Biochimica et Biophysica Acta 862 (1986) 127–133
Elsevier

BBA 73317

Characteristics of lysine uptake by isolated renal cortical tubule fragments from mature and immature dogs

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(Received 23 June 1986)

Key words: Amino acid transport; Renal development; Proximal tubule; Lysine transport; Cystinuria; (Dog kidney)

The uptake of L-lysine was examined in isolated renal cortical tubule fragments from adult and 1-week-old dogs. Lysine uptake by adult tubules was initially more rapid than that by the immature tubules. This uptake by mature tubules reached a steady state after 30 min of incubation, while the newborn tubules still had not reached a steady state by 90 min of incubation. Because a steady state of lysine uptake was not attained with the immature tubules, their uptake of lysine exceeded that of the adult after 60 min of incubation. Kinetic studies revealed that lysine was taken up by one saturable transport system with a K_m of 0.56 mM and V_{max} of 6.18 mmol/liter intercellular fluid per 5 min in the adult and one saturable transport system in the 1-week-old with a K_m of 0.38 mM and V_{max} of 3.66 mmol/l intracellular fluid per 5 min. Lysine also entered the renal tubule cells in both age groups via a diffusional pathway with a k_d of 0.35 min^{-1} in the adult and 0.30 min^{-1} in the newborn. Cystine competitively inhibited lysine uptake by adult dog tubules with a K_i of 0.61 mM. The other dibasic amino acids, ornithine and arginine, also inhibited lysine uptake in both the adult and the newborn.

Introduction

The interest in the human transport disorder, cystinuria, has focused attention on the nature of cystine and dibasic amino-acid transport. Several lines of evidence have indicated that a common transport system exists in kidney for the reabsorption of cystine and the dibasic amino acids, lysine, ornithine and arginine [1–12]. Patients afflicted with cystinuria typically demonstrate reabsorption defects only for these four amino acids [1,2,4]. Infusion of lysine into normal humans and animals leads to a reversible increase in the excretion of cystine, arginine and ornithine [2,4]. Cystine uptake in vitro by isolated renal cortical tubules from both rat [8] and dog [11] is inhibitible by dibasic amino acids. The locus of this interaction appears to reside on the brush-border membrane [6,7,9,10].

Dogs also inherit a disease which resembles human cystinuria [12–15]. Such animals frequently show a large defect in cystine reabsorption, although a defect in lysine reabsorption is not consistently observed. An alteration in lysine reabsorption can be uncovered with lysine loading [15].

Newborn animals, including man, dog and rat, have a decreased cystine reabsorption compared to mature animals [11,16,17]. In previous reports

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from this laboratory, we have examined the nature of cystine transport into renal tubule cells from mature and immature rats and dogs in order to identify factors underlying this maturational phenomenon [11,.17]. Slow exit of cystine from newborn renal tubule cells appears to be the explanation in the rat [17], whereas slow entry appears to be the more important mechanism in the dog [11].

In contrast, lysine reabsorption by the newborn dog is equal to that of the adult [18], in spite of the evidence for a common transport system with cystine. Because of this difference in reabsorption in vivo between these two amino acids, we have examined the nature of lysine transport into isolated cortical renal tubule fragments from the newborn and adult dog. The results of these studies form the basis of this report.

Methods

Isolated renal cortical tubule fragments were prepared from 5–7-day-old mongrel pups of either sex and dogs greater than 1 year old by a modification [19] of the method of Burg and Orloff [20]. Uptake studies of L-[^{14}C]lysine were performed to Krebs-Ringer bicarbonate buffer (pH 7.4), with 5% fetal calf serum and 10 mM sodium acetate (buffer 1) in Burg-Orloff flasks with continuous bubbling of a 95%O_2/5%CO_2 gas mixture as previously described [19]. Uptake was measured in terms of a distribution ratio of intracellular to extracellular radioactivity concentration as calculated by previous methods [8]. Intracellular volume was determined as the difference between the total tissue fluid, the wet weight minus the weight after overnight desiccation, and the fluid trapped between the tubules [19]. The trapped fluid in isolated dog renal tubules was measured with [^{14}C]poly(ethylene glycol) as reported in Ref. 19.

