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REGULATORY CHARACTERISTICS OF AMINO ACID TRANSPORT IN NEWBORN RAT RENAL CORTEX CELLS

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SUMMARY

The uptake of α-aminoisobutyric acid by slices of kidney cortex from newborn rats is enhanced by a preliminary incubation of the tissue in buffer at 37 °C. This effect is abolished by anaerobiosis, the presence of dinitrophenol or the removal of Na^+ during the preliminary incubation. Cycloheximide (50 μM) and purimycin (1 mM) as well as α-aminoisobutyric acid, glycine and proline (5 mM) in the pre-incubation buffer also abolish the effect, while actinomycin D (0.8 μM) partially inhibits the enhancement due to preliminary incubation. A kinetic examination of the phenomenon indicates that the enhanced uptake is due to an increased entry rate into the cells without a change in efflux. There is no alteration in the apparent transport K_m but an increase in the V for entry. The effect is dependent on tissue age being observed between birth and 22 days, after which there is a decrease in response to preliminary incubation with no effect seen in adult tissues.

INTRODUCTION

The developing rat kidney cortex has been used as a model system to determine the characteristics of sugar and amino acid transport mechanisms [1]. Examination of the in vitro uptake of these solutes by renal cortical slices from newborn and older animals has served to delineate differences in the transport processes for cystine and cysteine [2], lysine [3] and proline and glycine [4] and has aided in distinguishing the separate nature of the process for sugars and amino acids [1, 4, 5].

Recently, we reported a time-dependent regulatory process for amino acid transport in renal cortical cells of newborn rats [7]. The ability to accumulate α-aminoisobutyric acid, cycloleucine, glycine and proline was enhanced by a preliminary incubation of slices of newborn kidney cortex at 37 °C in saline buffer prior to the uptake measurement. The magnitude of the effect increased progressively with the length of time of the preliminary incubation, a three-fold increase in α-aminoisobutyric acid and cycloleucine uptake being observed after the cells were incubated for 2 h at 37 °C. The purpose of this paper is to further demonstrate the properties of the system involved in this enhanced transport. Employing as substrates α-amino-

isobutyric acid and cycloleucine, we have examined the requirement for energy and protein synthesis, the effects of Na^+ and amino acids in the incubation medium, the kinetics and concentration dependence of uptake and the relationship to tissue age. Results of these studies form the basis of the report.

METHODS

Preparation of tissues

Kidneys were obtained from Sprague-Dawley rats of various age after decapitation. From newborn (within 36 h of birth) to 15 days of age, cortical slices were made with a sharp razor blade from the surface of the kidney as described previously [2]. Three slices were made from each tiny newborn kidney, each slice weighing about 2 mg. This technique has been evaluated histologically and these slices have been found to contain only cortical cells. The slices from kidneys of one litter, about 10 animals, were pooled and three slices placed in each incubation vessel. It required about 15 min to prepare the slices, which were placed in a small petri dish containing Krebs-Ringer buffer at room temperature. Cortical slices of older animals were prepared with a Stadie-Riggs microtome as reported in our previous communications [1, 8]. Three slices weighing 10–20 mg each, one from each of three animals, were placed in each incubation vessel. No discrimination was made with regard to sex in the newborn animals. Thereafter only male rate were used.

Preliminary incubation

For preliminary incubation the slices were placed in 30-ml plastic bottles containing 2 ml of Krebs-Ringer bicarbonate buffer (118.5 mM NaCl, 4.75 mM KCl, 2.53 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$ and 25 mM $NaHCO_3$) pH 7.35, which were gassed 30 s with O_2/CO_2 95:5 % and incubated for periods of time up to 2 h at 37 °C in a Dubnoff shaker. In experiments where no additions were made to the buffer the incubation flasks were opened, radioactive substrate added, the contents regassed for 30 s, and incubated again at 37 °C to measure the uptake of the amino acid. Studies were also performed in which the slices were removed from the flasks after the preliminary incubation, washed, blotted and transferred to new flasks containing 2 ml of buffer and radioactive amino acid for the uptake measurement. The effect of preliminary incubation on uptake was identical under these two circumstances.

