THE POSSIBLE ROLE OF THE VESICLES IN RENAL AMMONIA EXCRETION

An Implication of Concentrated Glutamic Oxalacetic Transaminase

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In the kidney, the initial step in the release of ammonia from glutamine may be catalyzed by either glutaminase or glutamine ketoacid transaminase systems (4–6, 17). The glutaminase pathway leading to the formation of glutamate, which serves as the intermediate product and may undergo further oxidative deamination to form alpha ketoglutarate and ammonia, appears to be the major one (4). It has also been reported that glutamate, alpha ketoglutarate, and oxalacetate are among the most effective "feedback" inhibitors of glutaminase activity (3). Therefore, the study of the subcellular organelles whose function may determine the local concentrations of these metabolites in the renal tubular cells will be helpful in understanding the intracellular structure involved in the formation of ammonia.

One important mechanism which may control the cellular concentrations of glutamate and alpha ketoglutarate is the reversible enzymatic transamination reaction between these two dicarboxylic acids. Among the enzymes involved in this reaction, glutamic oxalacetic transaminase (GOT) is the most widely distributed in the mammalian tissues. A previous communication has briefly reported that the activity of this enzyme in the kidney is largely concentrated in the convoluted tubules of the renal cortex and certain cortical tubules near the corticomedullary junction (12). Subsequent work carried out in this laboratory has revealed that the latter are the ascending thick limbs of the loops of Henle. This report is concerned with the relationship of this enzyme activity to the subcellular organelles of these tubular cells, particularly the subapical vesicles. The possible significance of this enzyme to the interrelationship among renal ammonia excretion, gluconeogenesis, and anaerobic carbon dioxide production will be discussed.

MATERIALS AND METHODS

Under light ether anesthesia, the descending aorta of Wistar albino rats (weighing 150–200 g each) was perfused with 1% glutaraldehyde (Biological Grade, Fisher Scientific Company, Fair Lawn, N.J.) in 0.25 M sucrose containing 0.05 M imidazole as buffer at pH 7.2–7.4. Approximately 300 ml of perfusate was forced into the abdominal aorta in 2 min with the proximal end of the vessel clamped. After perfusion, the kidney tissue of the inner stripe of the outer medulla, where the ascending thick limbs of the loops of Henle are located, was selected and minced to small tissue blocks not more than 0.5 mm in diameter. These tissue blocks were then immersed in a fixative containing 3.7% formaldehyde in 0.05 M imidazole-buffered sucrose, pH 7.2–7.4, for 30 min. All fixative solutions were cooled to 0–4°C before use. After the immersion fixation in formaldehyde, the tissue blocks were washed overnight in cold imidazole-buffered sucrose, incubated for 20 min at room temperature (25 ± 3°C) in a GOT histochemical medium consisting of 20 mM L-aspartic acid, 2 mM alpha ketoglutaric acid, 6 mM lead nitrate, 0.05 M imidazole, and 0.25 M sucrose, pH 7.4–7.5. Thereafter, they were rinsed briefly in a postincubation washing solution which consisted of 20 mM L-aspartic acid and 0.25 M sucrose buffered at pH 7.4 with 0.05 M imidazole-nitric acid, osmicated in 1% OsO_4 in Veronal-acetate buffer (15) for 1 hr, rinsed in 0.05 M imidazole buffer, dehydrated in a buffered alcohol series (11), and embedded in Epon 812. The thin sections were cut with an MT-2 Porter-Blum ultramicrotome and examined in a Philips EM 300 electron microscope either without contrast staining or after brief double staining in saturated aqueous uranyl acetate and lead citrate solutions (23).

As controls, some fixed kidney tissue blocks were incubated in two media in which either (*a*) the alpha ketoglutaric acid of the GOT medium was withdrawn or (*b*) the L-aspartic acid was replaced with equimolar D-aspartic acid, and were processed in the same manner as outlined above except that, for rinsing the tissue blocks which were incubated in the control medium (*b*), the postincubation washing solution contained D-aspartic acid instead of L-aspartic acid. The aspartic acids, alpha ketoglutaric acid, and imidazole used in the experiment were purchased from Sigma Chemical Co., St. Louis, Mo.