In concentration dependence studies tubules were incubated for 5 min with 0.1 μCi/ml L-[^{14}C]lysine plus unlabeled lysine to give the desired final concentration over the range 0.01–20 mM. Distributions were calculated and the observed transport kinetic parameters were determined from a Hofstee plot of the transport data. The kinetic parameters were also calculated from Eqn. 1 to give the best fit to the observed total uptake using a direct grid search method described by Becsey et al. [21] and Hsu et al. [22].

$$V = \frac{V_{max1}(S)}{K_{m1} + (S)} + \frac{V_{max2}(S)}{K_{m2} + (S)} + k_d(S) \quad (1)$$

This method will determine all five of the above parameters (V_{max1}, V_{max2}, K_{m1}, K_{m2}, k_d) simultaneously from the V versus S data pairs. It operates by choosing values of the non-linear parameter (K_{m1} and K_{m2}) and then solving the resultant equation for the best values of the linear term (V_{max1}, V_{max2} and k_d) using standard least-squares equations. K_{m1} and K_{m2} are then iterated until the best fit is obtained. The best fit is defined as the minimum squared error after weighting the individual datum point as in Eqn. 2:

$$W = \frac{1}{V(S)} \quad (2)$$

This weighting factor, W, reflects the expected statistical uncertainty of each datum point, given the nature of the experiment.

All studies of statistical significance were made using Student's t-test [23].

Results

Lysine uptake with time

Adult renal tubule fragments actively took up 0.05 mM L-lysine, reaching steady state after 30 min of incubation (Fig. 1). After 5 min of incubation, lysine uptake by the tubules from the 1-week-old puppies was significantly less than that of the adult (9.73 ± 0.23 vs. 11.81 ± 0.37, $P < 0.01$). Similar data are shown in Table I, where the adult uptake is over 50% higher. After 15 min of incubation there was no difference until 60 min of incubation, when the uptake by tubules from the 1-week-old exceeded that by the adult (24.54 ± 0.66 vs. 22.55 ± 0.32, $P < 0.05$). A steady state was not reached by 90 min of incubation with the tubules from the 1-week-old puppies, a phenomenon observed previously with immature tissue. These radioactivity distribution ratios represent concentration gradients, since lysine has been shown not to be metabolized in renal tissue from the dog and rat over this time interval [24–26].

The effect of 1 mM cystine on the uptake of 0.05 mM lysine is also shown in Fig. 1. This

TABLE I

EFFECT OF OTHER DIBASIC AMINO ACIDS ON LYSINE UPTAKE

Isolated renal cortical tubules were prepared from adult and newborn dogs as described in Methods. These tubules were incubated for 5 min in buffer 1 with 0.05 mM L-[^{14}C]lysine plus a 1 mM concentration of the competitor. The distribution ratio (DR) represents the mean ± S.E. of at least four determinations, except for the effect of arginine on lysine uptake by adult tubules, where it represents two determinations.

Competitor	Newborn DR	Adult DR
Control	8.81 ± 0.44	14.04 ± 0.15 [c]
Ornithine	3.31 ± 0.14 [a]	5.26 ± 0.43 [b]
Arginine	2.44 ± 0.04 [a]	3.66

[a] $P < 0.01$ when compared to the newborn control.
[b] $P < 0.01$ when compared to the adult control.
[c] $P < 0.01$ comparing the newborn control with the adult control.

concentration of cystine constitutes a supersaturated solution but was used in order to maximize the effect on lysine uptake. Cystine significantly inhibited lysine uptake by tubules from both age groups, consistent with the hypothesis of a common transport system in the renal tubule. In similar experiments, lysine has been shown to inhibit cystine uptake by isolated dog renal tubules [11].

