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EFFECTS OF ETHYL HYDRAZINOACETATE ON GLUCONEOGENESIS AND ON ETHANOL OXIDATION IN RAT HEPATOCYTES

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Summary

Both ethyl hydrazinoacetate and aminooxyacetate strongly inhibit gluconeogenesis from L-lactate, but not from pyruvate or fructose, in rat hepatocytes. Ethyl hydrazinoacetate partially inhibits gluconeogenesis from polyols, and also partially inhibits ethanol oxidation. In contrast to results obtained with aminooxyacetate, increasing the ethyl hydrazinoacetate concentration from 0.2 to 2 mM does not tend to diminish the inhibitory effect of this transaminase inhibitor on ethanol or polyol utilization.

We intended to study the effects of hydrazinoacetate, the hydrazine derivative analogous to aminooxyacetate:

$H_2N\text{-}NHCH_2\text{-}COO^-$ hydrazinoacetate

$H_2N\text{-}OCH_2\text{-}COO^-$ aminooxyacetate

However, only the ethyl ester of hydrazinoacetate was commercially available Ethyl hydrazinoacetate is a somewhat more potent transaminase inhibitor than aminooxyacetate in rat liver cells. In some of the experiments, we have also compared the effects of these inhibitors with 2-amino-4-methoxy-*trans*-but-3-enoic acid, a more specific inhibitor of glutamate oxalacetate transaminase [1].

Hepatocytes were prepared as previously described [2] from Wistar rats which had been fasted for 24 h. The cells were incubated in 25-ml Erlenmeyer flasks at 38°C in Krebs-Henseleit buffer [3] with 5% CO_2/95% O_2 in the gas phase. The transaminase inhibitors were added to the medium containing substrates just prior to the addition of the cells. The incubations

were terminated by addition of 0.5 ml of 20% $HClO_4$; the medium was washed out, made to 10 ml, and centrifuged. Glucose and ethanol were estimated enzymically.

Aminooxyacetate was obtained from Eastman Organic Chemicals (Rochester, NY). Ethyl hydrazinoacetate was from Aldrich Chemical (Milwaukee, WI); 2-amino-4-methoxy-*trans*-but-3-enoic acid was generously supplied by Dr. W.E. Scott of Hoffmann-La Roche (Nutley, NJ).

Ethyl hydrazinoacetate was a very potent inhibitor of gluconeogenesis from lactate. Since the relative degree of inhibition with transaminase inhibitors has been found to vary considerably, depending upon the hepatocyte preparation, we tested the relative potency of aminooxyacetate and ethyl hydrazinoacetate on the same batch of cells (Fig. 1). Ethyl hydrazinoacetate was a more potent inhibitor of lactate gluconeogenesis than aminooxyacetate, with 50% inhibition of lactate gluconeogenesis being reached at about 4 μM ethyl hydrazinoacetate, and at about 6 μM aminooxyacetate. As discussed elsewhere [4], the shape of the inhibitor titration curves are typical of near-equilibrium enzymes. There is an initial region where the inhibitor shows only slight effects on overall gluconeogenesis, followed by a nearly linear, strongly inhibitory phase [4].

Ethyl hydrazinoacetate and aminooxyacetate at concentrations up to 2 mM, and 2-amino-4-methoxy-*trans*-but-3-enoate at concentrations up to 5 mM, all gave at the most, slight (6% maximal) inhibition of gluconeogenesis from fructose, suggesting that none of these transaminase inhibitors have any major inhibitory effects on pathways of energy generation in the cell. Also, none of these transaminase inhibitors showed any major inhibition of gluconeogenesis from pyruvate, when measured at short-time periods before lactate accumulated.

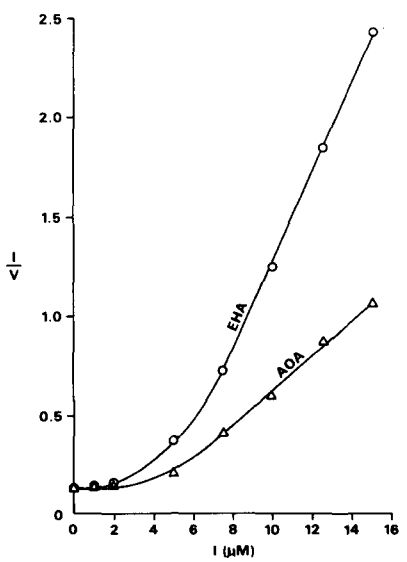

Fig. 1. Hepatocytes (30 mg dry wt.) were incubated for 60 min with 10 mM L-lactate with different concentrations of aminooxyacetate (AOA) or ethyl hydrazinoacetate (EHA). *V* represents the rate of glucose synthesis.

Ethyl hydrazinoacetate and aminooxyacetate inhibited gluconeogenesis from xylitol (10 mM) in hepatocytes from fasted rats. In bicarbonate-buffered medium, the extent of inhibition by both inhibitors was about 40% at 0.2 mM inhibitor concentration. As previously found [5], raising the concentration of aminooxyacetate to 2 mM decreased the extent of inhibition (for yet unknown reasons). However, raising the concentration of ethyl hydrazinoacetate to 2 mM slightly increased the inhibition of gluconeogenesis from xylitol. These opposite effects of inhibitor concentration were more pronounced in a non-bicarbonate buffered medium (buffered with 10 mM Hepes). The transaminase inhibitors are thought to inhibit gluconeogenesis from polyols by virtue of inhibition of the malate-aspartate cycle.

The malate-aspartate cycle also may participate in the transfer of excess cytosolic reducing equivalents into the mitochondria when ethanol is oxidized in hepatocytes. Ethyl hydrazinoacetate and aminooxyacetate, at 0.2 mM, and 2-amino-4-methoxy-*trans*-but-3-enoate, at 0.5 mM, inhibited uptake of ethanol (10 mM) by hepatocytes from fasted rats to nearly the same extent (about 65% inhibition). Again, as previously found [5], raising the aminooxyacetate concentration 10-fold caused a marked decrease (to 22%) in the extent of inhibition. However, a similar 10-fold increase in the concentration of ethyl hydrazinoacetate or 2-amino-4-methoxy-*trans*-but-3-enoate, caused a slight or no increase, respectively, in the extent of inhibition.

Glucose formation from xylitol, and also ethanol oxidation, were essentially linear for 60 min, in the absence and in the presence of the highest concentrations of the transaminase inhibitors used in these studies. These results indicate the lack of toxic effects of these compounds in these short-term experiments.

There remain marked quantitative discrepancies between these studies on the malate aspartate cycle and those of Berry and coworkers [6, 7]. These workers found only 5% inhibition of ethanol oxidation by 15 mM fluoromalate, a malate dehydrogenase inhibitor [6]. Also 5 mM difluorooxalacetate, a transaminase inhibitor, was found to inhibit ethanol oxidation by only 12% [7]. We are not able to compare the effects of these inhibitors with aminooxyacetate or ethyl hydrazinoacetate because of the unavailability of these fluoro compounds, and no such comparison has been made by these workers. In our opinion, the specificity and the potency of these fluoro compounds, for use in whole cell studies, has yet to be established.

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