Effect of Glycine Excess on Para-Aminohippurate Uptake by the Kidney* (33748)

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The organic acid transport system of the kidney has major significance in excretion of organic residues and drugs and, accordingly, has been the topic of extensive study. Para-aminohippurate (PAH) has frequently been used as a model substrate for this system. This transport of PAH appears to be carrier mediated and energy dependent, and is enhanced by acetate. Acetate increased the uptake of PAH by renal cortical slices *in vitro* (1) and increased the tubular maximum for PAH *in vivo* (2). Since long-chain acyl glycines inhibit PAH uptake by the renal cortical slice, but acetyl glycine does not (3), it has been thought that the acetate enhancement of PAH uptake (acetate effect) was due to the formation of acetyl glycine, decreasing glycine available for the production of the long-chain acyl glycines (4). In this manner, acetate could be said to decrease the normal inhibitory effect of the long-chain acyl glycines produced in the kidney. The present study utilizing glycine loading of renal cortical slices does not support the above explanation for the acetate effect.

Methods. Fifteen studies were conducted using rabbit renal cortical slices. Care was taken that the rabbits had not received a diet containing added para-aminobenzoic acid. Cortical slices were obtained using a Stadie-Riggs microtome. The slices were incubated in Erlenmeyer flasks with Cross and Taggart medium at 20° in a metabolic shaker under an oxygen atmosphere for 60 min (1). The medium contained 7×10^{-5} M PAH. The slice preparations were run in triplicate or quadruplicate. Two-thirds of the flasks contained 0.01 M acetate. Half of the flasks with acetate in the medium also contained 0.01 M glycine. The slices were weighted on a Roller-Smith balance. After incubation the renal cortical slices were removed from the medium and homogenized in 10% trichloroacetic acid. Analyses for PAH concentration were made on the medium and on the trichloroacetic supernatant of the renal cortical slice homogenate using the method of Bratton and Marshall (5) adapted to the Technicon AutoAnalyzer. The PAH concentration was expressed in milligrams per gram of wet weight, and the ratio was expressed as PAH concentration per gram of tissue to PAH concentration per gram of medium. Glass-distilled water was used to prepare all media and reagents.

The glycine content of renal cortical slices was determined by the method of Alexander (6) after incubation in Cross and Taggart medium containing PAH and acetate but not added glycine. A series of renal cortical slices incubated with PAH were incubated with or without acetate for prolonged periods to determine if the acetate effect would disappear with prolonged incubation. Flasks were removed from the metabolic shaker at 30, 60, 90, and 120 min, and handled as above.

Results. The control S/M ratios in slices from different animals varied from 3 to 10. Accordingly the acetate effect was expressed as percentage increase above control values. The addition of acetate to the medium resulted in the expected increase in PAH S/M by $83 \pm 40\%$ (p .001). The addition of large quantities of glycine to the medium did not change the acetate effect, and an $89 \pm 31\%$ increase in S/M occurred (p .001).

Glycine was demonstrable in renal cortical slices after incubation with PAH and acetate, even without glycine added to the medium.

The findings with prolonged incubation of slices revealed that the difference in PAH S/M between acetate-incubated and nonace-

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tate-incubated slices persisted even to the time when the S/M began decreasing, presumably due to deterioration of the slice preparation.

Discussion. The explanation of the acetate effect through the production of acetyl glycine has been a logical deduction. The demonstration that long-chain acyl glycines inhibit PAH uptake by the renal cortical slice, presumably by competing for the transport system, represents an endogenously existing competing mechanism. The finding that acetyl-CoA could react with glycine and form acetyl glycine that does not inhibit PAH uptake by the cortical slice completed the link for this logical deduction.

If acetate did have its effect through the consumption of glycine to form acetyl glycine, the addition of excess glycine should prevent the acetate effect since the excess glycine would still be available for the formation of long-chain acyl glycines. The finding that large quantities of glycine did not inhibit the acetate effect makes the hypothesis less tenable.

A simple explanation for the acetate effect representing an alteration of permeability of the slice to para-aminohippurate is not supported by the prolonged incubation studies. If acetate affected the para-aminohippurate uptake by increasing the permeability of the cell membrane to PAH, it would be expected that with increased time the difference in the acetate-incubated slices and the nonacetate-incubated slices would disappear. This was not found.

The exact mechanism of the acetate effect is not apparent. It could represent an increased affinity of carrier for PAH, but this has not been ascertained.

Summary. The PAH uptake by glycine loaded rabbit renal cortical slices was studied to evaluate further the acetate enhancement of PAH transport. The presence of excess glycine did not inhibit the acetate enhancement. This finding indicates that the mechanism of the acetate effect is not the postulated prevention of long-chain acyl glycines by consumption of available glycine to form aceglycine.

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