Concentration dependence of uptake

The concentration dependence of uptake was studied using tubules from both adult and newborn dogs over the concentration range of 0.01 to 20 mM. An Eadie-Hofstee transformation of the transport data from both age groups gave a curvilinear plot, indicating multiple pathways for entry into the renal tubule cell (Fig. 2). There was little change the in ratio of velocity over substrate concentration (V/S) with increasing concentrations of lysine over 2.5 mM, indicating a linear relationship between V and S. This suggested that there

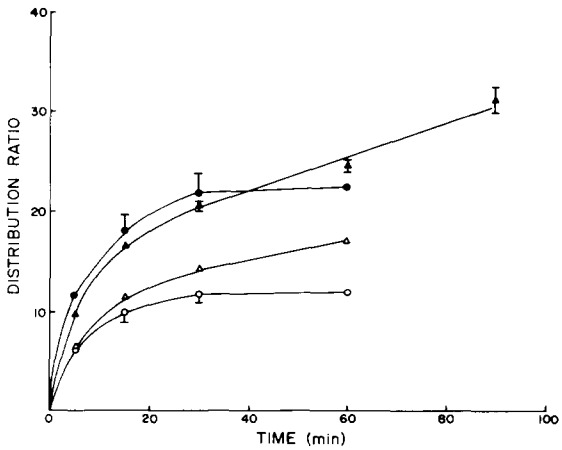

Fig. 1. Uptake of 0.05 mM L-[^{14}C]lysine with time. Isolated renal cortical tubules were prepared from adult (●) and 1-week-old (▲) dogs and incubated in Krebs-Ringer bicarbonate buffer with 5% fetal calf serum under a 95%O_2/5%CO_2 atmosphere. The effect of 1 mM L-cystine on the uptake of 0.05 mM L-[^{14}C]lysine with time by tubule from adult (○) and 1-week-old dogs (△). Each point represents the mean ± S.E. for four determinations in the adult and seven determinations in the 1-week-old. Standard errors not shown are within the size of the point.

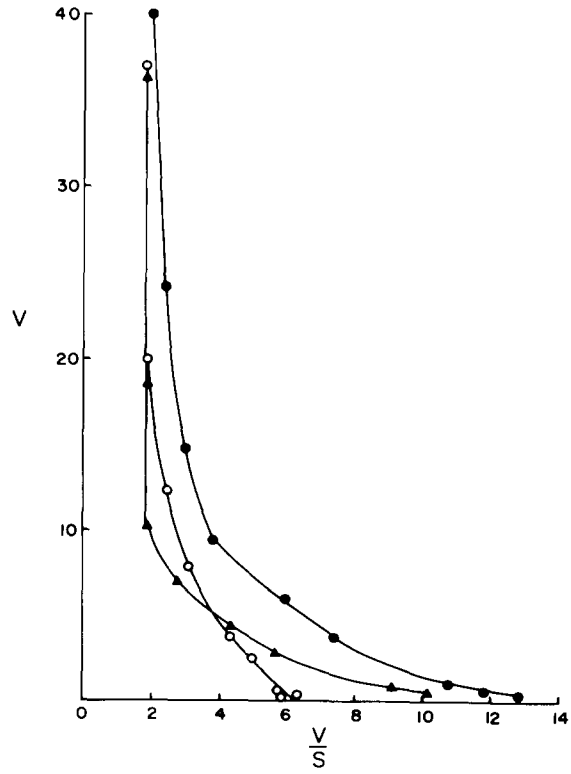

Fig. 2. Eadie-Hofstee plot of concentration dependence of L-lysine uptake by isolated renal cortical tubules from adult (●) and 1-week-old (▲) dogs. Tubules were incubated for 5 min with 0.1 μCi/ml L-[^{14}C]lysine plus sufficient unlabeled lysine to give the desired concentrations over the range of 0.1 to 20 mM. The effect of 1 mM cystine on the uptake of lysine over the same concentration ranges in adult dog tubules is also shown (○). V represents the velocity of uptake in mmol/liter intracellular fluid per 5 min and S represents the substrate concentration in mM.

