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Transport of α-aminoisobutyric acid by separated rabbit renal tubules

Suspensions of isolated rabbit kidney tubules have been prepared by BURG AND ORLOFF[1] and used for studies of cation transport. These preparations are able to accumulate from the medium and to maintain concentration gradients of Na^+, K^+, and *p*-aminohippuric acid. Advantages of a tubule preparation include: (1) Separation of the active kidney components from those not concerned with the secretion and reabsorption processes. (2) Tubules are uniformly bathed with the medium. (3) Steady-state concentrations in the intracellular fluid are more rapidly attained, and, also, subsequent rapid modifications of these concentrations are possible upon addition of appropriate agents. (4) The homogeneity of tubule suspensions permits rapid removal of uniform aliquots from the incubation medium at desired time intervals. (5) Comparative studies of solute fluxes in slices and in tubular fragments can provide a measure of the properties of the luminal membrane[2]. These advantages suggest the desirability of employing such preparations in studies of renal amino acid transport. Consequently, we desired to test the ability of separated tubules to transport amino acids, and for this purpose chose to use the normally transported, but essentially nonmetabolized, model amino acid, α-aminoisobutyric acid[3].

Rabbit kidney tubules were prepared essentially by the method of BURG AND ORLOFF[1]. Perfused rabbit kidneys were diced and incubated with 0.1 % collagenase (Sigma Chemical Co.) prepared from *Clostridium histolyticum*. Digestions were conducted for 45 min at room temperature. The digestion mixture was diluted with salt solution (described below) and filtered through cheese cloth. The suspended tubules were isolated by centrifugation at 50 times gravity for 90 sec. The isolated tubules were washed 3 times by resuspension and centrifugation. The salt solution used to isolate and wash the tubules contained 126 mM NaCl, 5 mM KCl, 10 mM sodium acetate, 1.2 mM $MgSO_4$, 1 mM $CaCl_2$, and 10 mM sodium phosphate buffer, pH 7.4.

The washed tubules obtained from the kidneys of two rabbits were resuspended in 30 ml of a medium of the following composition: 93.9 % (by vol.) of the above salt solution, 6.1 % (by vol.) bovine calf serum (Pentex), and 245 μg of creatinine per ml. Equal aliquots (9.0 ml) of the suspension were transferred to three Burg–Orloff incubation flasks[1] (designated A, B and C, respectively) placed in a 25° water bath. The tubules were kept in uniform suspension by bubbling oxygen through the inlet tubes. Dow Corning Antifoam A was used to prevent foaming. Salt solution (1.0 ml) was added to two of the flasks (A and C) and 1.0 ml of salt solution containing 1.9 μC of [^{14}C]inulin (New England Nuclear Corp.) was added to the third flask (B). After a preincubation period of 15 min, 1.0 ml of 3.88 mM α-amino[1-^{14}C]isobutyric acid (2.1 μC, Tracerlab) in salt solution was added to Flask A, and 1.0 ml of 3.88 mM nonradioactive α-aminoisobutyric acid (A grade, Calbiochem) was added to Flasks B and C. Equal aliquots (3.0 ml) of the tubule suspensions were removed from each of the flasks at three time intervals and centrifuged at 1000 × *g* for 10 min at 25° in Burg–Orloff centrifuge tubes[1]. Since the active transport process was not stopped prior to the isolation of the tubules by centrifugation, the true incubation time was estimated to be 2 min past sampling time for samples removed at 0 and 5 min, and 5 min longer than sampling time for subsequent samples.

The supernatants were decanted from the flasks, and 100-μl aliquots of the supernatants from Flasks A and B were counted for ^{14}C activity in a Tri-Carb liquid scintillation spectrometer (Packard Model 3003). (The counting solvent consisted of 5 g 2,5-diphenyloxazole, 0.05 g α-naphthylphenyloxazole and 80 g naphthalene per l dissolved in xylene–dioxane–ethanol (5:5:3, by vol.)). The tubes were rinsed with dilute salt solution (1:10 dilution with distilled water), wiped dry, and the tissue pellets extruded from the bottom. Pellets derived from Flasks A and B were resuspended in 3.0-ml volumes of dilute salt solution and placed in a boiling water bath for 5 min. The resulting suspension of nonviable tubules was cooled, centrifuged at 1000 $\times$ *g* for 10 min, and 100-μl aliquots taken for determination of ^{14}C activities. The pellets derived from Flask C were transferred into tared weighing bottles, weighed immediately and again after drying 24 h at 110°. In this manner, medium and pellet α-amino[1-^{14}C]isobutyric acid content was determined from Flask A, pellet-free extracellular space from Flask B and total pellet fluid from Flask C. Using these results the ratio of tubular fluid α-amino[1-^{14}C]isobutyric acid concentration to medium α-amino[1-^{14}C]isobutyric acid concentration was calculated for incubation periods of different lengths.

