BBA 75760

THE ONTOGENY OF AMINO ACID TRANSPORT IN RAT KIDNEY

II. KINETICS OF UPTAKE AND EFFECT OF ANOXIA*

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Received May 17th, 1971)

SUMMARY

Low-K_m transport of L-proline and glycine and high-K_m transport of α-aminoisobutyric acid is absent in newborn Long–Evans rat kidney cortex slices. Other modes of uptake found in mature kidney are present in postnatal kidney.

The deficient proline transport appears at one week of age while the systems for glycine and α-aminoisobutyric acid become active by the 3rd week. The v_{max} of systems present at birth increases 2- or 3-fold during maturation indicating that ontogeny affects both total and specific activity of membrane transport systems in kidney. The findings *in vitro* correlate with postnatal physiological transport *in vivo*.

Steady-state accumulation of amino acids *in vitro* is greater in newborn kidney than in mature tissue; reduced efflux accounts for this. Elevation of temperature stimulates efflux less in newborn kidney.

Anoxia primarily inhibits low-K_m transport of L-proline and glycine. Transport in newborn kidney by the high-K_m system is relatively protected from anoxia.

INTRODUCTION

Uptake of L-proline and glycine[1] and of α-aminoisobutyric acid[2], in rat kidney is served by several membrane transport systems *in vitro*. Under conditions of short incubation, low-K_m transport of proline and glycine and high-K_m transport of α-aminoisobutyric acid is deficient in newborn rat kidney slices compared to mature tissue[3–5]. However, net uptake after prolonged incubation is generally greater in newborn kidney[3–6]. The manner in which this ontogeny of amino transport expresses itself in kidney, is the subject of this paper. We found that the specific activity of membrane transport systems is modulated during postnatal maturation of renal function. There is evidence that systems already present at birth can increase their activity with age while other systems not present at birth can appear at specific times during development. The relationship of influx to efflux also appears to be different in newborn and mature kidney.

* Publication No. 249 from the McGill University-Montreal, Children Hospital Research Institute.

METHODS AND MATERIALS

Long–Evans rats were used as in the previous study[6]. Techniques for incubation of kidney cortex slices and measurement of uptake have been described[6]. Efflux was examined in the present study by an adaptation of the method of SEGAL AND CRAWHALL[7]. Measurements were performed at 1, 3, 5, 10 and 20 min; the first 10-sec period of efflux was taken to determine zero-time data. Efflux was expressed as the percent of initial radioactivity lost from the slice.

Concentration-dependent uptake of L-proline, glycine and α-aminoisobutyric acid was measured over a 100-fold range of initial substrate concentration. Correction for non-saturable uptake under the near steady-state conditions of these studies, was made according to AKEDO AND CHRISTENSEN[8]. Derivations of the Michaelis equation[1,2] were again employed to determine the kinetics of mediated uptake. Steady-state measurements will provide valid information about the apparent K_m for substrate binding to the transport site[2,9].

Materials

Labelled compounds of known specific activity and radiochemical purity were obtained from the sources and examined as described in the accompanying paper[6]. Other chemicals were obtained from Fisher Laboratories or Mann Chemical Corporation, New York.

RESULTS

Early time course

Initial uptake ratios after short incubation (<15 min) with L-proline, glycine at low initial concentrations, and of α-aminoisobutyric acid at high and low concentrations, are lower in kidney cortex slices obtained from unweaned rat pups when compared with the mature animal (Fig. 1). Uptake of glycine and α-aminoisobutyric