OBSERVATIONS

The tubular epithelial cells of the thick limb of Henle's loop are characterized by the presence of many long mitochondria arranged in a direction perpendicular to the basement membrane and by their bulging into the lumen of the tubule in the nuclear region (16, 19). In the apex, electron microscopy reveals a large population of vesicular profiles which may be partially filled with a flocculent material (22). After a 20 min incubation in the histochemical medium, the reaction product due to GOT activity is found to be localized to the subapical vesicles or related organelles, the mitochondria and the nuclear envelope (Fig. 1). The Golgi complex, multivesicular bodies, and the cell surface membrane are free of reaction product. No nonspecific lead deposition occurs in the tissue blocks incubated in either of the two control media.

The sequence of appearance of enzymatic reaction product is of particular interest. Unlike that in the liver cells (11) and in the cardiac myofibers (10), the electron-opaque enzymatic reaction product in these renal tubular cells appears first in the subapical vesicles, and is deposited most heavily there, instead of in the mitochondria (Figs. 1 and 2).

The majority of subapical vesicles are outlined by a membrane that is even evident in the unstained sections. They measure about 1500–2500 A in diameter. Within any group of vesicles the amount of reaction product deposited in each organelle varies considerably; it is abundant in some and practically absent in the others. In those vesicles which contain reaction product, the latter is usually visualized as well-defined electron-opaque aggregates often with a smooth contour as if it were in a small loculation or subcompartment. The number of these electron-opaque aggregates in each vesicle ranges from zero to five. When saturated with deposits, the aggregates are ovoid or round, but may be teardrop-like in appearance and are always separated from the outer surface of the vesicle by a rather clear zone. They may range from 300 to 800 A in diameter, frequently occupy two to five symmetrical positions in the vesicle, and may be arranged in a rosette pattern with a faintly electron-opaque material in the center (Fig. 3). No continuity between these GOT-positive vesicles and the tubular lumen is noted.

DISCUSSION

For histochemical demonstration of glutamic oxalacetic transaminase activity, L-aspartic acid and alpha ketoglutaric acid are used as substrates. As a result of an enzymatic amino transfer reaction between the two substrates, two reaction products are formed, namely, oxalacetic acid and glutamic acid. The oxalacetate is captured as an insoluble lead salt which serves as a marker to indicate the site of enzyme activity (9), whereas the glutamate, although formed at the same time, is not precipitated.

In a previous study (12), it was reported that GOT activity is largely localized to the convoluted tubules of the renal cortex and a segment of the nephron at the corticomedullary junction, corresponding to the thick limbs of Henle's loop. It has been suggested by Rhodin (20) that the latter may be the major site for ammonia production in the kidney and that the subapical vesicles in the cytoplasm of these cells may be involved directly in this specific function. The results of the present study have demonstrated that in these vesicles there is a reversible enzymatic transamination mechanism, the function of which is probably to maintain appropriate concentration ratios among four metabolically active chemical substances. Of these four metabolites, three, i.e. glutamate, alpha ketoglutarate, and oxalacetate, are known to be initimately related to ammonia production or the control of this process.

Glutamate is a major intermediate metabolite during glutamine degradation and is a potential ammonia donor (4–6, 17). The close relationship between renal ammonia production and renal gluconeogenesis has been well documented (3–6). Goodman et al. (5) and Goldstein (3, 4) have demonstrated that metabolic acidosis causes an increase in renal gluconeogenesis and an enhanced ammonia production. They suggest that the increased renal gluconeogenesis may accelerate the removal of glutamate and alpha ketoglutarate, thereby stimulating the deamination and deamidation of glutamine. Glutamate, alpha ketoglutarate, malate, and oxalacetate have been shown to be the most effective inhibitors of glutaminase, causing a marked decrease in the rate of ammonia excretion in acidotic rats (3).

