DELINEATION OF SEPARATE TRANSPORT SYSTEMS IN RAT KIDNEY CORTEX FOR L-LYSINE AND L-CYSTINE BY DEVELOPMENTAL PATTERNS

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L-Cystine uptake by newborn rat kidney cortex in vitro is impaired and does not reach the normal adult level until fifteen days of age. L-lysine uptake by young cortex, on the other hand, is comparable to that observed in adults. Exchange diffusion of lysine in the newborn's cortex is also similar to adult tissue. Cystine uptake by newborn cortex is impaired further with sodium deprivation while that of lysine is markedly increased in the absence of sodium. These developmental differences argue against the concept of a common transport process for these two amino acids.

The concomitant findings of normal plasma levels and increased urinary excretion of the amino acids, L-cystine, L-lysine, L-arginine and L-ornithine in patients with human cystinuria has led to the postulate that these amino acids share a common transport mechanism for the reabsorptive process in the renal tubule cell (1) which is deficient in the disease. Several recent observations have, however, cast some doubt that cystine participates in the same transport process with the dibasic amino acids. In vitro determinations of the uptake of these amino acids by kidney cortex from cystinuric patients have revealed a clear-cut defect in the accumulation of lysine and arginine but no impairment of cystine uptake (2). Moreover, in neither human (2) nor rat kidney cortex (3) in vitro has a mutual inhibition of cystine transport by lysine nor lysine transport by cystine been seen, expected interactions for a common transport system. Different responses of cystine and lysine transport in rat kidney to such parameters as anaerobiosis, pH alteration and sodium deprivation have tended to reinforce the conclusion that there is a mechanism for cystine transport which differs from that for lysine (4). Seeking a parameter

associated with transport phenomena that would more clearly distinguish between the cystine and lysine uptake mechanism, we have examined in vitro the transport capabilities of kidney cortex from rats of various ages for these amino acids. This report demonstrates differences in the developmental patterns of cystine and lysine uptake which indicate the separate nature of the underlysing processes.

METHODS

The technique for the assessment of intracellular accumulation of radioactive amino acids by kidney cortex slices of both adult and newborn rats in Krebs-Ringer bicarbonate buffer (KRB), pH 7.35 has been reported (5,6,7). Conditions for the determination of efflux rates (6) and for the demonstration of exchange diffusion have also been published (8). Previous experiments have indicated the validity of the comparison of transport in small segments of renal cortex weighing 1 mg with larger slices or segments of slices (9). Radioactive L-cystine-S^{35} (Schwartz Bioresearch) was made up as a 2 mM solution in dilute NaOH weekly to protect from contamination with endogenously formed cysteic acid. Lysine-C^{14} was purchased from New England Nuclear. These compounds were found to be pure by high voltage electrophoresis and paper chromatography.

RESULTS

The uptake of cystine-S^{35} and lysine-C^{14} by kidney slices from various age rats is shown in Figure 1. The uptake of cystine varied considerably with age of the animal, the ability to accumulate S^{35} in intracellular fluid (ICF) of newborn and 5 day old cortical cells being markedly impaired. Ten day old tissues showed better ability to take up cystine and 15 day old functioned like the adult. Cystine uptake by newborn slices was not enhanced in 5 mM glucose or succinate. Efflux of S^{35} from newborn and adult tissue was the same, thus indicating that the poor uptake by newborn cortical tissue was due to impaired influx of the amino acid. On the other hand, the pattern of lysine uptake by kidney cortex of various aged rats is entirely

