inhibitions of mitochondrial respiration by AA and NAPQI may both contribute to the toxicity observed following AA overdose.

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Metabolic alterations resulting from the inhibition of mitochondrial respiration by acetaminophen *in vivo*

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Acetaminophen (AA) is considered to be a relatively safe drug when taken in therapeutic doses which generally range from 650 to 1000 mg taken every 4 hr. Toxic doses of AA vary depending on a variety of conditions such as the blood levels of AA attained after overdose, the rate of disposition of the drug, the activity of the mixed-function oxidase system and the level of glutathione stores in the liver at the time of the AA overdose. In general, a single dose of over 15 g will lead to hepatotoxicity in most individuals [1].

Within the first 24 hr following AA overdose, symptoms such as pallor, anorexia, right hypochondrial tenderness, nausea and vomiting may occur [1, 2]. Often, however, early clinical signs and symptoms are absent making AA overdoses extremely difficult to diagnose in the absence of patient cooperation. In cases of severe overdose (plasma AA concentrations > 800 mg/l. or approximately 5 mM), coma and metabolic acidosis have been reported in the hours immediately following the overdose in the absence

of overt signs of hepatic damage. The observed coma and metabolic acidosis do not appear to be causally related as correction of the acid-base balance in the blood does not cause the immediate reversal of the coma [2–5]. The underlying mechanism of this acute toxicity has not been established. However, it appears to be independent of the development of hepatotoxicity following AA overdose.

We believe that the coma and metabolic acidosis seen following a severe overdose of AA are the result of the inhibitory action of the drug on mitochondrial respiration. To test this hypothesis, we have examined the ability of the meta-isomer of AA, 3-hydroxyacetanilide (3HAA), to inhibit mitochondrial respiration. In addition, the abilities of both isomers to alter carbohydrate metabolism *in vitro* and *in vivo* have been compared. Since AA is hepatotoxic and 3HAA has been shown to be nonhepatotoxic [6, 7], metabolic alterations common to both isomers should be the consequence of a commonly shared mechanism of action which would be independent of the hepatotoxic potential of AA.

Materials and methods

Materials. Female Sprague–Dawley rats, weighing between 150 and 225 g, were purchased from Taconic Farms (Germantown, NY). Acetaminophen, ATP, ADP, $NADP^+$, NAD^+, glucose-6-phosphate dehydrogenase, hexokinase, lactate dehydrogenase, glucose, lactate, pyruvate, malate, succinate, and β-hydroxybutyrate were purchased from the Sigma Chemical Co. (St Louis, MO). 3-Hydroxyacetanilide was purchased from the Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were of the highest grade commercially available.

Experimental methodology. All rats used in the *in vivo* studies were fed and were treated prior to 1:00 p.m., receiving i.p. injections of either AA or 3HAA at a dose of 1 g/kg. The solutions were prepared as 3 g of AA or 3HAA per 40 ml of 0.9% saline. Control rats received i.p. injections of 0.9% saline. All solutions were heated in a water bath to a temperature of 80° to facilitate AA and 3HAA dissolution immediately prior to injection.

Concentration of glucose and lactate in serum were determined using a Rotochem Autoanalyzer (Travenol Laboratories Inc., Deerfield, IL) with appropriate kits adapted for use with the instrument. Glucose levels were measured using the Worthington Statzyme™ Glucose 16 assay kit from Cooper Biomedical Inc. (Malvern, PA). Lactate levels were determined using the Statpack™ Rapid Lactate Test assay kit from Behring Diagnostics (La Jolla, CA). Serum levels of AA and 3HAA were determined using the Sigma Diagnostics Acetaminophen Kit, Procedure No. 430 (Sigma Diagnostics, St Louis, MO).

Glucose and lactate concentrations in the effluent perfusate were determined using the methods of Bergmeyer [8, 9].

Rat livers were perfused using a modification of the method of Scholz *et al.* [10]. The perfusate used was Krebs-Henseleit bicarbonate buffer, pH 7.4, at 37°, saturated with 95% O_2/5% CO_2. Rat liver mitochondria isolation and acceptor control ratio determinations were performed using the methods of Pedersen *et al.* [11].

Statistical analyses. Data were statistically analyzed using Student's *t*-test. Results were considered to be statistically significant if the probability of erroneously inferring a significant difference between the two groups of data was less than 5% ($P \leq 0.05$) [12].

Results and discussion

3HAA caused an inhibition of respiration in mitochondria utilizing NAD-linked respiratory substrates such as β-hydroxybutyrate (Fig. 1) or pyruvate and malate (data not shown) in a manner identical to AA. The inhibition of mitochondrial respiration was the result of a concentration-dependent inhibition of state 3 respiration which was

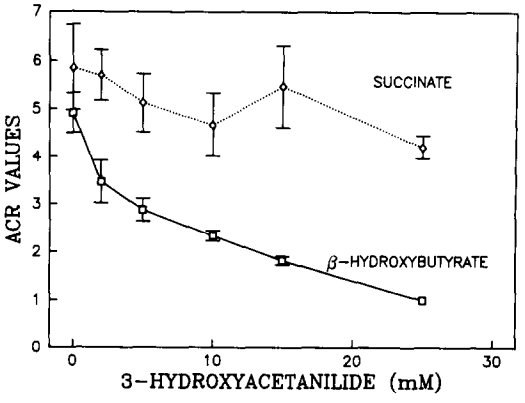

Fig. 1. Effect of 3HAA (0–25 mM) on the acceptor control ratios (ACR) of mitochondria, utilizing β-hydroxybutyrate or succinate as respiratory substrate. ACR values were determined as described in Materials and Methods and are the means ± SE of three experiments.