was either a diffusional pathway or a carrier mediated system with a very high K_m. The calculated best fit of the adult dog transport data using Eqn. 1 was to one saturable system with a K_m of 0.56 mM and V_{max} of 6.18 mmol/l intracellular fluid per 5 min and a non-saturable system with a k_d of 0.35 min^{-1}. Lysine uptake by renal brush-border membranes vesicles from the adult rat also appeared to enter via one saturable and one non-saturable system [27]. A similar analysis of the newborn transport data was best described by one saturable system with a K_m of 0.38 mM and a V_{max} of 3.66 mmol/l intracellular fluid per 5 min and a non-saturable system with a k_d of 0.30 min^{-1}.

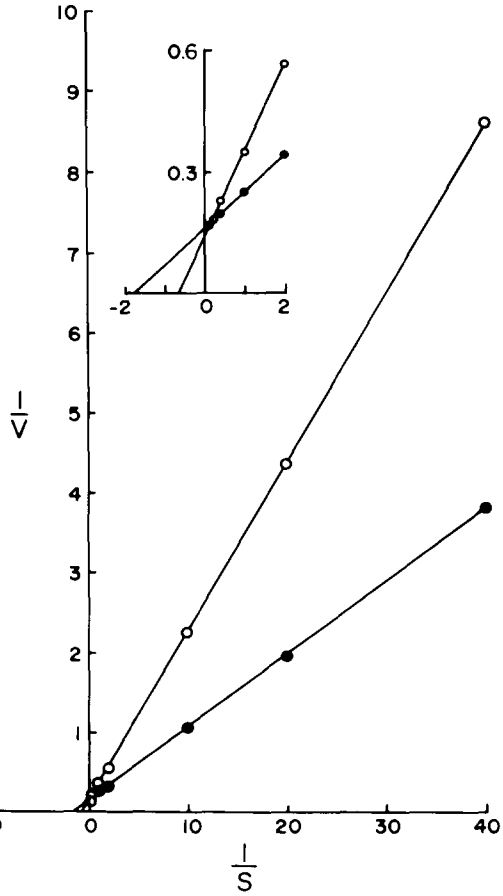

Fig. 3. Lineweaver-Burk plot of the effect of 1 mM cystine on the concentration dependent uptake of L-lysine. Tubules from adult dogs were incubated in the presence (○) and absence (●) of 1 mM cystine with 0.1 μCi/ml L-[^{14}C]lysine plus sufficient unlabeled lysine to give the desired concentrations over the range of 0.1 to 20 mM for 5 min. A diffusion component was subtracted from each uptake velocity using a k_d of 0.35 per min and the resulting value was then graphed as a Lineweaver-Burk plot. S is the concentration in mM and V is the velocity of uptake in mmol/liter intracellular fluid per 5 min.

In Fig. 2, the effect of 1 mM cystine on the concentration dependence of lysine uptake by adult dog tubules is shown. When 1 mM cystine was present in the media, the Eadie-Hofstee plot of the concentration dependence of lysine became more linear, with a marked attenuation of the lower limb of the plot observed when cystine was not in the media. In Fig. 3, these data are graphed as a Lineweaver-Burk plot after subtracting the diffusional component based on the k_d previously calculated. This led to a linear plot of both the inhibited and uninhibited lysine uptake data. As can be seen with this plot, cystine had no effect on the V_{max} for lysine uptake, but did change the K_m to 1.48 mM, indicating competitive inhibition. The K_i of cystine inhibition on lysine uptake was 0.61 mM.

Competition with other dibasic amino acids

The effect of the other dibasic amino acids on 0.05 mM lysine uptake is shown in Table I. Arginine and ornithine significantly inhibited lysine transport in isolated renal tubules from both the adult and newborn dog. This is supportive of the concept that dibasic amino acids are transported by a shared carrier.