The medium of the preliminary incubation phase was altered in several different ways. Additions were made of dinitrophenol, puromycin, cycloheximide, actinomycin D, ouabain and various amino acids, Na^+ was removed and Tris was substituted [9] and anaerobic conditions were imposed by gassing the buffer and flask with N_2:CO_2 95:5 %. When these manipulations were made the slices were removed after the preincubation period, washed in 0.9 % saline, blotted, placed in bottles containing only buffer and radioactive substrate, gassed for 30 s and returned to the shaker bath for measurement of uptake. The effect of temperature during the preliminary incubation was studied by placing the vessels in water at 4 and 22 °C.

Amino acid uptake without preincubation

The technique for determining the in vitro uptake and intracellular concentration of ^{14}C-labeled amino acids in kidney cortex slices from newborn and adult

animals has been described [1, 8, 9]. For the direct measurement of uptake, three slices not treated by preliminary incubation were placed into 30-ml plastic bottles containing 2 ml of Krebs-Ringer-bicarbonate buffer (pH 7.35) or in modified buffer, as indicated, with 0.065 mM ^{14}C-labeled α-aminoisobutyric acid or cycloleucine, 0.1 μCi/ml. The vessels were gassed 30 s with O_2:CO_2 95:5 %, or N_2:CO_2 if anaerobiosis was imposed, sealed and incubated at 37 °C in a Dubnoff metabolic shaker for the times indicated. These same conditions prevailed during the uptake measurements in those tissues exposed to the preliminary incubation.

Measurement of labeled amino acid uptake

At the end of the uptake incubation, all slices were removed, rinsed quickly in 0.9 % saline, blotted, weighed and placed in tubes containing 1 ml of water, which were heated in boiling water for 6 min. 0.2 ml of the tissue extracts and medium were assayed for radioactivity by liquid scintillation techniques [1, 8].

The uptake is expressed as the distribution ratio, the ratio of cpm/ml intracellular fluid to cpm/ml of medium [8]. For non-metabolizable substrates this represents a concentration gradient. The inulin space of slices was used to determine the portion of the tissue radioactivity in extracellular fluid [10]. Total tissue water was determined by the difference between wet and dry weight of the slices heated in a vacuum oven for 18 h at 100 °C. The difference between total tissue water and inulin space was used as an assessment of the intracellular fluid. Total tissue water and inulin space were calculated for each age and ranged from 76 % and 20 %, respectively, of wet weight in the newborn [1, 2] to 80 % and 25 %, respectively, in the adult [8–10].

Exodus of intracellular α-aminoisobutyric acid

Exodus of amino acid was studied as reported previously [2] with modification to include the preliminary incubation phase. Twenty-five slices from newborn rats were incubated for 15 min at 37 °C in 2 ml of Krebs-Ringer-bicarbonate buffer (pH 7.35) containing 0.065 mM α-aminoisobutyric acid, 1 μCi/ml without preliminary incubation and after 60 and 120 min of prior incubation. Following the 15 min uptake, slices were rinsed quickly in 0.9 % saline, blotted and transferred to new flasks containing 3 ml of buffer, which were gassed and returned to the shaker bath. At 3 min intervals aliquots of medium were removed and assayed for radioactivity. The flasks were regassed, sealed between sampling, and returned to the Dubnoff incubator. The total counts effluxed into the medium after 18 min and the counts in the tissue were summed to give the radioactivity in the tissue at the start of the efflux phase. The percent of the initial radioactivity in the tissue at each time interval when plotted semi-logarithmically gives a linear plot from which a rate constant can be calculated.

MATERIALS

Unlabeled amino acids were purchased from either Mann Research Laboratories or Nutrition Biochemical Company. Puromycin, cyclohexamide and ouabain were obtained from Sigma Chemical Company. [*Carboxyl*-^{14}C]Inulin, α-amino-[3-^{14}C]isobutyric acid and 1-aminocyclopentane-1-[*carboxy*-^{14}C]carboxylic acid (cycloleucine) were products of New England Nuclear Corporation.