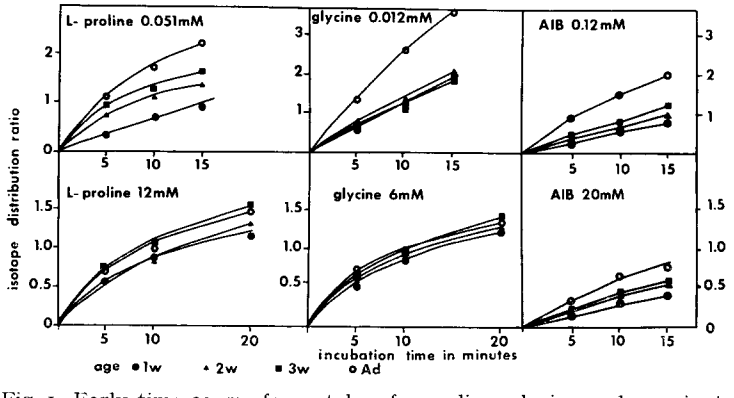

Fig. 1. Early time course for uptake of L-proline, glycine and α-aminoisobutyric acid (AIB) at low and high concentrations in the initial medium. The isotope distribution ratio is (counts/min per ml intracellular fluid per unit time): (counts/min per ml initial medium). Preliminary studies have shown[3] that differences in oxidation of substrate do not explain age-dependent differences in uptake of L-proline and glycine. Each point is the mean of at least three observations. w = week(s); Ad = adult.

acid is depressed for 3 weeks after birth while that for L-proline improves after the first week. At high concentrations of proline and glycine, there is little difference in the initial uptake between immature and adult kidney. These findings suggest that postnatal development of transport is both substrate and concentration specific.

Late time course

Newborn kidney achieves a higher isotope distribution ratio than mature kidney after prolonged incubation with L-proline and glycine at high or low concentrations and with α-aminoisobutyric acid at low concentrations (Fig. 2); mean values and standard deviation at 120 min are given in the preceding paper[6]. These findings indicate that net uptake of amino acids newborn kidney is influenced by more than the initial uptake rate[3,5,6,10,11].

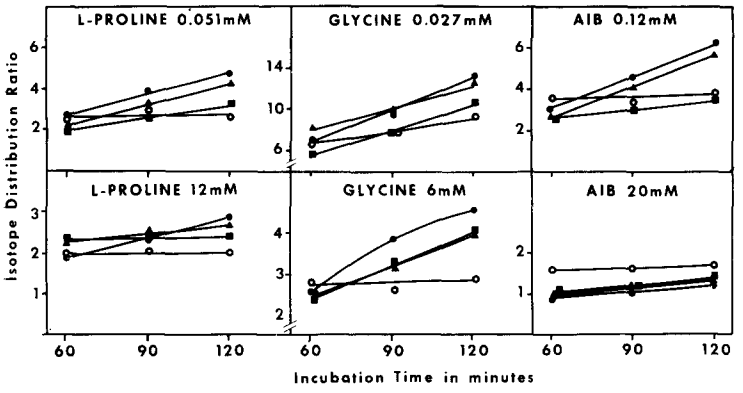

Fig. 2. Late time course for uptake of L-proline, glycine and α-aminoisobutyric acid (AIB) at low and high concentrations in the initial medium. Isotope distribution ratio as described in Fig. 1. Values are the mean of at least three observations. Symbols are as in Fig. 1.

TABLE I

ONTOGENY OF EFFLUX IN RAT KIDNEY

Data are percent of initial internal label lost to medium after 20 min incubation in efflux medium at 37°. Slices were incubated at 37° at the indicated concentration of ^{14}C-labelled substrate in the uptake medium for a period chosen so that the isotope distribution ratio after preloading was the same in slices taken from animals of different ages[3] (*e.g.* 30 min preincubation for 0.051 mM L-proline, and 60 min for 12 mM L-proline). Efflux was then performed at 37° into 5 ml of fresh medium containing no amino acid according to the technique described in METHODS. The data on efflux are the mean and S.D. of at least triplicate observations.

