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Re-evaluation of Amino-oxyacetate as an Inhibitor

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Data are provided which indicate that pyruvate and/or acetaldehyde can reverse the inhibition of alanine aminotransferase and aspartate aminotransferase by amino-oxyacetate. It was shown that acetaldehyde could reverse the inhibition of gluconeogenesis from alanine and that pyruvate could reverse the inhibition of urea synthesis by amino-oxyacetate.

Amino-oxyacetate has been useful as an inhibitor of pyridoxal phosphate-requiring enzymes and has been utilized for studying urea synthesis and gluconeogenesis (Meijer *et al.*, 1975; Rognstad & Clark, 1974). Unless isolated rat hepatocyte preparations are preincubated with amino-oxyacetate before the addition of ketone or aldehyde substrates, the inhibitor forms a complex with the ketone or aldehyde and is rendered ineffective (Meijer & Van Dam, 1974). However, to date it has not been shown conclusively whether ketones or aldehydes can reverse inhibition by amino-oxyacetate even after preincubation. Therefore we undertook a study with isolated rat hepatocytes, with pyruvate as our model ketone and acetaldehyde as our model aldehyde. Since acetaldehyde is not glucogenic, it causes no interference in the measurement of glucose. Glucose production could not be the criterion for studying reversal of inhibition by pyruvate, since pyruvate is not only glucogenic, but remains so even in the presence of amino-oxyacetate (Rognstad & Clark, 1974). Therefore we chose the process of urea synthesis to examine the reversal of amino-oxyacetate inhibition by pyruvate. In the present paper we show that even after preincubation of hepatocytes with amino-oxyacetate some reversal of inhibition may occur in the presence of pyruvate and/or acetaldehyde.

Isolated rat hepatocytes were prepared from male rats by the method of Berry & Friend (1969), as modified by Krebs *et al.* (1974), and gluconeogenesis was determined as described by Cornell *et al.* (1973). Urea synthesis was measured as described by Briggs & Freedland (1976). In experiments with acetaldehyde, cells were preincubated for 15min either with no additions or with 0.2mM-amino-oxyacetate. At the end of the preincubation period, 10mM-alanine or 10mM-alanine plus 5mM-acetaldehyde was added to cell suspensions. The results are shown in Fig. 1. As expected, amino-oxyacetate completely inhibited gluconeogenesis from alanine. However, even after preincubation with the inhibitor, the addition of acetaldehyde partially restored the production of glucose from alanine. Since alanine must undergo transamination before being converted

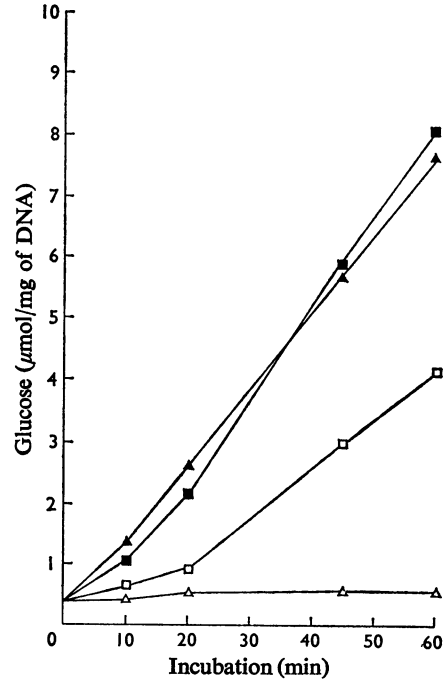

Fig. 1. *Effect of acetaldehyde and amino-oxyacetate on gluconeogenesis from* L-*alanine by isolated rat hepatocytes*
Hepatocytes were preincubated either with 0.2mM-amino-oxyacetate (open symbols) or without amino-oxyacetate (solid symbols) for 15min before the addition of either 10mM-L-alanine (▲ or △) or 10mM-L-alanine plus 5mM-acetaldehyde (■ or □). Values are means for two experiments, each run in duplicate. Rates of glucose production did not vary by more than 8% from the mean values.

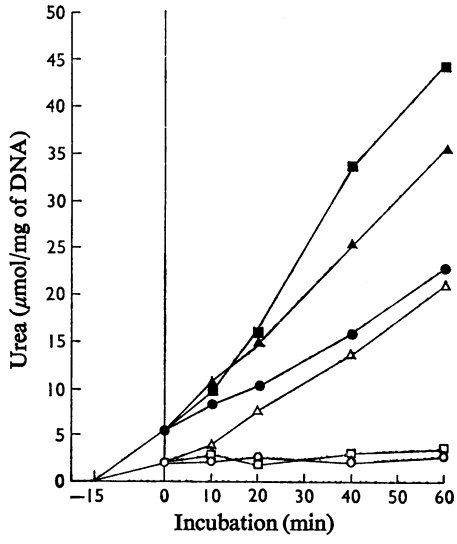

Fig. 2. *Effect of pyruvate, lactate and amino-oxyacetate on urea synthesis from NH_4Cl and ornithine by isolated rat hepatocytes*

Hepatocytes were preincubated with either 10mM-NH_4Cl and 5mM-ornithine (solid symbols) or with 10mM-NH_4Cl and 5mM-ornithine plus 0.2mM-amino-oxyacetate (open symbols) for 15min before the addition of 10mM-pyruvate (▲ or △) or 10mM-lactate (■ or □). Samples in which neither pyruvate nor lactate was added were included (● or ○). Values are the means of two experiments, each run in duplicate. Rates of urea production did not vary by more than 12% from the mean values.

into glucose, acetaldehyde must have reversed the complete inhibition of the alanine aminotransferase.

