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ELECTROGENIC TRANSPORT OF 5-OXOPROLINE IN RABBIT RENAL BRUSH-BORDER MEMBRANE VESICLES

EFFECT OF INTRAVESICULAR POTASSIUM

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The Na^+-dependent transport of 5-oxoproline into rabbit renal brush-border vesicles was stimulated by a K^+ diffusion potential (interior-negative) induced by valinomycin. Na^+ salts of two anions of different epithelial permeabilities also affected 5-oxoproline transport. These results show that the Na^+-dependent 5-oxoproline transport in renal brush-border vesicles is an electrogenic process which results in a net transfer of positive charge. Maximum transport of 5-oxoproline occurred at an extravesicular pH of 6.0 to 8.0 and over that pH range, 5-oxoproline exists completely as an anion with a negative charge. The simplest stoichiometry consistent with this process is, therefore, the cotransport of one 5-oxoproline anion with two sodium ions. The presence of K^+ inside the vesicles stimulated the Na^+-dependent transport of 5-oxoproline. This stimulatory effect was specific for K^+ and required the presence of Na^+. The presence of Na^+ gradient was not mandatory for the K^+ action. The stimulation by the intravesicular K^+ was seen in the presence as well as in the absence of a K^+ gradient. Therefore, the increased influx of 5-oxoproline was not coupled to the simultaneous efflux of K^+. The presence of K^+ in the extravesicular medium alone did not affect the Na^+-dependent transport of 5-oxoproline, showing that the site of K^+ action was intravesicular. Glutamate did not interact with the Na^+-dependent 5-oxoproline transport even in the presence of an outward K^+ gradient.

Introduction

5-Oxoproline is an intermediate in the γ-glutamyl cycle, involved in the synthesis and degradation of glutathione [1]. Chemically, it is a cyclic lactam of glutamic acid. It is present in mammalian tissues and body fluids, and mammalian kidney possesses an effective mechanism to reabsorb this compound from the filtrate. We have recently demonstrated that the transport of 5-oxoproline in rabbit renal brush-border membrane vesicles is Na^+-dependent and occurs against a concentration gradient [2]. Many neutral amino acids cause significant inhibition of its transport while the acidic amino acids, glutamate and aspartate, have no effect. Earlier studies from many laboratories have shown that neutral amino acids, which exist as zwitterions at physiological pH, are transported in renal brush-border membrane vesicles by a Na^+-dependent mechanism that is electrogenic [3–7]. On the other hand, acidic amino acids which exist as anions with a net negative

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Abbreviations: Tris, tris(hydroxymethyl)aminomethane; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

charge at pH 7.4 are also transported by a Na^+-dependent mechanism, but the process is electroneutral [8]. 5-Oxoproline, like glutamate and aspartate, exists as an anion at physiological pH. However, these acidic amino acids do not compete with 5-oxoproline for renal transport, whereas neutral amino acids do. This suggests that the mechanism of 5-oxoproline transport may be similar to the mechanism involved in the transport of neutral amino acids.

Recently, it has been reported that, in addition to an inward Na^+ gradient, an outward K^+ gradient can stimulate the transport of glutamate in rabbit [9] and rat [10] renal brush-border membrane vesicles. Since 5-oxoproline is a cyclic lactam of glutamic acid, it was of interest to study the effect of K^+ on 5-oxoproline transport and to compare it with that on glutamate transport. The present paper reports that, in rabbit renal brush-border membrane vesicles, (a) 5-oxoproline transport is Na^+-dependent and the process is electrogenic, (b) the presence of K^+ inside the vesicles stimulates the Na^+-dependent 5-oxoproline transport and (c) this stimulation is not affected by the presence or absence of an outward K^+ gradient.

Methods and Materials

Rabbit renal brush-border membrane vesicles were isolated using the $CaCl_2$ precipitation technique as described [11]. In experiments in which the intravesicular medium was varied, the membrane vesicles were preloaded by resuspending the initial 43 000 × *g* pellet and carrying out the entire washing procedure in the desired medium. Unless otherwise indicated, the media were prepared in 1 mM Hepes-Tris buffer, pH 7.5, containing the required salts and mannitol was used to adjust the osmolality of the media to 300 mosM. The purity of the membrane vesicles was routinely determined by assay of maltase and alkaline phosphatase, marker enzymes for brush-border membranes. Contamination by basal-lateral membranes was assessed by $(Na^+ + K^+)$-ATPase. Brush-border membrane vesicles were consistently enriched at least 10-fold [12,13]. Though traces of $(Na^+ + K^+)$-ATPase activity were occasionally present in the preparations, only those membranes completely free of this contaminant were employed in transport studies.