Amino acid	*Concn. of substrate during preincubation (mM)*	*Age of animal:*			
		1 week	*2 week*	*3 week*	*Adult*
L-Proline	0.05	50.3 ± 5.2	57.6 ± 2.6	64.6 ± 2.5	73.9 ± 2.6
	12.0	60.0 ± 2.1	71.3 ± 1.26	67.8 ± 3.6	82.4 ± 1.2
Glycine	0.03	30.1 ± 1.3	37.7 ± 2.4	38.5 ± 4.2	49.6 ± 4.2
	6.0	47.2 ± 5.6	63.0 ± 3.4	66.4 ± 1.0	74.7 ± 1.4
α-Aminoisobutyric acid	0.12	44.5 ± 0.8	58.7 ± 2.0	68.3 ± 2.8	77.7 ± 6.4
	20.0	54.3 ± 4.5	69.7 ± 4.8	74.6 ± 2.4	75.8 ± 2.9

Efflux

Efflux of L-proline, glycine and α-aminoisobutyric acid at 37° is impaired in newborn kidney, at both low and high concentrations of internal substrate (Table I); the rate of efflux increases with age after birth. Efflux is temperature-sensitive at all ages, but less so in newborn kidney. α-Aminoisobutyric acid efflux at 20 min is 234% greater at 37° than at 17° in adult kidney, but only 180% greater in the one-week kidney (mean of 6 observations at each temperature; $P < 0.05$ by Students t test); the corresponding values for L-proline efflux are 173% and 148% ($P < 0.02$).

Concentration-dependent uptake

The Michaelis Constant. Uptake of iminoacids, glycine and α-aminoisobutyric acid in mature kidney is saturable[1,2] (Fig. 3). However, L-proline uptake for example, observes a different pattern of saturation in newborn kidney (Fig. 3). A striking difference characterizes the kinetics of uptake by newborn and mature kidney. Augustinssen plots (u *vs.* $u/[S]$) reveal only one mode of transport for each amino acid in the kidney of 1-week-old animals (Fig. 4), in contrast to the more complex transport

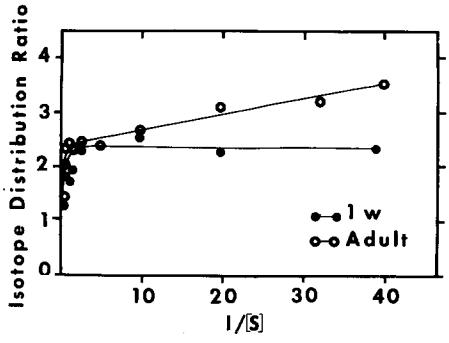

Fig. 3. Akedo–Christensen plot[8] of L-proline uptake by newborn and adult rat kidney cortex slices. Uptake undergoes saturation over the whole concentration range in adult kidney suggesting more than one type of uptake whereas uptake at low concentrations is different in the newborn

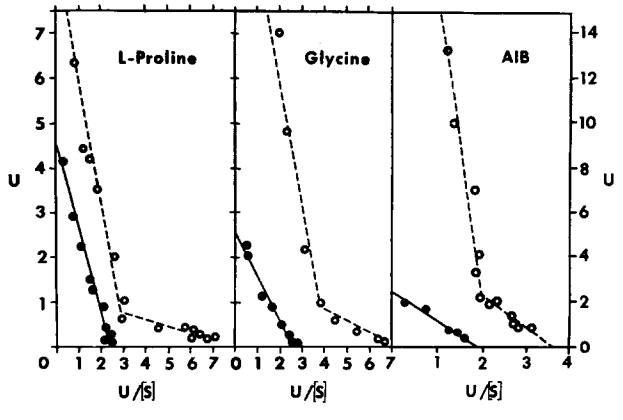

Fig. 4. Eadie–Augustinsson plots (u *vs.* $u/[S]$) for uptake of L-proline, glycine and α-aminoisobutyric acid (AIB) by renal cortex slices from 1-week-old animal (●) and adult rats (○). A low-K_m mode of uptake is missing for L-proline and glycine in the newborn; the high-K_m mediation for α-aminoisobutyric acid is also absent. The apparent K_m's are given in Fig. 5.

found in adult kidney[1,2]. The respective low-K_m components for L-proline and glycine transport and the high-K_m component for α-aminoisobutyric acid transport are each missing in kidney of 1-week-old animals. The low-K_m component of L-proline transport becomes active by the second postnatal week (Fig. 5), whereas the corresponding system for glycine transport appears only in the third postnatal week. The high-K_m mode of α-aminoisobutyric acid uptake also appears during the third postnatal week.