In experiments to examine the reversal of amino-oxyacetate inhibition by pyruvate, isolated rat hepatocytes were preincubated with either 10mM-NH_4Cl and 5mM-ornithine or 10mM-NH_4Cl, 5mM-ornithine and 0.2mM-amino-oxyacetate. After 15min, either 10mM-pyruvate or 10mM-lactate was added to the cell suspension. Results for these experiments are shown in Fig. 2. Lactate and pyruvate have both been shown to stimulate urea synthesis from NH_3 and ornithine in isolated rat hepatocytes (Briggs & Freedland, 1976). In the isolated cell system, amino-oxyacetate inhibited urea production from NH_3 with ornithine and from NH_3 with ornithine plus lactate added after preincubation. However, pyruvate added after preincubation of the cells with amino-oxyacetate reversed the inhibition of urea synthesis. Since continued availability of aspartate is necessary to maintain urea production, it seems very probable that reversal of amino-oxyacetate inhibition was occurring in the presence of pyruvate. Our studies with the inhibitor 2-amino-4-methoxy-*trans*-but-3-enoic acid, which is specific for aspartate aminotransferase (Rando *et al.*, 1976), have shown that the inhibition of urea synthesis in cells preincubated with the inhibitor is not reversed by the addition of pyruvate (results not shown). These results further support the contention that the reversal of amino-oxyacetate effects by pyruvate is due to reversal of amino-oxyacetate inhibition of the transaminases.

Table 1. *Effect of amino-oxyacetate and 2-amino-4-methoxy-trans-but-3-enoic acid on gluconeogenesis from lactate and pyruvate by rat hepatocytes*

Hepatocytes were preincubated with 0.2mM-amino-oxyacetate or 1mM-2-amino-4-methoxy-*trans*-but-3-enoic acid for 15min before the addition of 10mM-lactate or 10mM-pyruvate. Control rates were: 10mM-lactate, 0.185±0.014; 10mM-pyruvate, 0.170±0.023 μmol/min per mg of DNA (means±S.E.M. for eight observations). DNA was measured by the method of Burton (1956). Experimental values are given as percentages of control values, as the means±S.E.M. of four observations from two separate preparations.

	Rate of glucose production (% of control)	
Substrate	+Amino-oxyacetate	+2-Amino-4-methoxy-*trans*-but-3-enoate
Lactate	47.0±9.1	32.8±0.9
Pyruvate	97.7±7.8	104.1±2.2

Studies of gluconeogenesis by using 2-amino-4-methoxy-*trans*-but-3-enoic acid as an inhibitor have yielded results similar to those with amino-oxyacetate. Glucose production was inhibited by 2-amino-4-methoxy-*trans*-but-3-enoic acid in cell suspensions with lactate as the substrate, whereas gluconeogenesis from pyruvate was unaffected (Table 1). As shown by the present results, the lack of inhibition of gluconeogenesis from pyruvate in hepatocytes preincubated with aminooxy-acetate could have been due to reversal of amino-oxyacetate inhibition of the transaminases on addition of pyruvate. The treatment with 2-amino-4-methoxy-*trans*-but-3-enoic acid is not subject to this misinterpretation, since pyruvate does not reverse the inhibition by 2-amino-4-methoxy-*trans*-but-3-enoic acid of aspartate aminotransferase. These results provide new and better evidence that the major C_4 intermediate transported out of the mitochondria is malate when pyruvate is the substrate for gluconeogenesis, whereas when lactate is the substrate the C_4 intermediates are transported out of the mitochondria predominantly as aspartate (Rognstad & Clark, 1974).

The addition of 2-amino-4-methoxy-*trans*-but-3-enoic acid to the incubation medium did not prevent or change the rate of alanine formation after the addition of NH_3 and pyruvate to the medium.

It is apparent, therefore, that 2-amino-4-methoxy-*trans*-but-3-enoic acid does not markedly affect the activities of glutamate dehydrogenase and alanine aminotransferase.

Longshaw *et al.* (1972) considered the possibility that amino-oxyacetate might form substituted oximes by interaction with carbonyl compounds such as pyruvate or 2-oxoglutarate. The equilibrium value for the formation of the pyruvate–amino-oxyacetate complex might be high enough to favour some reversal of the pyridoxal phosphate–amino-oxyacetate complex. Presumably, acetaldehyde forms a similar complex with amino-oxyacetate. Fig. 1 shows an initial period of low glucose production after the addition of alanine and acetaldehyde to cells preincubated with amino-oxyacetate. Also, a period of low rates of urea production is evident after the addition of pyruvate to cells preincubated with NH_4Cl, ornithine and amino-oxyacetate (Fig. 2). These lag times presumably represent the time necessary for acetaldehyde or pyruvate to reverse formation of the pyridoxal phosphate–amino-oxyacetate complex.

We have presented evidence that acetaldehyde and pyruvate can reverse inhibition of at least two transaminases, aspartate aminotransferase and alanine aminotransferase. Therefore care must be taken in interpreting results from experiments in which a slight reversal of inhibition may have been sufficient to yield maximum rates. If amino-oxyacetate was used in conjunction with an aldehyde or ketone substrate, even with preincubation, reaction rates obtained could have been artifacts due to reversal of inhibition. 2-Amino-4-methoxy-*trans*-but-3-enoic acid is not subject to these effects and therefore is a superior choice if one desires to inhibit only aspartate aminotransferase.

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