The major pathway of gluconeogenesis involves the formation of phosphoenolpyruvate. Although this step takes place in the extramitochondrial compartment, the precursor, oxalacetate, is

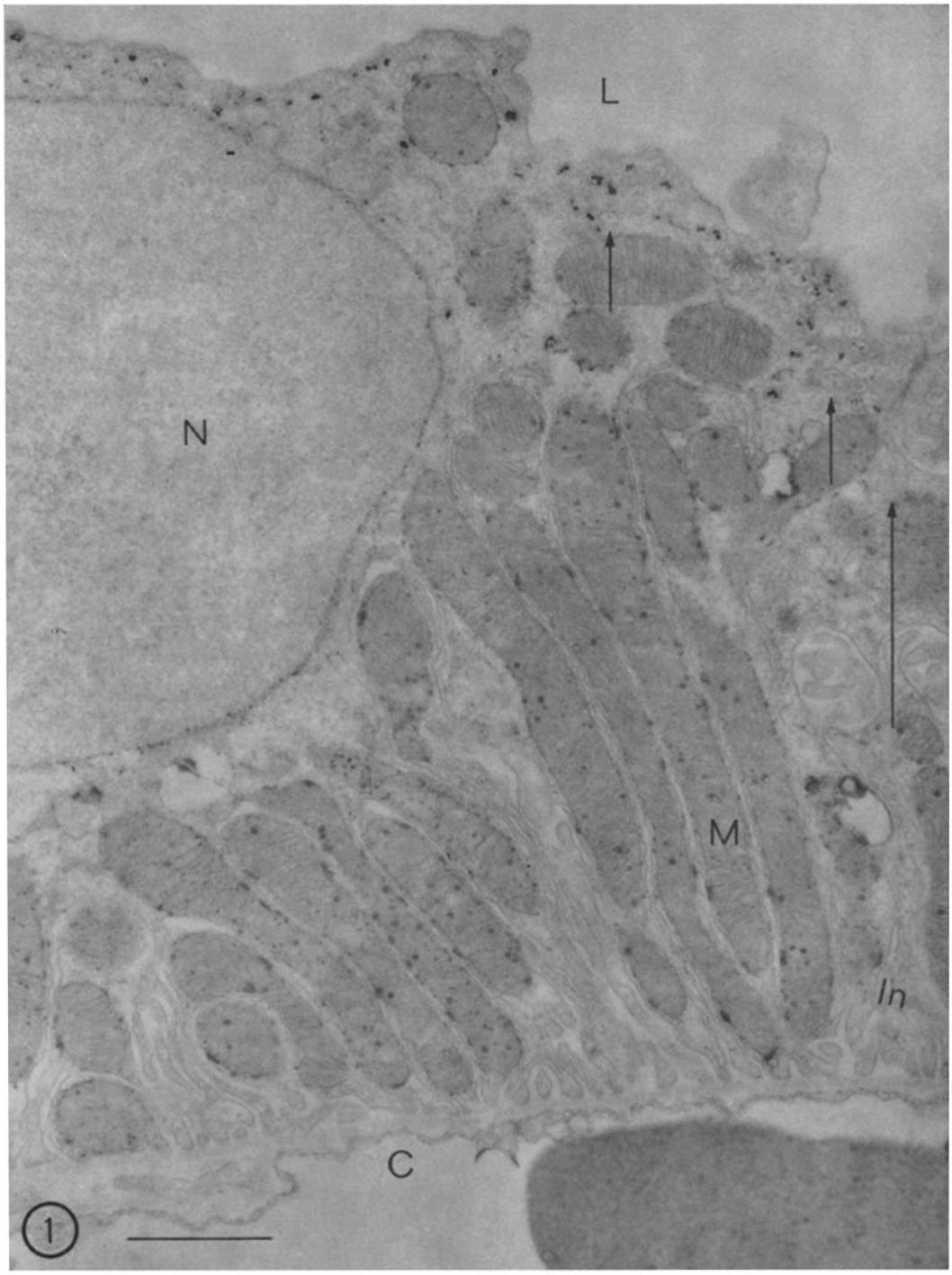
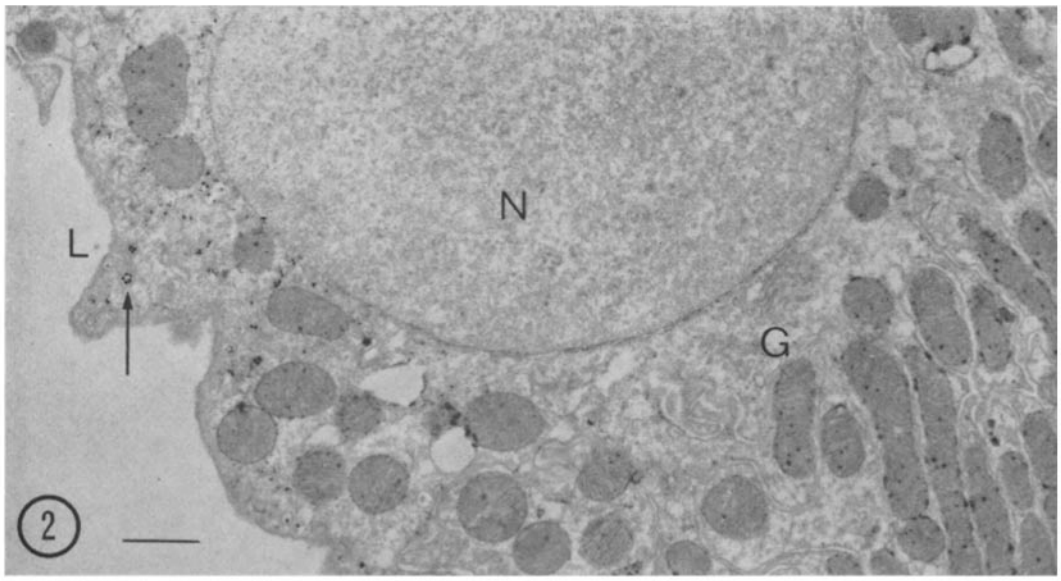