Discussion

We have examined lysine transport with isolated renal cortical tubules prepared from the mature and immature dog using a modification [19] the method of Burg and Orloff [20]. This procedure yields a mixture of nephron segments, although, the predominant segment is from the proximal tubule. Previous studies have noted the close correlation between findings in isolated renal cortical tubules and brush-border membranes [6,8,9,28,29]. Newer methods have been devised for isolating purer preparations of proximal tubules based on differences in buoyant density between the various nephron segments [30,31]. However, no difference was noted in uptake between tubules isolated by the Burg-Orloff technique and these other methods [31]. In addition, these methods have not been attempted in immature animals,

TABLE II

AFFINITY CONSTANTS FOR RENAL DIBASIC AMINO ACID UPTAKE

Technique	Animal species	Number of systems	K_m (mM)
Lysine			
Cortical tubules	dog	1	0.6
Cortical slices [34]	rat	2	2.4, 50
Cortical slices [33]	man	2	0.2–0.4, 2–5
Brush-border vesicles [27]	rat	1	0.04
Brush-border vesicles [32]	rat	1	0.3–0.7
Arginine			
Microperfusion [35]	rat	1	1.2
Brush-border vesicles [7]	rat	2	0.07–0.1, 3.5–4.0
Brush-border vesicles [38]	rat	1	0.03–0.06
Brush-border vesicles [37]	rabbit	1	0.16
Ornithine			
Microperfusion [36]	rat	1	1.8

where the differences in nephron segments are not as pronounced as in the adult. Exploration into adapting these methods to the newborn should, however, be made in the future.

Our data indicate that lysine transport in the isolated dog renal cortical tubule is by a single mediated system with a significant diffusional component (Table II). This is similar to findings in our laboratory using isolated rat renal brush-border membrane vesicles, where only a single mediated system with a K_m of 0.04 and a large diffusional pathway were noted [27]. Stieger et al. [32] also noted a single system with a K_m of 0.3–0.7 mM in rat brush-border vesicles. Earlier studies with human [33] and rat [34] renal cortical slices indicated that there were two saturable transport systems for lysine uptake. However, the K_m for the second system for lysine was 50 mM, which is markedly higher than the concentration in the plasma and luminal fluid, calling into question its physiologic relevance (Table II).

Only one mediated transport system has been observed for the other dibasic amino acids, arginine and ornithine, by most investigators. Silbernagl et al. [35] found only a single mediated system for arginine with a K_m of 1.2 mM in microperfused rat proximal tubules, and Ullrich et al. [36] only noted a single mediated system for ornithine with a K_m of 1.8 mM using similar techniques in the rat. Busse [7], using rabbit brush-border vesicles, noted two mediated systems for arginine transport with a K_m of 0.07–0.01 mM for the high-affinity system and 3.5–4.0 mM for the low-affinity system. However, Hammerman [37], using rabbit brush-border vesicles, and Jean et al. [38], using rat brush-border vesicles, both identified only a single mediated system for arginine uptake, with a K_m of 0.16 mM and 0.03–0.06 mM, respectively.

The carrier-mediated portion of lysine uptake was inhibited by other dibasic amino acids and cystine, as noted in previous studies [8,27,32]. Cystine inhibition was competitive, consistent with the concept of a shared system for cystine and dibasic amino-acid transport in the kidney. A genetic defect in this system would explain human and canine cystinuria. Unlike classical human cystinuria, canine cystinuria is not always associated with lysinuria [14], but an alteration in renal handling of lysine can be brought by loading experiments [15].

There are a number of studies which lead us to believe that the uptake of lysine and other amino acids by tubule fragments reflects brush-border transport activity, although in this preparation as in other intact renal cell systems a contribution at the antiluminal membrane cannot be excluded. The characteristics of uptake of cystine [8], proline [40] glycine [41] and taurine [42] by renal cortical tubule cells mirrors that observed with isolated brush-border vesicles for these same amino acids [9,43,44]. In addition, lysine inhibition of cystine uptake occurs at the luminal membrane in isolated brush-border vesicle experiments [9] and cystine inhibition of lysine uptake, which is demonstrated in the present study with tubules, has recently been shown to occur on the brush-border membrane [27]. There have been no studies of cystine or lysine entry with renal basolateral membrane vesicles. Schafer and Watkins [45], however, have published that there is very little flux of cystine from the basal-lateral side of isolated perfused renal proximal tubules.