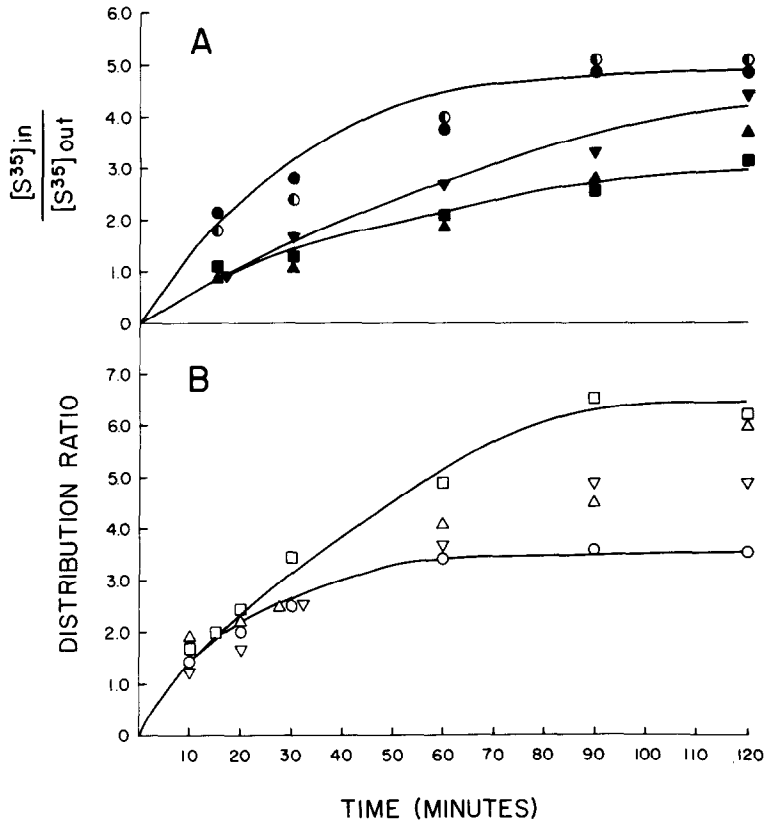

Figure 1. The uptake of cystine-S^{35} (A) and Lysine-C^{14} (B) by rat kidney cortex slices. Incubations were performed in plastic flasks with 2 ml of Krebs-Ringer bicarbonate buffer, pH 7.35 containing 0.07 μM/ml of the amino acid and 0.25 μC/ml of label at 37° in a Dubnoff shaker. Free-hand slices of cortex from young animals were pooled and three slices added per flask to give a total weight ranging from 3 to 7 mg wet weight. Three small segments of adult slices made with a Stadie-Riggs microtome weighing 10-15 mg were employed in adult incubations. Solid and open circles represent tissues from adult animals; squares, newborn; triangles, 5 day old; inverted triangles, 10 day old; half filled circles, 15 day old. Each point is an average of from 6 to 20 determinations. The uptake of lysine is designated by the distribution ratio, the ratio of cpm/ml ICF to cpm/ml of media, a true concentration gradient. Uptake of cystine is denoted by $[S^{35}]$ in/$[S^{35}]$ out, the ratio of S^{35} in ICF to that in the media. This is not a concentration gradient since the intracellular S^{35} in the ICF is predominantly cysteine and glutathione (6,7).

different. Lysine uptake by the newborn kidney is not deficient either in initial rate of uptake or the steady state reached[1]. Indeed, the latter is higher in the newborn and decreases as the tissue matures. Efflux experiments reveal that the high steady state lysine concentration in the newborn is due to slower efflux, the efflux rate constant being 35% lower than that of the adult.

Also, present in the newborn is the ability to form a concentration gradient of 1.7 with 30 mM lysine, evidence of the existence of a second lysine transport process with a high Km found in adult tissue (4).

Exchange diffusion (8) of lysine in the newborn kidney is similar to that in the adult tissue since preloading the tissues with unlabeled lysine accelerates the influx of radioactive lysine to the same extent in both. Arginine and ornithine demonstrated heteroexchange with lysine in newborn tissue but cystine, as in adult tissue (8), did not participate in this exchange system. The presence of exchange diffusion or counterflow may be construed to be evidence of a functioning "carrier" mechanism (10).

The uptake of lysine and cystine by newborn slices incubated in sodium free media serves to further emphasize differences in the transport systems. The low cystine uptake by newborn slices is further slowed without sodium but lysine uptake shows a paradoxically marked increase (Figure 2), especially in the initial rate, when sodium is omitted. Cystine uptake by adult slices has been shown previously to be markedly impaired while lysine uptake is only slightly impeded in sodium deficient media (4,6).