RESULTS

Requirement for energy and metabolism

The effect of preliminary incubation on the uptake of α-aminoisobutyric acid and cycloleucine by newborn and adult kidney cortex slices is shown in Table 1. When the cortical slices from newborn rats are incubated for 60 min in buffer at 37 °C prior to measuring amino acid uptake, the cortex showed a two-fold increase in the ability to accumulate these substrates ($P < 0.001$). The adult cortical cells were unaffected by the prior incubation. The enhanced uptake of α-aminoisobutyric acid and cycloleucine by slices from newborn rats was dependent on the temperature at which the preliminary incubation was carried out. No increase in the distribution ratio was observed if the temperature of the preliminary incubation was 4 or 22 °C, thus suggesting that this effect is related to optimal metabolic function of the cells at 37 °C. Without preincubation, the 60 min uptake of both amino acids is higher in newborn tissue, an observation explained previously by slower exodus from cells of the newborn kidney [1].

Confirmation of the need for oxidative metabolic energy to produce the

TABLE 1

EFFECT OF TEMPERATURE DURING PRELIMINARY INCUBATION ON UPTAKE OF NEWBORN AND ADULT KIDNEY CORTEX SLICES

Cortical slices were incubated for 60 min in 2 ml Krebs-Ringer-bicarbonate buffer (pH 7.35) at the temperature shown. At the end of this period radioactive α-aminoisobutyric acid was added to give 0.1 μCi/ml and 0.065 mM and the flasks were incubated for another 60 min to measure the uptake of the amino acid. "None" for preincubation temperature indicates that there was no preliminary incubation, the slices being incubated with labeled α-aminoisobutyric acid for 60 min immediately after preparation to determine uptake directly. Distribution ratio is given as the mean ± S.E. with the number of determinations in parentheses.

Age of animal	Labeled amino acid	Temperature of preliminary incubation (°C)	Distribution ratio
Newborn	α-Aminoisobutyric acid	None	7.02 ± 0.24 (6)
		4	6.27 ± 0.38 (6)
		22	6.96 ± 0.19 (6)
		37	12.90 ± 0.60 (6)*
Adult	α-Aminoisobutyric acid	None	3.01 ± 0.18 (12)
		4	2.75 ± 0.11 (12)
		22	2.81 ± 0.13 (12)
		37	2.93 ± 0.16 (12)
Newborn	Cycloleucine	None	2.18 ± 0.11 (6)
		4	2.38 ± 0.16 (5)
		22	2.43 ± 0.11 (6)
		37	4.96 ± 0.54 (5)*
Adult	Cycloleucine	None	1.81 ± 0.07 (3)
		4	1.78 ± 0.00 (3)
		22	1.48 ± 0.14 (3)
		37	1.88 ± 0.22 (3)

* Different from no preliminary incubation ($p < 0.001$).

TABLE II

EFFECT OF VARIOUS INCUBATION CONDITIONS ON α-AMINOISOBUTYRIC ACID UPTAKE BY SLICES OF KIDNEY FROM NEWBORN RATS

For direct uptake determination slices were incubated for 60 min with labeled α-aminoisobutyric acid under the conditions noted. Preliminary incubation for 60 min was carried out under the same circumstances prior to rinsing and transferred to oxygenated Krebs-Ringer buffer containing only labeled α-aminoisobutyric acid for a 60 min uptake measurement. The distribution ratio is the ratio of cpm/ml intracellular fluid to cpm/ml medium. Details are described under Methods. Mean ±S.E. is shown. Numbers in parentheses are the number of determinations.