FIGURE 1 Electron micrograph of part of an epithelial cell lining the thick ascending limb of Henle's loop. The tissue block was fixed in glutaraldehyde and formaldehyde, incubated in a GOT histochemical medium, postfixed in OsO_4, and embedded in Epon. The thin section was examined without contrast staining. The most concentrated GOT activity appears to be in the subapical vesicles. The electron-opaque enzymatic reaction product is deposited as well circumscribed aggregates which are separated from the vesicle surface by a rather clear zone. There is a small quantity of reaction product deposited in the nuclear envelope and in the mitochondria. About half the number of the vesicles in this part of the cell do not show enzyme activity, but may contain one to three collections of faintly electron-opaque material in the center (short arrows). Lumen of the renal tubule (*L*); nucleus (*N*); mitochondria (*M*); multivesicular body (long arrow); basal cytoplasmic membrane infoldings (*In*); and capillary (*C*). Marker, 1 μ. $\times$ 21,000.



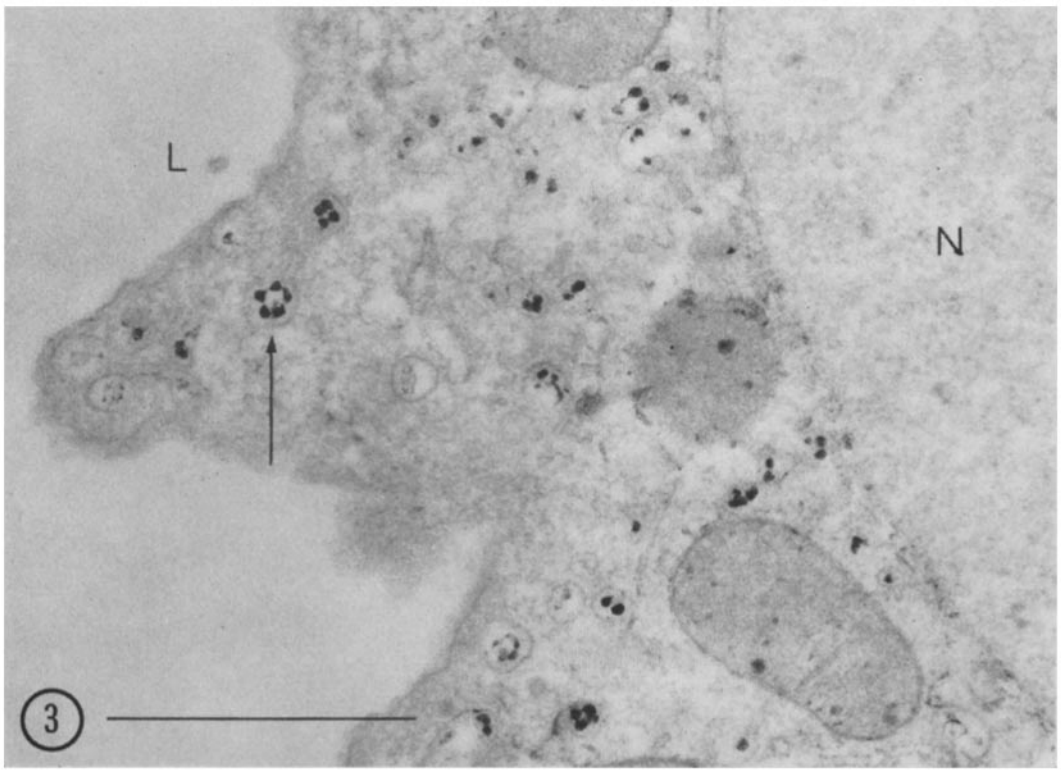


FIGURE 2 Electron micrograph of part of another epithelial cell in the thick limb, showing subapical vesicles filled with aggregates of electron-opaque reaction product. Some of the deposits may be arranged in a rosette pattern (arrow). A Golgi complex is shown to be free of reaction product deposits. Thin section unstained. Lumen of tubule (*L*); nucleus (*N*); and Golgi complex (*G*). Marker, 1 μ. × 10,000.

FIGURE 3 A higher magnification of the apical portion of the cell shown in Fig. 2. Aggregates in a vesicle arranged in a rosette pattern are indicated by an arrow. Lumen of tubule (*L*); nucleus (*N*). Thin section unstained. Marker, 1 μ. × 41,000.

primarily generated in the mitochondria and diffuses out into the extramitochondrial compartment in the forms of aspartate or malate, since the mitochondrial membrane is relatively impermeable to oxalacetate (8). It seems likely that one of the functions of the extramitochondrial GOT activity is to provide oxalacetate by