2). It has been proposed that decreased ATP production leads to enhanced degradation of adenine nucleotides and, therefore, increased levels of xanthine. Xanthine could serve

as substrate for xanthine oxidase (EC 1.2.3.2), resulting in generation of superoxide anion and subsequently other reactive oxygen species that could damage tissue. Indeed, allopurinol, an inhibitor of xanthine oxidase, and cianidanol, a radical scavenger that can stimulate ATP production, diminished menadione bisulfite-induced toxicity (4). Alternatively, both allopurinol and cianidanol may have afforded protection against menadione bisulfite simply by acting as radical scavengers.

Cell death due to menadione bisulfite occurred exclusively in oxygen-rich periportal regions of the liver lobule in this (Table 2) and a previous (4) study. These data suggest that high oxygen tension is required for menadione bisulfite-induced toxicity. One possibility to explain the zone-specific nature of toxicity is a greater direct formation of deleterious reactive oxygen species in regions of the liver lobule where O_2 tension is high. This has been observed for menadione in isolated hepatocytes; the stimulation of oxygen uptake is less sensitive to inhibition by KCN at high than at low oxygen pressures (29). However, it is unlikely that this occurs in periportal regions of the perfused liver, because menadione bisulfite-induced oxygen uptake was inhibited nearly completely by KCN (Table 1), indicating a requirement for an active mitochondrial electron transport system. Another explanation for the periportal nature of toxicity may be that mitochondria in regions of high oxygen tension are more active and, therefore, more sensitive to menadione bisulfite. Alternatively, regions with low oxygen tension may be protected. For example, respiration was stimulated by the uncoupler dinitrophenol only in regions of high oxygen tension (30). Menadione bisulfite also stimulated oxygen uptake only in oxygen-rich regions of the liver lobule (4). Thus, it is possible that mitochondria in regions of low oxygen tension cannot be stimulated by menadione bisulfite. Considering the direct association between oxygen uptake due to menadione bisulfite and toxicity (see Results), this may explain why only periportal regions of the liver lobule were damaged during perfusion in the anterograde direction. Even in the presence of ethanol, cell death was confined to periportal regions of the liver lobule (Table 2).

In conclusion, ethanol enhances menadione bisulfite-induced oxygen uptake and toxicity in perfused rat liver. This effect requires metabolism of ethanol via ADH, which increases the NADH redox state in mitochondria and most likely acts by providing reducing equivalents for redox cycling.

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