The results obtained in three separate experiments using different pairs of rabbits have been pooled and are shown in Fig. 1. Steady-state conditions are attained after 25 min. The distribution ratio at this time was about 2.1. A steady-state distribution ratio of 1.0 would be expected if the amino acid accumulation was simply caused by free diffusion across the cell membrane. Occasionally, a low distribution ratio was found for a sample possibly arising from a rapid loss of tubule viability after an extended incubation period or from a poorly formed tissue pellet.

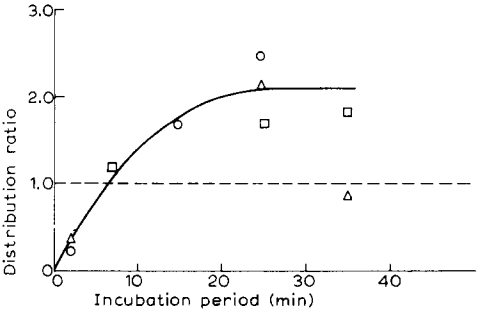

Fig. 1. Uptake of α-amino[1-^{14}C]isobutyric acid into intracellular rabbit kidney tubular fluid in an O_2 atmosphere at 25°. Medium α-aminoisobutyric acid concentration was 0.35 mM. Different symbols represent separate experiments. Distribution ratio = (counts/min α-amino[1-^{14}C]isobutyric acid per ml intracellular H_2O)/(counts/min α-amino[1-^{14}C]isobutyric acid per ml medium).

For purposes of comparison, ROSENBERG, BLAIR AND SEGAL[4] using kidney cortex slices found that 0.1 mM α-aminoisobutyric acid reached equilibrium conditions in approx. 90 min at 37° (95 % O_2–5 % CO_2 atmosphere). At a temperature of 27°, equilibrium conditions apparently had not yet been attained after incubation for 120 min. A distribution ratio of 3.8 was found after 90 min at 37°. However, *p*-aminohippuric acid distribution ratios obtained by MURTHY AND FOULKES[2] with both slice and tubule preparations indicate that distribution ratios found with tissue slices should run somewhat higher than those found using isolated kidney tubules.

We were interested in determining the effect of a reduction in the oxidative

metabolism of the tubules upon the accumulation of α-aminoisobutyric acid. Tubules prepared from the kidneys of one rabbit were suspended in 30 ml of the incubation medium. Aliquots (9.0 ml) of this suspension were transferred to three incubation flasks in a 25° water bath. Oxygen was bubbled through two of the flasks (A and B) and nitrogen through the third (C). Salt solution (1.0 ml) was added to Flasks A and C, and 1.0 ml of $1.1 \cdot 10^{-3}$ M 2,4-dinitrophenol (Eastman Kodak Co.) in salt solution was added to Flask B. After a 15-min preincubation period, 1.0 ml of 2.2 mM α-amino[1-^{14}C]isobutyric acid (1.7 μC) in salt solution was added to each flask. Aliquots (4.0 ml) were removed immediately upon mixing and after a 5-min incubation period. The aliquots were quickly mixed with 3.5 vol. of ice-cold incubation medium contained in Burg–Orloff centrifuge tubes in order to stop the active transport process. The tubes were then centrifuged at 1000 × *g* for 10 min. Supernatants were decanted and an aliquot counted in the scintillation spectrometer for ^{14}C activity; another aliquot was analyzed for creatinine content[5]. The pellets were resuspended in 3.0 ml of diluted salt solution and placed in a boiling water bath for 5 min. The tubes were then cooled and centrifuged at 1000 × *g* for 10 min. Aliquots (100 μl) were counted in the scintillation spectrometer for ^{14}C activity. Aliquots (2.7 ml) of the remaining supernatants from the boiled pellets were also analyzed for creatinine content. The free extracellular water contents of the pellets were calculated from the creatinine analyses[6]. The accumulated α-amino[1-^{14}C]isobutyric acid after 5-min incubation in an N_2 atmosphere was 52 % that accumulated in an atmosphere of O_2, and the α-amino[1-^{14}C]isobutyric acid accumulated in the presence of 10^{-4} M 2,4-dinitrophenol (O_2 atmosphere) was 15 % of that accumulated in its absence.

The findings that the distribution ratio of tubular α-aminoisobutyric acid to that of the medium reaches values greater than 1.0, and that accumulation of α-aminoisobutyric acid is diminished in the presence of 2,4-dinitrophenol or in the absence of O_2 indicates that the active transport process for the amino acid is still functioning in the separated rabbit kidney tubules. A portion of the accumulated α-aminoisobutyric acid probably is present in the intraluminal space. Presumably, however, the amino acid is primarily accumulated into the intracellular fluid.

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Note added in revision: A communication on transport of amino acids by isolated rabbit renal tubules by HILLMAN, ALBRECHT AND ROSENBERG has recently appeared[7].

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