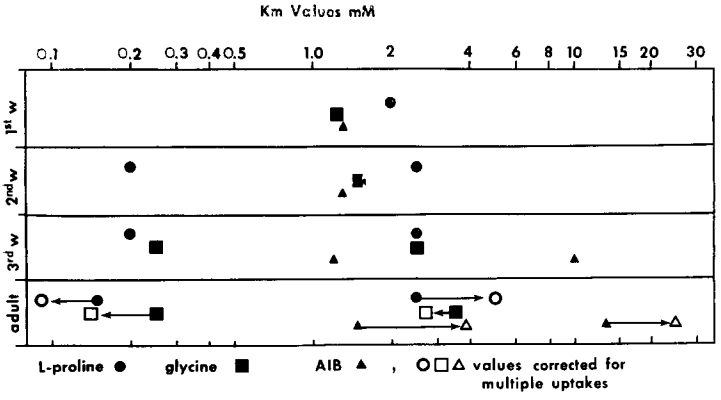

Fig. 5. Graph of apparent K_m values for uptake of L-proline, glycine and α-aminoisobutyric acid (AIB) by rat kidney cortex slices prepared from animals of different ages. A second low-K_m mode of entry for L-proline appears at 2 weeks while the low-K_m system for glycine enters in the third week. A high-K_m mode for α-aminoisobutyric acid uptake appeared in the third week. These data are given as apparent K_m values (filled symbols). Open symbols for the adult indicate corrected K_m values when simultaneous entry by multiple systems is accounted for in estimating the K_m as described previously[1,2].

Maximum uptake rates v_{max}. The v_{max} values for uptake mechanisms already present at birth (the shared high-K_m transport for L-proline and glycine, and the low-K_m transport for α-aminoisobutyric acid) increase with age (Table II). This finding

TABLE II

MAXIMAL RATES (v_{max}) FOR TRANSPORT OF L-PROLINE, GLYCINE AND α-AMINOISOBUTYRIC ACID IN NEWBORN AND MATURE KIDNEY

The average v_{max} for the saturable component was determined from the Michaelis equation substituting data from all experiments performed in this series of investigations (approx. 15 determinations were made at each concentration). Incubation times for L-proline, glycine and α-aminoisobutyric acid were, 30, 30 and 40 min respectively. The concentrations of L-proline and glycine used in these experiments favor uptake on their shared high-K_m system[1] whereas that chosen for α-aminoisobutyric acid favors uptake on its low-K_m system[2].

Substrate	*Concn. range (mM)*	*v_{max} per age of animal (μmoles/ml intracellular fluid per unit time)*		
		1 week	*Adult*	*v_{max} ratio; adult/newborn*
L-Proline	1–12	4.8	10.0	2.1
Glycine	1–10	3.1	10.5	3.3
α-Aminoisobutyric acid	0.12–8	2.5	5.2	2.2

suggests that the number of these transport sites or their capacity to transport, increases during postnatal development.

Inhibition studies

Interaction between amino acids. The respective low-K_m systems for transport of L-proline and glycine cannot be inhibited by each other's substrate in mature kidney[1]. These two amino acids inhibit each other's uptake in newborn kidney (Table III). High concentrations of L-proline and glycine will stimulate each other's uptake if entry of the substrate is assigned predominantly to its own low-K_m system[1]. No stimulation is apparent in newborn kidney under these conditions. L-Alanine inhibits α-aminoisobutyric acid transport by its high-K_m system in mature kidney[2] while L-proline stimulates α-aminoisobutyric acid entry by its low-K_m system[2]. L-Alanine is a poor inhibitor of α-aminoisobutyric acid uptake in newborn kidney and L-proline does not stimulate α-aminoisobutyric acid uptake (Table III). These findings imply that the sites at which inhibition or exchange and counterflow occur in mature kidney are different in newborn kidney.