Condition	Distribution ratio	
	Direct uptake	After preliminary incubation
Control (Krebs-Ringer buffer)	7.15±0.20 (25)	12.73±0.36 (26)
Puromycin (1 mM)	6.40±0.39 (4)**	6.11±0.25 (8)*
Cycloheximide (50 μM)	6.12±0.16 (6)**	5.34±0.35 (10)*
Actinomycin D (0.8 μM)	6.29±0.64 (4)**	9.89±0.79 (4)*
Dinitrophenol (50 μM)	6.48±0.19 (7)**	8.82±0.54 (7)*
Anaerobiosis	5.12±0.28 (8)*	9.53±0.12 (3)*
Ouabain (1 mM)	3.12±0.32 (4)*	14.23±0.69 (8)**
Na^+-free buffer	1.09±0.21 (13)*	6.64±0.87 (6)*

* $p < 0.01$.
** Not significant comparing treated slices within its own column.

effect is given in Table II. Dinitrophenol, which uncouples oxidative phosphorylation, has no effect and anaerobiosis only a slight effect on direct uptake of α-aminoisobutyric acid by slices from newborn kidney. This contrasts with adult tissue, where both conditions markedly impair uptake [8], and is consistent with the generally observed ability of young tissue to withstand anoxia better than adult tissues. When the slices from newborn kidney were incubated at 37 °C in either dinitrophenol-containing buffer, or under anaerobiosis prior to transfer and measurement of α-aminoisobutyric acid uptake in Krebs-Ringer oxygenated buffer without dinitrophenol, the mechanism of enhancing uptake after preliminary incubation in Krebs-Ringer buffer (control) was significantly diminished (distribution ratio of 8.82±0.54 with dinitrophenol and 9.53±0.19 with anaerobiosis during preincubation vs. 12.73±0.36 for controls). It thus appears that the underlying mechanism for enhanced uptake of α-aminoisobutyric acid after preliminary incubation at 37 °C is much more dependent on oxidative metabolism than is the transport process itself.

Requirement for protein synthesis

Table II demonstrates that the addition of cycloheximide in the preliminary incubation buffer at a concentration that almost totally inhibits protein synthesis obliterates the effect usually observed after a 60 min pretreatment of the tissues at 37 °C. This effect of cycloheximide is shown graphically in Fig. 1. With increasing time of the preliminary incubation there is a progressively greater ability of the cells to accumulate α-aminoisobutyric acid with the distribution ratio increasing from about 7 to 18. When cycloheximide is present during the preliminary incubation no increase is observed. We have drawn a line through the data points, where cyclo-

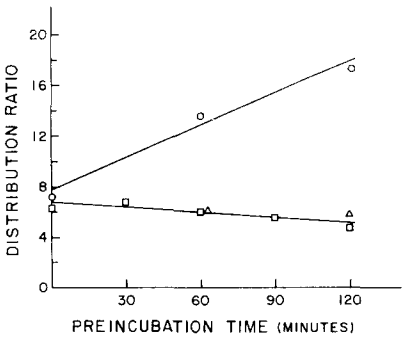

Fig. 1. The effect of cycloheximide in the preliminary incubation on the subsequent uptake of α-aminoisobutyric acid of newborn rat kidney cortex slices. The abscissa shows duration of incubation prior to the addition of labeled α-aminoisobutyric acid. Tissue was incubated in the presence (□) or absence (○) of cycloheximide (0.05 mM) for varying lengths of time prior to the addition of 0.065 mM labeled α-aminoisobutyric acid, after which all tissue were further incubated for 1 h to measure uptake. At two time points (△) slices previously incubated with cycloheximide were removed, rinsed and placed into fresh buffer with labeled α-aminoisobutyric acid and allowed to incubate for 1 h. Uptake is designated by the distribution ratio, that is the cpm/ml of intracellular fluid to cpm/ml medium. Each point represents the mean of at least triplicate determinations.

heximide was present, with a slightly decreasing slope, but this could be drawn as a flat curve. There is, indeed, little effect of cycloheximide itself on the uptake process. Elsas and Rosenberg reported that between 2 and 3 h of incubation of cortical slices with puromycin was required before a depression in amino acid uptake is observed in adult tissue [11].