The influx of lysine into renal tubule cells appears to be slower in the 1-week-old dog compared to the adult, in spite of similar fractional reabsorption rates. Of course, the filtered load is

much lower in the immature animals, since the glomerular filtration rate is reduced. We have previously noted lower influx rates for glycine [19], cystine [11] and methyl α-glucoside [39] in the immature dog compared to the mature dog. This slower influx is not due to the lack of specific transport systems which later appear with maturation. In contrast, uptake by newborn rat tubules is equal to or faster than that of mature animals [17,40,41], except for taurine [42] and methyl α-glucoside [43]. A characteristic of uptake by immature animals from both species is slower efflux, which results in higher distribution ratio at later time points [17,19,39–42,46–48]. From these data, it would appear that the physiologic aminoaciduria of immaturity in the dog is due to impaired influx and decreased efflux, while in the rat decreased efflux appears to be the major determinant. Recent data from isolated brush-border membranes from the rat, however, have indicated that there is an increase in the influx rate of proline with maturation across the luminal membrane [49]. This is in contrast to events noted with isolated renal tubules [40]. Future studies using isolated membranes may provide further insights into this complex phenomenon.

Acknowledgements

This work has been supported by Grant AM 10894 from the National Institutes of Health, Bethesda, MD, and Grant 841122 from the American Heart Association.