The procedure for the transport assay was essentially the same as described previously [12]. The assay was initiated by adding 40 μl of the brush-border membrane suspension to 200 μl of transport buffer. The transport buffer contained traces of ^{14}C-labeled 5-oxoproline and sufficient unlabeled 5-oxoproline to give a final concentration of 25 μM. The experiments were regularly done in duplicate and the variation between duplicate experiments was always less than ± 10% of the mean value.

Protein was determined by the method of Lowry et al. [14], using crystalline bovine serum albumin as the standard.

5-Oxo-L-[U-^{14}C]proline (specific radioactivity, 278 mCi/mmol; radiolabel purity, > 99%) was obtained from New England Nuclear. All other chemicals were of the highest commercial grade available. The membrane filters (pore size, 0.45 μm) used in transport assays were from Gelman Scientific Inc., Ann Arbor, MI.

Results

Role of membrane potential in 5-oxoproline transport

5-Oxoproline transport in renal brush-border membrane vesicles has been shown to be Na^+-dependent [2]. The role of a membrane potential in the Na^+-dependent transport of 5-oxoproline was studied by examining the effect of Na^+ gradients using sodium salts of two anions (SO_4^{2-} and Cl^-) with different epithelial permeabilities. The permeability coefficient of Cl^- is greater than that of SO_4^{2-} [15]. The magnitude of an interior-negative membrane potential produced by movement of these anions into the intravesicular space would be directly proportional to their permeability coefficients and the difference in their concentrations across the membrane. If 5-oxoproline/Na^+ cotransport is electrogenic, as is the Na^+-dependent transport of neutral amino acids, then the use of these anions would affect the initial rates of 5-oxoproline transport. On the other hand, if 5-oxoproline/Na^+ cotransport is electroneutral, as is the Na^+-dependent transport of glutamate, then these anions would have no effect on 5-oxoproline transport.

The effects of these anions on the Na^+-dependent transport of 5-oxoproline are shown in Fig. 1. The initial rate of 5-oxoproline transport measured at 15 s was significantly greater in the presence of a NaCl gradient than in the presence of a Na_2SO_4 gradient. These results, therefore, suggest that 5-oxoproline/Na^+ cotransport is significantly affected by changes in the membrane potential. Since the induction of a greater interior-negative membrane potential by the more permeant anion Cl^- than by SO_4^{2-} resulted in stimulation of Na^+-dependent transport of 5-oxoproline, the 5-oxoproline/Na^+ cotransport across the brush-border membrane should be associated with a net transfer of positive charge.

When Na^+ was replaced by Li^+, the transport of 5-oxoproline was very slow and the overshoot phenomenon was absent. However, the initial rates of transport were significantly greater in the presence of Cl^- than in the presence of SO_4^{2-}. This shows that at least a part of 5-oxoproline transport under these conditions is electrogenic and carrier-mediated. Li^+ may substitute for Na^+ in the 5-oxoproline/Na^+ cotransport system to some extent. Alternatively, it is possible that traces of Na^+ were present as contamination in the lithium salts.

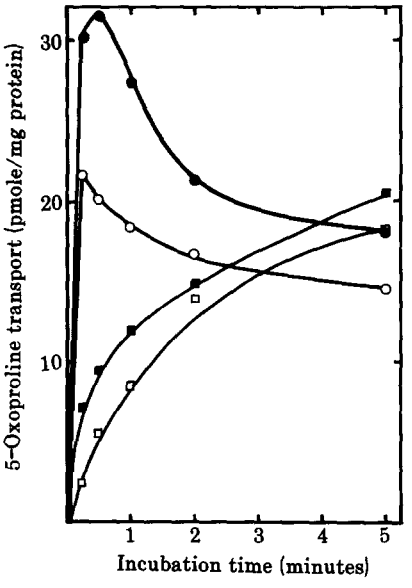

Fig. 1. Effect of anions on 5-oxoproline transport. The membrane vesicles were preloaded with 1 mM Hepes-Tris buffer, pH 7.5 containing 300 mM mannitol. The transport buffer contained 120 mM NaCl (●), 60 mM Na_2SO_4 (○), 120 mM LiCl (■) or 60 mM Li_2SO_4 (□) and 30 μM labeled 5-oxoproline in 1 mM Hepes-Tris buffer, pH 7.5. Mannitol was used to adjust the osmolality.