Table III reveals additional data on the influence of cycloheximide on the preliminary incubation effect. In this experiment, there were two preliminary in-

TABLE III

INFLUENCE OF CYCLOHEXIMIDE ON THE ENHANCEMENT OF α-AMINOISOBUTYRIC ACID UPTAKE

Tissues were incubated for 60 min in Krebs-Ringer buffer with or without cycloheximide (50 μM) (1st preliminary incubation) after which they were removed, rinsed with 0.9 % saline, blotted and placed in new flasks in the presence or absence of cycloheximide (50 μM) for another 60 min incubation (2nd preliminary incubation). After the second hour the slices were rinsed again and transferred to Krebs-Ringer buffer containing only ^{14}C-labeled α-aminoisobutyric acid for a 60 min uptake measurement. Mean ± S.E. is indicated with the number of determinations in parentheses.

Cycloheximide in preliminary incubation		Distribution ratio
First	Second	
−	−	12.57±0.14 (10)
+	−	8.16±0.38 (9)*
−	+	7.74±0.33 (10)*
+	+	5.29±0.27 (10)*

* $p < 0.001$.

cubation phases each for 1 h, after which the slices were transferred a second time to new flasks for the 60 min uptake period. In the first set of circumstances cycloheximide was placed in the flask only during the first hour of preliminary incubation, the second hour of preincubation being in buffer alone. The distribution ratio of 8.16 ± 0.38 differed significantly ($P < 0.001$) from the control value (12.57 ± 0.64) where buffer alone was used in the first 2 h, and suggests that the effect of cycloheximide on the phenomena is exerted after it is removed. It appears to inhibit the enhancement effect that would be expected to result from the second hour of preliminary incubation at 37 °C in buffer alone. When cycloheximide was present only during the second hour of preliminary incubation the effect of the first hour of incubation was obliterated, which seems to point to the need for continued protein synthesis to maintain the enhanced uptake once initiated by preincubation [distribution ratio 7.74 ± 0.33 compared to 12.57 ± 0.64 ($P < 0.001$)). Cycloheximide in the buffer during both periods of preincubation causes a slight reduction in subsequent uptake and corresponds to the effect seen in Fig. 1.

Effect of the presence of amino acids in the preliminary incubation

Table IV shows the results of adding α-aminoisobutyric acid, proline or glycine to the buffer during the preliminary incubation. At 2 mM concentration the presence of all three amino acids caused a diminution of the expected increase in uptake due to the preliminary incubation, the most profound effect due to α-aminoisobutyric acid whose distribution ratio was the same as seen without preincubation. When the concentration of amino acids was increased to 5 mM the added proline resulted in a greater decrease in the distribution ratio of radioactive α-aminoisobutyric acid which was similar to that observed with tissue not exposed to the preliminary incubation. Glycine, however, produced essentially the same effect at both 2 and 5 mM

TABLE IV

UPTAKE OF α-AMINOISOBUTYRIC ACID BY NEWBORN KIDNEY AFTER ADDITION OF AMINO ACIDS TO THE PRELIMINARY INCUBATION

Tissue slices were subjected to preliminary incubation for 60 min in buffer with or without added amino acids. Subsequently slices were rinsed in saline, blotted and placed in buffer containing labeled α-aminoisobutyric acid for a 60 min uptake measurement. Distribution ratio is given as the mean ±S.E. with the number of determinations in parentheses.

Amino acid added	Concn. (mM)	Distribution ratio
None		12.43 ± 0.55 (12)
None (no preincubation)		6.48 ± 0.19 (12)*
α-Aminoisobutyric acid	2	7.41 ± 0.32 (4)*
	5	6.40 ± 0.92 (8)*
Glycine	2	8.50 ± 0.56 (4)*
	5	9.18 ± 0.93 (8)**
Proline	2	10.48 ± 0.78 (3)***
	5	6.08 ± 0.47 (8)*

* $p < 0.001$.
** $p < 0.01$.
*** $p < 0.02$.

with the uptake of α-aminoisobutyric acid not being lowered to the level seen without preincubation.