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[1] Our curve of lysine uptake by newborn kidney cortex with time differs somewhat from that published by Webber, W. and Cairns, J. A. Can. J. Physiol. Pharmacol. 46, 165 (1968) who found a decrease in the early uptake of lysine compared to adults but higher than adult lysine levels after thirty minutes of incubation. This may be due to differences in technique especially our use of higher substrate concentrations. Our 10 and 20 minute newborn data consist of 11 determinations obtained in four different experiments. No single determination was lower than data obtained at comparable times with adult tissue.

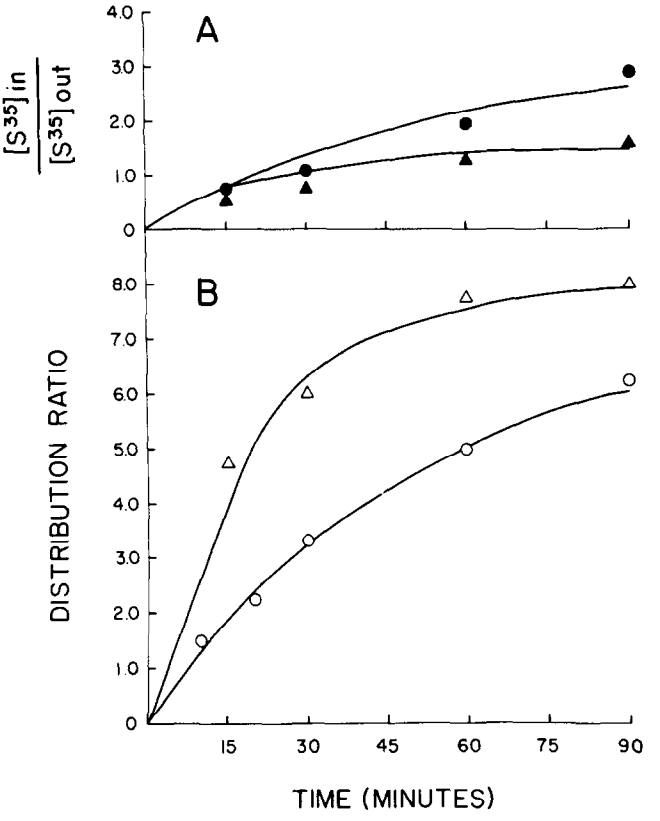

Figure 2. Effect of sodium deprivation on the uptake of Cystine-S^{35}(A) and Lysine-C^{14}(B) by newborn cortex slices. Sodium was replaced by Tris (17). Circles designate KRB buffer and triangles, sodium free buffer.

DISCUSSION

It is difficult to envision a single transport process for cystine and lysine which at the same time is markedly impaired for the one and is normal or above normal for the other in the newborn period. The slow uptake of cystine by newborn cortex does not appear to be related to intracellular reductive fates of this amino acid for the patterns of intracellular metabolites of cystine are essentially the same in newborn and adult cortex (7). The present data indicate that the cystine transport mechanism undergoes a maturation with age while that for lysine functions like the adult at birth. The observed differences may be related to maturation of microvillae of the

kidney tubule cell (11). The diverse effect of sodium deprivation on cystine and lysine uptake by newborn cortex also indicates a difference in the two processes. The reason for enhanced uptake of lysine in Na free media in young tissue is at present unknown.

The results of in vitro experiments appear to be against the concept that cystine shares a common transport system with dibasic amino acids in the kidney. Recent clinical evidence of hyperexcretion of cystine without lysinuria (12) and dibasic aminoaciduria without hyperexcretion of cystine (13) supports the dual nature of the transport process in the kidney. There is sufficiently conclusive evidence, however, that cystine does share the dibasic transport process in the intestine (14), thus indicating the different transport characteristics of kidney and intestine (15).

The basis of hyperexcretion of cystine by human cystinurics is still enigmatic. There is some evidence that lysine interacts with the reduced sulfur amino acid, cysteine, by sharing a common cellular efflux mechanism. An hypothesis to explain hyperexcretion of cystine in cystinuria on this basis has recently been made (16).

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