significant starting at 3HAA concentrations of 5 mM (data not shown). As was the case with AA, 3HAA did not affect succinate-supported respiration (Fig. 1) or NAD-linked state 4 respiration (data not shown). An additional similarity between the actions of AA and 3HAA was in their ability to inhibit NADH oxidase activity in submitochondrial particles in a concentration-dependent manner, indicating that both compounds inhibit respiration at the level of the mitochondrial electron transport chain (data not shown). The fact that both compounds equally inhibited mitochondrial respiration suggests that this inhibition cannot account for the differences in the hepatotoxic potential of the two compounds. However, this observation does not rule out a contribution by the inhibition of mitochondrial respiration to the hepatotoxic action of AA.

A comparison of the effects of infusing increasing concentrations of either AA or 3HAA into the isolated perfused rat liver demonstrated that both compounds stimulate glycolysis and glycogenolysis in a concentration-dependent manner as indicated by increased levels of lactate and glucose in the effluent perfusate (Fig. 2). The release of lactate and glucose coincided with a concentration-dependent inhibition of hepatic respiration following the infusion of either AA or 3HAA (data not shown), suggesting that the alterations in carbohydrate metabolism were secondary to the inhibition of NAD-linked mitochondrial respiration by the two isomers.

The administration of 1 g/kg of either isomer of acetaminophen i.p. to fed rats resulted in pronounced changes in serum glucose and lactate levels 1 hr after dosing (Table 1). The resulting concentrations of AA and 3HAA in the serum (approximately 5 mM) were sufficient to inhibit mitochondrial respiration *in vivo*, resulting in increased serum lactate and glucose levels, which would have been predicted based on the results obtained in the isolated perfused rat liver (Fig. 2).

Therefore, the ability of both AA and the nonhepatotoxic 3HAA to increase the serum levels of lactate and glucose suggests that large doses of AA (and 3HAA) can inhibit mitochondrial respiration *in vivo*, thereby causing toxicity by a mechanism which is independent of the classical hepatotoxic action of the drug. The cases of coma and metabolic acidosis that have have been associated with acetaminophen overdoses were in patients with plasma acetaminophen levels greater than 800 mg/l. (approximately 5 mM) [2–5]. We suggest that the inhibition of NAD-linked mitochondrial respiration by AA is the underlying mechanism of the metabolic acidosis and coma and that this inhibition of respiration constitutes an additional threat to life following a severe overdose of acetaminophen.

Table 1. Effects of AA and 3HAA on serum glucose and lactate levels

Treatment	AA or 3HAA (mM)	Lactate (mg/dl)	Glucose (mg/dl)
Control	0	33.0 ± 2.8	189.2 ± 4.1
AA (1 g/kg)	5.44 ± 0.45	84.0 ± 5.4*	460.1 ± 35.1*
3HAA (1 g/kg)	5.86 ± 0.53	78.8 ± 4.5*	392.6 ± 9.0*

One hour following dosing with AA or 3HAA, the concentrations of the two compounds as well as lactate and glucose were determined in the serum of the rats. All treatment groups consisted of six rats with the values reported as the mean ± SE.
* Significantly different from control ($P \leq 0.5$).

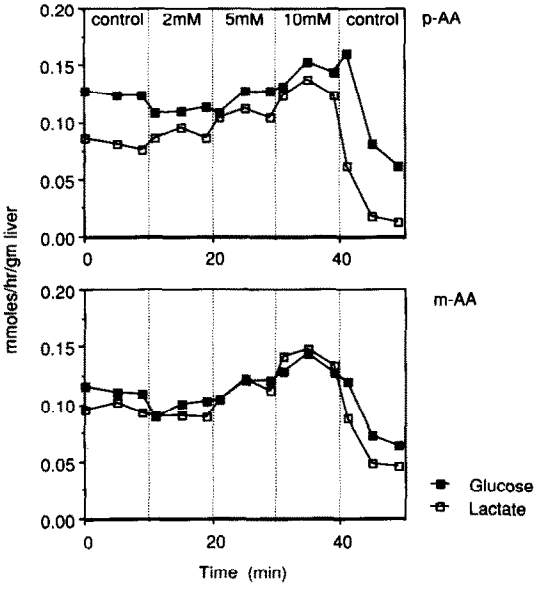

Fig. 2. Alterations in carbohydrate metabolism following the perfusion of increasing concentrations of AA (upper panel) and 3HAA, (lower panel) through the isolated rat liver. The livers were perfused with Krebs–Henseleit buffer for 15 min prior to beginning experimentation to allow for equilibration. Following a 10-min control perfusion, increasing concentrations (2–10 mM) of AA or 3HAA in Krebs–Henseleit buffer were perfused through the liver for 10-min intervals. At the end of the 10 mM perfusion period, control buffer was perfused through the liver for another 10 min. Perfusate samples were taken three times over the course of each 10-min perfusion period. Glucose and lactate concentrations were determined as described in Materials and Methods. The results are expressed as the mean rate of glucose or lactate released (mmol/hr/g wet liver) into the perfusate of three livers.

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