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P-aminohippurate Accumulation in Kidney Cortex Slices: Stimulation by Dicarboxylates, Amino Acids and Their Oxoanalogues

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Summary

The effect of various amino acids and oxoacids on the accumulation of PAH in rat kidney cortex slices was determined. The following compounds were found to increase the PAH tissue to medium ratio (T/M_{PAH}): a) dicarboxylic acids: glutarate, 2-oxoglutarate and oxaloacetate, b) amino acids: glutamate, isoleucine, leucine, valine, methionine, tryptophane, histidine, threonine and glycine, c) monocarboxylates: hydroxymethionine, oxovaline, oxoisoleucine and oxoleucine. There were no marked concentration/effect differences to glycine, glutamate, glutarate and oxovaline. Ouabain inhibited T/M_{PAH} only slightly, but abolished its increase by pyruvate, 2-oxoglutarate and histidine. Oxygen hyposaturation abolished the T/M_{PAH} increase caused by 2-oxoglutarate, pyruvate, glutamate and histidine. It is concluded that various substrates stimulating the organic anion transport system (OATS) do so namely by improving the energy supply, although the direct participation of dicarboxylates in OATS could be of relevance namely in short-lasting variations.

Key words:
Organic anion transport system – Dicarboxylates – Aminoacids – Monocarboxylates

Introduction

Various organic anions are accumulated in patients with renal insufficiency as a result of decreased organic anion transport system (OATS) activity in basolateral membranes of proximal tubular cells. Recently, it has been postulated that OATS is a tertiary active transport system coupled with the countertransport of di-/tricarboxylates (Ullrich and Rumrich 1988, Burckhardt and Ullrich 1989, Pritchard 1988) which are transported into the proximal tubular cells by Na^+-dependent transport system through both the basolateral and luminal membranes. Consequently, the presence of glutarate or 2-oxoglutarate in the incubation medium stimulate PAH transport through the isolated basolateral vesicles (Pritchard 1988).

The possibility of stimulating the OATS is of outstanding clinical relevance. The stimulation could improve the excretion of organic anions, which share various untoward interactions. This possibility prompted our search for other metabolites stimulating OATS and a definition of the limiting steps in this stimulation.

Methods

Rat kidney cortex slices: The experiments were performed on male Wistar rats weighing 150–200 g fasted for 24 hours before the experiment. They were sacrificed by decapitation, the kidneys were removed, chilled in cold saline and kidney cortex slices were prepared by "free hand" dissection with a razor blade. Each experiment was performed from randomized slices obtained from 3–4 rats and each point is the mean of six samples.

Incubation: The incubation was performed in medium (Maxild *et al.* 1980) containing NaCl 140 mmol/l, $CaCl_2$ 0.5 mmol/l, $MgSO_4$ 0.7 mmol/l, TRIS-HCl 25 mmol/l, PAH 0.075 mmol/l (optimal PAH concentration) and the tested compound 5.0 mmol/l (usual concentration of substrates which are used also for energetical processes). The incubation was performed at 25 °C and oxygen atmosphere for 1 hour. The recently developed adaptation (Maxild *et al.* 1980) was used because of high T/M_{PAH} which is probably a result of the used buffer and its capacity. The effect of oxygen hyposaturation was evaluated in a gas mixture of 40 % nitrogen + 55 % oxygen and 5 % carbon dioxide.

Analyses: PAH was determined spectrophotometrically (Brun 1951). Statistical analyses were performed according to standard procedures (Tallarida and Murray 1981) on a personal computer.