References

1 Dent, C.E. and Rose, G.A. (1951) Q. J. Med. 20, 205–219
2 Robson, E.B. and Rose, G.A. (1951) Clin. Sci. 16, 75–93
3 Webber, W.A., Brown, J. and Pitts, R.F. (1961) Am. J. Physiol. 200, 380–386
4 Cusworth, D.C. and Dent, C.E. (1960) Biochem. J. 74, 550–561
5 Silbernagl, S. and Deetjen, P. (1972) Pflügers Arch. 337, 227–284
6 Segal, S., McNamara, P.D. and Pepe, L.M. (1977) Science 197, 169–171
7 Busse, D. (1978) Arch. Biochem. Biophys. 191, 551–560
8 Foreman, J.W., Hwang, S.M. and Segal, S. (1980) Metabolism 29, 53–61
9 McNamara, P.D., Pepe, L.M., and Segal, S. (1981) Biochem. J. 194, 443–449
10 Hilden, S.A. and Sactor, B. (1981) Arch. Biochem. Biophys. 210, 289–297
11 Foreman, J.W., Medow, M.S., Bovee, K.C. and Segal, S. (1986) Pediatr. Res., in the press
12 Brand, E. and Cahill, C.F. (1936) J. Biol. Chem. 114, 15
13 Holtzapple, P., Bovee, K.C., Rea, C. and Segal, S. (1971) Metabolism 20, 1016–1022
14 Bovee, K.C., Thier, S.O., Rea, C. and Segal, S. (1974) Metabolism 23, 51–58
15 Bovee, K.C. and Segal, S. (1984) Metabolism 33, 602–607
16 Brodehl, J. (1976) in Amino Acid Transport and Uric Acid Transport (Silbernagl, S., Lang, F. and Greger, R., eds.), pp. 128–135, Georg Thieme, Stuttgart
17 Hwang, S.M., Foreman, J.W. and Segal, S. (1982) Biochim. Biophys. Acta 690, 145–153
18 Bovee, K.C., Jezyk, P.F. and Segal, S. (1984) Am. J. Vet. Res. 45, 830–832
19 Medow, M.S., Foreman, J.W., Bovee, K.C. and Segal, S. (1982) Biochim. Biophys. Acta 693, 85–92
20 Burg, M. and Orloff, J. (1962) Am. J. Physiol. 203, 327–330
21 Becsey, J.G., Berke, L., and Callan, J.R. (1968) J. Chem. Educ. 45, 728–730
22 Hsu, B.Y.-L., Corcoran, S.M., Marshall, S.M. and Segal, S. (1983) Biochim. Biophys. Acta 735, 40–52
23 Colton, T. (1974) Statistics in Medicine, pp. 91–151, Little Brown, Boston.
24 Rosenberg, L.E., Downing, S.J. and Segal, S. (1962) J. Biol. Chem. 237, 2265–2270
25 Holtzapple, P.G., Bovee, K.C., Rea, C. and Segal, S. (1969) Science 166, 1526–1527
26 Ausiello, D.A., Segal, S. and Thier, S.O. (1972) Am. J. Physiol. 226, 1473–1478
27 McNamara, P.D., Rea, C.T. and Segal, S. (1986) Am. J. Physiol., in the press
28 Chesney, R.W., Friedmen, A.L., Albright, P.W. and Gusowski, N. (1982) Proc. Soc. Exp. Biol. Med. 170, 493–501
29 Chesney, R.W., Gusowski, N. and Theissen, M. (1984) Pediatr. Res. 18, 611–618
30 Balaban, R.S., Soltoff, S.P., Storey, J.M. and Mandel, L.J. (1980) Am. J. Physiol. 238, F50–F59
31 Vinay, P., Gougoux, A. and Lemieux, G. (1981) Am. J. Physiol. 241, F403–F411
32 Stieger, B., Stange, G., Biber, J. and Murer, H. (1983) Pflügers Arch. 397, 106–113
33 Rosenberg, L.E., Albrecht, I. and Segal, S. (1967) Science 155, 1426–1428
34 Segal, S., Schwartzman, L., Blair, A. and Bertoli, D. (1967) Biochim. Biophys. Acta 135, 127–135
35 Silbernagl, S. and Deetjen, P. (1972) Pflügers Arch. 336, 79–86
36 Ullich, K.J., Rumrich, G. and Kloss, S. (1974) Pflügers Arch. 351, 49–60
37 Hammerman, M.R. (1982) Biochim. Biophys. Acta 685, 71–77
38 Jen, T., Ripoche, P. and Poujeol, P. (1983) Membrane Biochem. 5, 1–18
39 Foreman, J.W., Medow, M.S., Wald, H., Ginkinger, K. and Segal, S. (1984) Pediatr. Res. 12, 1127–1131

40 Hwang, S.M., Serabian, M.A., Roth, K.S. and Segal, S. (1983) Pediatr. Res. 17, 42–46
41 Roth, K.S., Hwang, S.M., London, J.W. and Segal, S. (1977) Am. J. Physiol. 233, F241–F246
42 Friedman, A.L., Jax, D.K. and Chesney, R.W. (1981) Pediatr. Res. 15, 10–13
43 McNamara, P., Ozegovic, B., Pepe, L. and Segal, S. (1976) Proc. Natl. Acad. Sci. USA 73, 4521–4525
44 Chesney, R.W., Gusowski, N. and Friedman, A.L. (1983) Kidney Int. 24, 588–594
45 Schafter, J.A. and Watkins, M.L. (1984) Pflügers Arch. 401, 143–151
46 Roth, K.S., Hwang, S.M., Yudkoff, M. and Segal, S. (1978) Pediatr. Res. 12, 1127–1131
47 Baerlocher, K.E., Scriver, C.R. and Mohyuddin, F. (1970) Proc. Natl. Acad. Sci. USA 65, 1009–1016
48 Segal, S., Rea, C. and Smith, I. (1971) Proc. Natl. Acad. Sci. USA 68, 372–376
49 Medow, M.S., Roth, K.S., Ginkinger, K. and Segal, S. (1983) Biochem. J. 214, 209–214