Non-competitive inhibition. Cyanide (10^{-2} M) inhibits low-K_m steady-state uptake of L-proline more than its high-K_m transport in adult kidney[1]; proline uptake at low and high concentrations is inhibited equally (85 %) in newborn kidney. Anoxia

TABLE III

INTERACTION BETWEEN AMINO ACIDS DURING STEADY-STATE UPTAKE BY RAT KIDNEY CORTEX SLICES FROM NEWBORN (1 WEEK OLD) AND ADULT RATS

Slices were paired from the same animal. Control slices were incubated in the presence of substrate alone while experimental slices were incubated in the presence of substrate and the inhibitor. Uptake (μmoles/ml intracellular fluid per unit time at steady state) in the presence of inhibitor is expressed as percentage of control uptake. Values are mean of six paired observations. All values unless indicated otherwise are significantly different from control ($P<0.01$ by Student's t test) and all newborn data are significantly different from adult data ($P<0.01$) unless indicated otherwise. Underlined values indicate stimulation.

Substrate	*Concn. (mM)*	*% of transport occurring on designated system in adult kidney*	*Inhibitor*	*Concn. (mM)*	*Uptake (% of control isotope distribution ratio)*	
					1 week	*Adult*
Pro	0.026	71 (low-K_m)*	Glycine	0.25	69	110
			α-Aminoisobutyric acid	0.25	68	96***
Pro	0.2	50 (high-K_m)*	α-Aminoisobutyric acid	100	4	18
Pro	8.0	92 (high-K_m)*	α-Aminoisobutyric acid	80	65§	70
Gly	0.026	43 (low-K_m)*	L-Proline	0.25	73	170
Gly	0.012	48 (low-K_m)*	L-Proline	100	12	40
α-Aminoisobutyric acid	0.8	58 (high-K_m)**	L-Proline	8.0	62	145
			L-Alanine	8.0	65§	76
α-Aminoisobutyric acid	8.0	72 (high-K_m)**	L-Alanine	80	65	38

* Data derived from ref. 1.
** Data derived from ref. 2.
*** No significant difference from control.
§ No significant difference between newborn and adult.

inhibits net uptake of glycine or L-proline at low or high concentrations only modestly but equally in newborn kidney (Fig. 6). Maturation of low-K_m transport of proline renders uptake at low concentrations sensitive to anoxia. Glycine entry at low concentrations remains comparatively resistant to anoxia throughout the newborn period (Fig. 6) but sensitivity to anoxia occurs when the low-K_m system for glycine transport eventually appears. These findings indicate that concentrative uptake of proline and glycine in newborn kidney is partially protected from anoxia by membrane transport which is coupled to anaerobic metabolism.

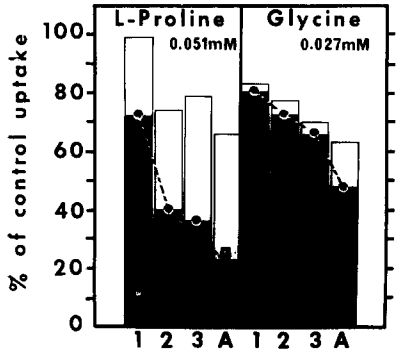

Fig. 6. Effect of anoxia (circles and bar graphs) and cyanide (squares) upon uptake of L-proline and glycine at low concentrations by kidney cortex slices from animals of different ages. Solid area of bar indicates effect of anoxia on isotope distribution ratio; open area above indicates total soluble isotope uptake ratio obtained when counts in $^{14}CO_2$ are included in calculating uptake. Paired slices from same animal were incubated either in oxygenated buffer or in buffer gassed with N_2. In experimental flasks, the incubation was also carried out under N_2. Incubations were 30 min with L-proline and 60 min with glycine. Data are presented for slices exposed to inhibitor expressed as percent of control uptake. Results are mean of at least triplicate observations. A = adult. Abscissa, age in weeks.