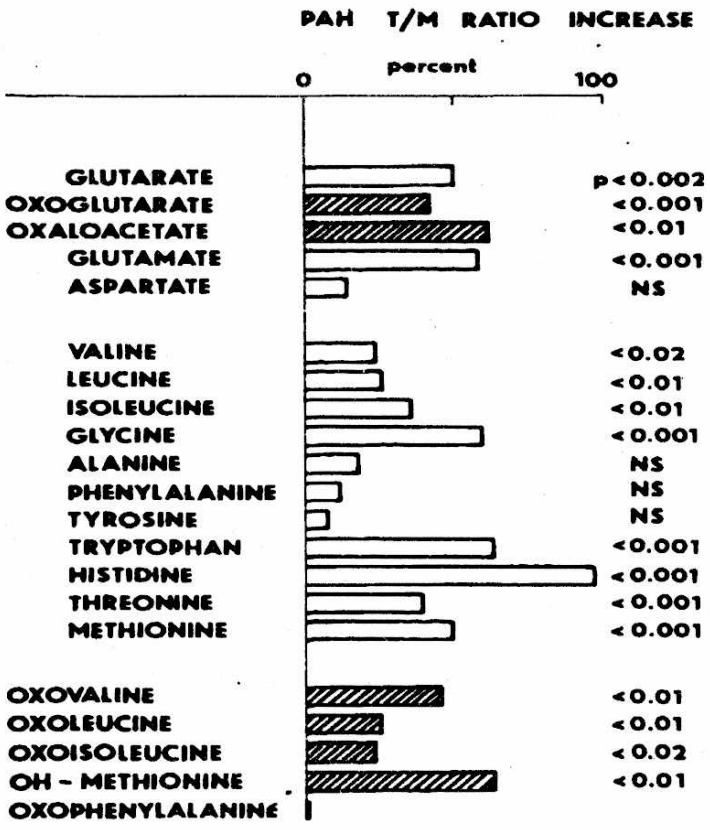

Fig. 1
The effect of various substrates on T/M_{PAH}. Hatched bars – oxocompounds, open bars – other compounds (the results are expressed in relative values).

Results

Dicarboxylates: The increase of T/M_{PAH} by glutarate, 2-oxoglutarate and oxaloacetate (Fig. 1) was predictable and verified the suitability of the experimental model for the study. However, the effects of corresponding acidic amino acids differed. Glutamate increased T/M_{PAH} equally as 2-oxoglutarate, aspartate increased T/M_{PAH} only slightly, while oxaloacetate increased it markedly. This was the reason why we extended our search for other amino acids and monocarboxylates.

Neutral amino acids: Branched chain amino acids increased the T/M_{PAH} only slightly (Fig. 1) whereas other neutral amino acids increased T/M_{PAH} variably. The effect of glycine was very marked in comparison with alanine and the effect of phenylalanine and tyrosine was relatively smaller in comparison with the marked effect of tryptophane and histidine.

Other monocarboxylates: Monocarboxylates and oxomonocarboxylates increased also T/M_{PAH}, although they did not participate in OATS countertransport.

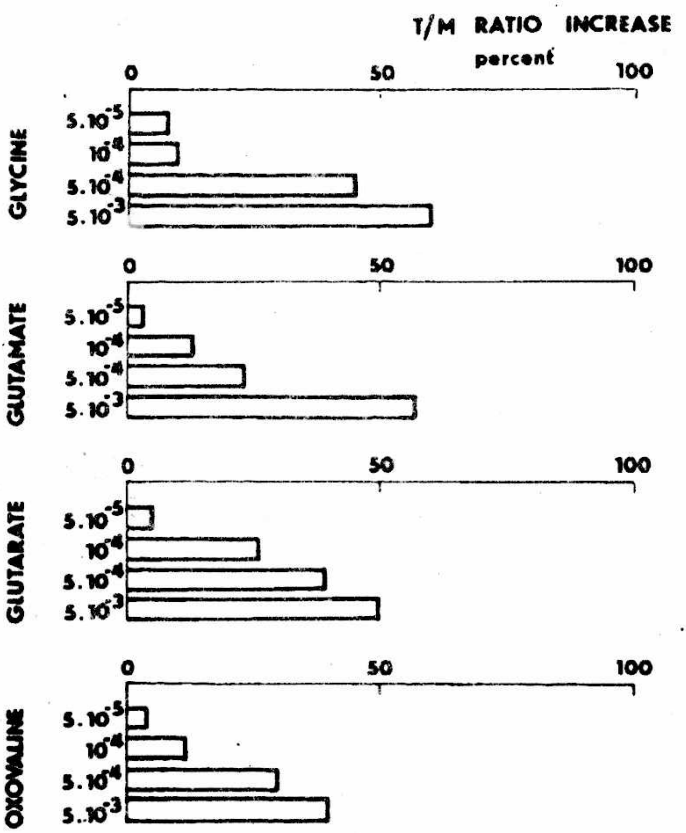

Fig. 2
The relationship between the concentration of model compounds and the stimulation of T/M_{PAH} (substrate concentrations are expressed in mol/l).

Concentration dependence: Some representative substrates, e.g. glycine, glutamate, glutarate and oxovaline, were tested for their enhancing effect on T/M_{PAH} at various concentrations in the incubation medium. All of them increased T/M_{PAH} in a concentration-dependent manner and no marked differences were found among them (Fig. 2).

