Inhibition of gluconeogenesis and urea synthesis in isolated rat hepatocytes by acetazolamide

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Citrulline generation in isolated rat liver mitochondria is markedly inhibited by the carbonic anhydrase inhibitor acetazolamide (Dodgson et al., 1983). This finding suggests that hepatic mitochondrial-matrix carbonic anhydrase may exert an important controlling effect on ureogenesis by supplying bicarbonate for carbamoyl phosphate synthesis.

Three different carbonic anhydrase isoenzymes have been identified in mammals and designated CAI, CAII and CAIII. In liver CAII is uniquely sensitive to inhibition by acetazolamide and is present in a 4-fold higher concentration than CAIII (Jeffrey et al., 1984), with higher cytosol concentration of CAII being found in males (Shiels et al., 1984). Sex differences in mitochondrial carbonic anhydrase have not been reported. We have extended these findings by examining the effect of acetazolamide on rates of ureogenesis and gluconeogenesis in isolated rat hepatocytes from male and female rats.

Isolated rat hepatocytes were prepared from 48 h-starved male and female Sprague-Dawley rats by collagenase perfusion and suspended in Krebs bicarbonate buffer (Krebs & Henseleit, 1932) gassed with O₂/CO₂ (19:1). Samples (2 ml) of cell suspension (30-60 mg wet wt.) were incubated with 1.8 mM-NH₄Cl, 5 mM-L-(-)-lactate and 0.2 mM-ornithine in the presence or absence of different concentrations of acetazolamide. Incubations were terminated after either 10 or 20 min by the additions of 4.5 ml of 0.6 M-HClO₄. Urea and glucose were determined in the deproteinized supernatant by standard spectrophotometric methods. Rates of ureogenesis and gluconeogenesis were determined between the 10 and 20 min time points.

Urea and gluconeogenesis in male rat hepatocytes were markedly inhibited by acetazolamide (Fig. 1). Maximum inhibition of ureogenesis (75%) occurred at acetazolamide concentrations between 0.5 and 1.0 mM, with 25% inhibition evident at 0.005 mM. Gluconeogenesis was inhibited by a maximum of 40%, at 1.0 mM-acetazolamide, with 12% inhibition at 0.05 mM. An identical degree of inhibition of ureogenesis was obtained with hepatocytes from female rats.

These data suggest that inhibition of carbonic anhydrase by acetazolamide may decrease the supply of intramitochondrial bicarbonate for both the carbamoyl-phosphate synthetase and pyruvate carboxylase reactions. It is thus possible that bicarbonate availability may be a limiting factor in both urea and glucose production. This conclusion is supported by the demonstrations of inhibition of ureogenesis by metabolic acidosis at a site proximal to citrulline synthesis (Monson et al., 1984). The observed similarity between acetazolamide inhibition of urea production in hepatocytes from male and female rats suggests the presence of similar mitochondrial concentrations in males and females of one or more susceptible carbonic anhydrase isozymes.

The possibility of mechanisms other than carbonic anhydrase inhibition being responsible for the effects of acetazolamide cannot be entirely excluded, but intracellular ATP concentrations were unaltered.

**Fig. 1.** Effect of acetazolamide on (a) urea and (b) glucose production from NH₄Cl (1.8 mM), lactate (5 mM) and ornithine (0.2 mM) in isolated hepatocytes from male rats.

<table>
<thead>
<tr>
<th>[Acetazolamide] (mM)</th>
<th>Inhibition of urea production (%)</th>
<th>Inhibition of glucose production (%)</th>
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<tbody>
<tr>
<td>0.005</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>0.05</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>0.1</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>1.0</td>
<td>75</td>
<td>80</td>
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The mean rates of ureogenesis and gluconeogenesis in the absence of acetazolamide (± S.E.M.) were 1.2 ± 0.21 (n = 10) and 0.44 ± 0.1 (n = 9) μmol/min per g wet wt., respectively. Numbers in parentheses indicate number of experiments.

**References**


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