DISCUSSION

Reabsorption of amino acids from urine occurs predominantly in the straight portion of the proximal tubule *in vivo*[12,13]. The reabsorptive process for the iminoacids and glycine is saturable[14,15] and there are many similarities between *in vivo* and *in vitro* transport of these substances by kidney[1,15,17]. The failure of mammalian kidney to reabsorb iminoacids and glycine efficiently after birth[18,19] might be attributed to the small mass of the proximal tubule relative to the glomerulus in the newborn[20–22]. However, a simple change in glomerular–tubular balance during postnatal development does not explain how proline and glycine reabsorption change independently after birth[3,6].

The behaviour of proline and glycine reabsorption *in vivo* during postnatal maturation is explicable when the kinetics of their concentration-dependent uptakes *in vitro* are considered. Initial rates of uptake for proline, glycine and α-aminoisobutyric acid *in vitro* are depressed in kidney of the newborn Long–Evans rat. The low-K_m uptake for L-proline and glycine are deficient at birth. However, their appearance *in vitro* in the 1st and 3rd weeks, respectively, after birth, corresponds precisely with the timing of improved reabsorption *in vivo* of the two substances. This finding suggests independent control of the two low-K_m transport systems in kidney.

The segregation of low-K_m and high-K_m transport for the iminoacids and glycine and also for α-aminoisobutyric acid in the newborn suggests that these two types of uptake[1,2] are also controlled independently. Ontogeny thus supports the genetic evidence[17] for discrete high-K_m and low-K_m systems transport mechanisms for iminoacids and glycine in kidney. If membrane transport proteins[23] account for solute binding during transport in renal tubular membranes[24] it should be possible eventually to isolate the appropriate targets of mutation and ontogeny relevant to membrane transport in kidney.

Absence of transport activity in postnatal kidney might reflect either the absence of particular membrane transport proteins or non-functioning mechanisms which bind substrate but cannot transport it. The failure to obtain stimulation of uptake in newborn kidney, under conditions where simple exchange with an internal solute stimulates uptake from the extracellular fluid in mature tissue[1,25], suggests that the carrier necessary for exchange is absent. Studies of exchange or interaction between solutes and the carrier, are helpful to discriminate between loss of carrier and lack of coupling of intact carrier with energy metabolism for active transport of amino acids[26].

Newborn kidney can achieve steady-state uptake ratios *in vitro* for proline and glycine and α-aminoisobutyric acid which are higher than in mature kidney. Impaired efflux in the newborn appears to explain this phenomenon, as shown here and by others[7,10,11]. Because ontogeny affects initial uptake (influx) and efflux independently, one is tempted to consider that they may be separate and identifiable processes.

The foregoing indicates changing specific activity of transport sites in kidney during postnatal maturation but there is also increased "total" membrane activity with maturation. This probably reflects enlargement of tubular mass[20–22] and development of a mature brush border configuration[20]. The greater membrane area at maturity is likely to contain a larger number of membrane binding sites to serve transport.

The shared high-K_m transport for proline and glycine in newborn kidney is apparently coupled to glycolysis and can maintain concentrative uptake under anaerobic conditions. When the low-K_m systems for uptake of proline and glycine become active after birth, anoxia can then inhibit transport. This finding indicates an adaptive advantage in the presence of anoxia held by immature kidney over mature tissue.

Some of our data are at variance with those of others. SEGAL's group[11] was unable to find deficient low-K_m transport of L-proline and glycine in newborn Sprague–Dawley rats; these authors did not present any corresponding data on tubular reabsorption *in vivo*. It is not known whether there is a species difference in the renal transport ontogeny of Sprague–Dawley and Long–Evans rats, or whether methodological differences account for this discrepancy.

ACKNOWLEDGEMENTS

The assistance of Mrs. Audrey Shannon in providing rat pups of known age was invaluable in this study. We are also grateful to Dr. Stanton Segal for helpful discussions. This work was supported by grant MT-1085 from the Medical Research Council of Canada. Dr. Baerlocher was a Medical Research Council Research Fellow. He is

presently at the Kinderspitel Zurich, Switzerland. Dr. Scriver is an Associate of the Medical Research Council.

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