Requirement for Na^+ and effects of ouabain

The ability of the slices to accumulate α-aminoisobutyric acid is essentially abolished in the absence of sodium (Table II). Inhibition of the sodium pumping mechanism by ouabain produces a marked diminution of accumulation but does not abolish the concentration gradient altogether. When the preliminary incubation is carried out in Na^+-free medium for 60 min, the subsequent uptake of α-aminoisobutyric acid is significantly less than it would have been had the tissue been preincubated in Krebs-Ringer-bicarbonate buffer with 145 mM Na^+ (8.24 ± 0.97 as 12.86 ± 0.35 distribution ratio, Table II). The presence of ouabain during the preliminary incubation, however, does not affect the subsequent uptake of α-aminoisobutyric acid (Table II). The mechanism for enhanced uptake of α-aminoisobutyric acid after preliminary incubation is Na^+ dependent, since elimination of Na^+ from the uptake phase results in a complete inability to accumulate the amino acid.

Kinetics of α-aminoisobutyric acid and cycloleucine uptake

The time course of 0.065 mM α-aminoisobutyric acid and cycloleucine uptake with and without a 60 min preliminary incubation is shown in Figs. 2 and 3. In Fig. 2 the uptake of α-aminoisobutyric acid by cortical slices from adult kidney is shown and is unchanged by prior incubation of the tissues. The uptake by newborn tissue differs kinetically from the adult under these experimental conditions by not achieving a steady state and maintaining a linear accumulation of amino acid. This difference between newborn and adult tissue has been explained by a slower rate of exodus from newborn tissues [1, 4, 13]. The preliminary incubation at 37 °C results in a significantly increased rate of α-aminoisobutyric acid uptake. The data of Fig. 3 show the increased rate of cycloleucine uptake resulting from preliminary tissue incubation.

Since the kidney slice may be considered a two-compartment system comprising the intracellular fluid and medium [12], the level of accumulation of amino

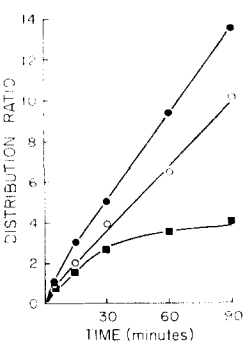

Fig. 2. The time course of accumulation of α-aminoisobutyric acid by newborn rat and adult rat renal cortical slices. The newborn rat slices were either not incubated (○) or were incubated at 37 °C for 60 min (●) prior to the addition of labeled α-aminoisobutyric acid, 0.065 mM. The incubation continued for the times indicated. The adult tissue (■) did not have prior incubation. Each point represents the mean of at least triplicate determinations.

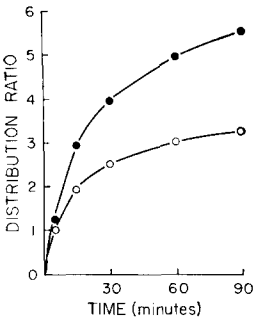

Fig. 3. The time course of accumulation of cycloleucine by newborn rat renal cortical slices. The newborn rat slices were either incubated at 37 °C for 60 min (●) or not incubated (○) prior to the addition of labeled cycloleucine, 0.065 mM. The incubation continued for the time indicated. Each point is the mean of 9 determinations.

TABLE V

EFFECT OF PRELIMINARY INCUBATION ON THE INITIAL UPTAKE OF α-AMINOISOBUTYRIC ACID

Renal cortical slices from newborn rats were subjected to preliminary incubation in buffer for varying times followed by measurement of labeled α-aminoisobutyric acid uptake measurements for the time shown. Distribution ratio is the mean ±S.E. of five determinations.

Duration of preliminary incubation	Distribution ratio	
	5 min	15 min
None	0.43±0.03	2.00±0.03
60	0.80±0.14*	3.46±0.26***
120	1.47±0.17**	4.24±0.18***

* $p < 0.05$.
** $p < 0.02$.
*** $p < 0.001$, compared to no preincubation value within its own column.

acids in the intracellular pool is dependent on both the rates of influx and efflux. The enhanced uptake we have observed could be due to increased entry or decreased exit. We therefore examined these processes. Table V shows the results of preliminary incubation on the early entry of α-aminoisobutyric acid at 5 and 15 min. Slices that were preincubated for 60 and 120 min displayed distribution ratios after 5 and 15 min uptake which were statistically higher than the controls. No difference in the exodus rate constant, K of 0.025 min^{-1}, was observed after 60 and 120 min of prior incubation. These results indicate that, indeed, the preliminary incubation at 37 °C enhances the entry of α-aminoisobutyric acid and this accounts for the increased accumulation of the amino acid.