transferring the amino group from asparate to alpha ketoglutarate. Further metabolism of the glutamate thus formed probably depends in part on the local concentration of alpha ketoglutarate. It is relevant at this point to note that both alpha ketoglutarate and oxalacetate are also known to participate in an effective system for anaerobic carbon dioxide production (18). In the gluconeogenic route as well, when the phosphoenolpyruvate is formed from oxalacetate, carbon dioxide is released at the same time. The enzyme, phosphoenolpyruvate carboxykinase, which catalyzes this reaction, has been found to be located in the extramitochondrial compartment of the rat liver cell (14). If this is also the pattern of enzyme distribution in the renal tubular cells, it is conceivable that in these cells, the three intimately related processes, i.e. ammonia formation, renal gluconeogenesis, and anaerobic carbon dioxide production, may take place in the cytoplasm near the cell apex. The demonstration of GOT activity concentrated in the subapical vesicles is compatible with this hypothesis.

Recently the biological properties of the specialized cytoplasmic vesicles have attracted much attention. It was Roth and Porter who first suggested that these vesicles may have specialized functions, and who postulated that the coated vesicles are specialized for the cellular uptake of protein (21). In the proximal renal tubular cells, many cytoplasmic vesicles have been shown to be involved in absorption and digestion of protein (1, 7, 13). On the other hand, Friend and Farquhar suggested that certain specialized coated vesicles in the rat vas deferens may be concerned with the transport of lytic enzymes from the Golgi complex to lysosomes (2). By demonstrating a local concentration of GOT activity, the findngs presented in this paper have provided evidence to suggest that a distinct group of vesicles in the distal renal tubular cells may be involved with excretion of ammonia and hydrogen ion or with the control of these processes.

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