Na^+ transport inhibition: Ouabain, a classical Na^+,K^+ – ATPase inhibitor, at 5 mmol/l concentration in the incubation medium decreased T/M_{PAH} only slightly. However, it completely abolished the T/M_{PAH} increase induced by pyruvate, 2-oxoglutarate and histidine (Tab. 1).

Table 1
The effect of ouabain on T/M_{PAH}

Control	6.0±0.25
Ouabain	5.3±0.22
2-oxoglutarate	7.3±0.09[a]
2-oxoglutarate + ouabain	4.9±0.12[b]
Histidine	8.0±0.25[a]
Histidine + ouabain	5.5±0.26[b]

The values represent means ± S.E.M. (6 experiments).
Significant differences ($p<0.01$): a – from control, b – effect of ouabain.

Oxygen supply: The incubation of slices in a gas mixture containing only 55 % oxygen also abolished the T/M_{PAH} increase by some representative stimulators (Tab. 2). The lower oxygen saturation already decreased the basal values of T/M_{PAH}.

Table 2
The effect of oxygen hyposaturation on T/M_{PAH}

Control	5.90±0.37
2-oxoglutarate	5.85±0.37
Pyruvate	5.68±0.27
Glutamate	5.20±0.27
Histidine	5.01±0.17

The values represent means ± S.E.M. (6 experiments)

Discussion

Physiological aspects: Single nephron studies (Ullrich and Rumrich 1988) were performed within periods of several seconds and isolated vesicle studies at several second to minute periods (Pritchard 1988) to measure the transport at its

maximal velocity, practically uninfluenced by the activity of simultaneous energy processes. However, this is not the case *in vivo*, where the processes are continuous with the dynamic balance built by the transport velocity, feedback mechanism of the transported substrates and the activity of energy processes. This is the reason, why the experiments on kidney cortex slices were performed at the prolonged incubation period up to one hour.

The experiments on kidney cortex slices confirm the previous studies with dicarboxylates (Ullrich and Rumrich 1988, Pritchard 1988) even under these experimental conditions. Moreover, they extend them for dicarboxylic amino acids. There are two alternatives of dicarboxylic amino acid participation in T/M_{PAH} increase: a) Dicarboxylic amino acids could be used in OATS countertransport. The necessary energy requirement would be covered by Na^+-dependent transport systems (Zelikovic and Chesney 1989). b) Dicarboxylic amino acids could be completely oxidized and increase the amount of produced ATP used in the $Na^+,K^+-ATPase$ transport system. This means the covering of energy processes.

To decide between these alternatives, further experiments were performed: a) Mono- and dicarboxylic amino acid concentration-dependent increase of T/M_{PAH} with no marked concentration differences, though monocarboxylates do not participate in OATS. Moreover, the required amount of dicarboxylates necessary for OATS is very low, because after extrusion they are immediately transported into the cell by a Na^+-dependent transport system (Ullrich and Rumrich 1988, Burckhardt and Ullrich 1989, Pritchard 1988). Thus, in the case of different mechanism of participation of mono- and dicarboxylates the concentration dependence would be different. b) Ouabain, an inhibitor of $Na^+,K^+-ATPase$, abolished the T/M_{PAH} increase caused by histidine and 2-oxoglutarate. c) Inhibition of oxidative processes by the decreased oxygen content in the incubation atmosphere abolished also the T/M_{PAH} increase by various dicarboxylates. d) Last, but not least, the capacity of OATS calculated *per* residual nephron unit in renal insufficiency patients is increased (Gajdoš *et al.* unpublished data).

Clinical aspects: The OATS activity appears to be of extreme interest namely in renal insufficiency. The accumulation of a model substance, hippurate, in the blood is more than 100 times higher in comparison with healthy subjects (Spustová *et al.* 1988) and in fact there is no other substance known to be accumulated to such an extent. The accumulation of organic anions appears to be a cause of metabolic acidosis with the increased anion gap (Dzúrik *et al.* 1989), decreased protein binding of drugs (Gulyassy *et al.* 1987), competitive inhibition of excretion of various drugs, such as penicillins or cefalosporins (Ullrich *et al.* 1989) and abnormal glucose utilization (Spustová *et al.* 1987). On the other hand, the therapeutic significance of essential amino acids or their oxoanalogue supplementation in renal insufficiency received a new impetus and the effect of their administration on the serum level of organic anions is to be studied in patients with renal insufficiency.

In conclusion, it could be suggested that the ability to stimulate the activity of organic anion excretion would be of both physiological and clinical relevance especially in the case of their accumulation such as metabolic acidosis with the increased anion gap or renal failure. This was the reason for broad screening of various substances in the present study.

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