Concentration dependence of α-aminoisobutyric acid uptake

All of the results thus far described were obtained with amino acid concentrations of 0.065 mM. When the substrate concentration was increased to 5 mM there was no enhancement of uptake of α-aminoisobutyric acid, cycloleucine, glycine or

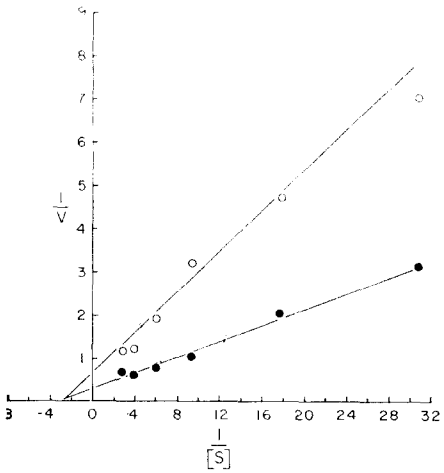

Fig. 4. Lineweaver-Burk plot of velocity of α-aminoisobutyric acid accumulation as a function of substrate concentration. The velocity, v, is mmol/l per 30 min, and [S] is substrate concentration in mM. Slices were either incubated at 37 °C for 60 min (●) or not incubated (○) prior to the addition of labeled α-aminoisobutyric acid (0.1 Ci/ml). Incubation was continued for 30 min. Points are averages of triplicate determinations. Lines are statistically different ($P < 0.001$) using the formula for t-test given by Goldstein [23].

proline. Since there are at least two transport systems which have been delineated for these amino acids, which differ in their K_m values [1, 4, 13], our data suggest that it is the low K_m system which is affected by preliminary incubation. We therefore studied the effect of 0.033–0.34 mM (low K_m system) on the velocity of uptake by pretreated newborn renal cortex. The results are shown in Fig. 4. Preliminary incubation at 37 °C did not alter the K_m of 0.31 mM but increased V from 0.33 to 1.67 mmol/l per 30 min. The lines were fitted to data by a Monroe model 1775 computer. The lines were statistically significant ($P < 0.001$).

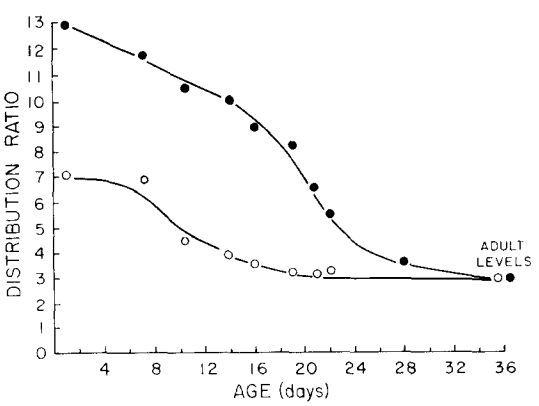

Fig. 5. The influence of animal age on the effect of prior incubation on α-aminoisobutyric acid uptake. Kidney cortical slices from animals of different ages either had no prior incubation (○) or had a 60 min incubation (●) before the addition of labeled α-aminoisobutyric acid, 0.065 mM. All tissues were incubated for 60 min after the addition of α-aminoisobutyric acid and distribution ratios calculated. Each point is the mean of at least 4 determinations.

Influence of animal age

The uptake of α-aminoisobutyric acid after a 60 min preliminary incubation was determined utilizing renal cortical slices from animals of varying age, from newborn to adults (36 days and older). These results are shown in Fig. 5. The high level of stimulation, reflected in the high level of uptake, was maintained to about 15 days of age. The ability to respond to preincubation lasted until 22 days of age. The response continued to decrease after this time so that at 36 days no effect of preliminary incubation is observed. Additional data with 44-day-old adult rats substantiate the lack of response to preliminary incubation. The distribution ratio for α-aminoisobutyric acid was 3.01 ± 0.18, mean $\pm$S.E., for untreated tissue and 2.83 ± 0.16 for those undergoing preliminary incubation (12 determinations).

DISCUSSION

Our data indicate the presence of an adaptive regulatory transport process for amino acids in immature kidney cortical cells which is initiated on incubating the tissue in a defined buffer at 37 °C. The resulting enhanced uptake of amino acids such as α-aminoisobutyric acid appears to be explained kinetically by an increase in the entry rate without change in the exit rate for the amino acid. Substrate concentration dependence experiments indicate that the increase in entry rate is not associated with a change in the K_m for transport but is characterized by an increase in the V of the saturable transport process. The latter finding is consistent with the interpretation either that there is an increase in the number of saturable binding sites or that those already present function with greater efficiency.

From both knowledge of bacterial transport of amino acids [14] and observations of genetically determined renal transport disorders [15], protein "carriers" appear to be involved in the transport process. All of our data, i.e. effects of cycloheximide, actinomycin D, Na^+, temperature and increased medium amino acid concentration, present a constellation of findings consistent with synthesis of new carrier protein. One could envisage, however, induced synthesis of other proteins involved in the rate of activation of carrier molecules or in their placement and proper orientation within the membrane. Since the rate of carrier protein turnover is important in determining the number of binding sites, another possibility is the inhibition of the degradative process being a determinant of transport capacity. Induced synthesis of an inhibitor of degradation would result in an increase in carrier protein and be consistent with our findings. Both of these alternatives really describe an activation-inactivation mechanism regulating the transport carriers. Recently, Gazzola and associates [16] have indicated that relatively high concentrations of amino acid in the incubation medium may be associated with an increase in turnover rate of carrier protein of chick embryo heart cells. The effect we see on adding amino acids may not be part of a repression-derepression mechanism but may be due to greater instability of carrier protein.

It has been clearly established that the amino acids involved are transported into cortical cells by at least two systems with differing K_m values, one with a low K_m of about 1 mM or below and another about 10-fold higher. Our experiments were carried out at 0.065 mM substrate concentration, where the main transport system utilized would be the one with low K_m. When 5 mM amino acids were studied to

assess the high K_m system no response to preliminary incubation was observed. These observations, indeed, strengthen the data showing the existence of the two systems with differing K_m for α-aminoisobutyric acid. Our data with proline and glycine participation in the response again indicate the presence of a low K_m system in the newborn for these amino acids in Sprague-Dawley rats and differ with that reported by Scriver's group [4, 13], indicating its absence in newborn kidney cortex of Long-Evans rats.

Besides the effect on the low K_m transport process, there are two other important specificities involved. The first is that the group of amino acids responding by enhanced uptake conform to the "A" transport system of Oxender and Christensen [17]. The second is that of animal age. The lack of response of adult tissue indicates that potential for response exists only in young tissues and that the aging process involves an unknown mechanism for turning off the effect of preliminary incubation.

Similar enhancement of transport of amino acids by preliminary incubation has been observed in other tissues: chick embryo heart cells [16, 18, 19], immature rat uterus [20] and human placenta [21, 22]. The best studied system has been the chick embryo heart cell. The data for all of these systems have a common pattern; response in immature tissues involving amino acids of the A mediation, dependent on protein synthesis, metabolic energy and presence of Na^+. Guidotti and colleagues, cognizant of the effect in rat uterus, postulated that the phenomenon was characteristic of immature muscle cells. The observations with human placenta and our own with kidney suggest that this is a more generalized response of immature cells.

Whether the phenomena of enhanced transport resulting from preliminary incubation has any physiological meaning remains to be determined. Perhaps the importance of this phenomenon may be in the use of immature kidneys or other tissue as a model system for the continued search for transport-related membrane proteins in mammalian cells. If there is, indeed, increased synthesis of carrier protein as the basis for our observations, it may be possible to manipulate the system so as to isolate and characterize it. This remains the goal of future experiments.

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