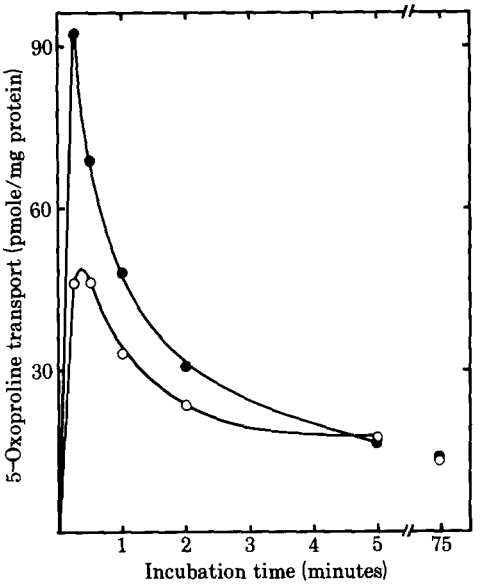

Fig. 2. Effect of valinomycin-induced K^+ diffusion potential (interior-negative) on the Na^+-gradient-dependent 5-oxoproline transport. The membrane vesicles were preloaded with 100 mM K_2SO_4 in 1 mM Hepes-Tris buffer, pH 7.5. The transport buffer contained 100 mM Na_2SO_4 and 30 μM labeled 5-oxoproline in 1 mM Hepes-Tris buffer, pH 7.5. Pretreatment with valinomycin was done in ethanol. Final concentrations in the incubation medium of valinomycin and ethanol were 10 μg/mg membrane protein and 0.17%, respectively, (●) or 0.17% ethanol alone (○).

The question of whether the Na^+-dependent transport of 5-oxoproline is electrogenic or electroneutral was further probed by studying the effect of K^+ diffusion potentials generated by valinomycin on 5-oxoproline transport. Fig. 2 shows the effects of a K^+ diffusion potential (interior-negative) on Na^+-dependent transport of 5-oxoproline. In this experiment, the membrane vesicles were preloaded with K_2SO_4 and the transport was initiated by adding 40 μl of the membrane suspension to 200 μl of the transport buffer containing Na_2SO_4. This resulted in an outwardly directed K^+ gradient. Addition of valinomycin under these conditions resulted in a 2-fold increase in 5-oxoproline transport at the overshoot. Valinomycin specifically induces the efflux of K^+ down its chemical gradient, transiently generating

an interior-negative membrane potential. These results, together with the data on the anion effects on 5-oxoproline transport, strongly indicate that the Na^+-dependent transport of 5-oxoproline is electrogenic, and the process results in a net transfer of positive charge. The data obtained in the absence of valinomycin also indicate that preloading the vesicles with K^+ may have a stimulatory effect on the Na^+-dependent 5-oxoproline transport. This particular aspect was further investigated and is discussed in the latter part of the paper.

If this is the case, induction of an interior-positive membrane potential would be expected to decrease the initial rates of 5-oxoproline transport. For this experiment, the membrane vesicles were preloaded with mannitol buffer and the transport buffer contained Na_2SO_4 and K_2SO_4. Addition of valinomycin would increase the influx of K^+ down its electrochemical gradient, inducing a transient interior-positive membrane potential. The results of the experiment is given in Fig. 3. The initial rates of 5-oxoproline transport were the same in the presence and in the absence of valinomycin. At longer periods of incubation, there was more 5-oxoproline inside the vesicles in the presence of valinomycin than in absence of valinomycin. However, the equilibrium level of 5-oxoproline was not altered. These results show that induction of an interior-positive membrane potential does not alter the initial transport rates of 5-oxoproline and at longer periods of incubation, it stimulates the transport. This is not in agreement with the results presented in Fig. 2. These apparently paradoxical data can be explained, however, if it is assumed that the increased influx of K^+ in the presence of valinomycin stimulates 5-oxoproline transport and that this stimulation masks the inhibitory effects of an interior-positive membrane potential on 5-oxoproline transport.

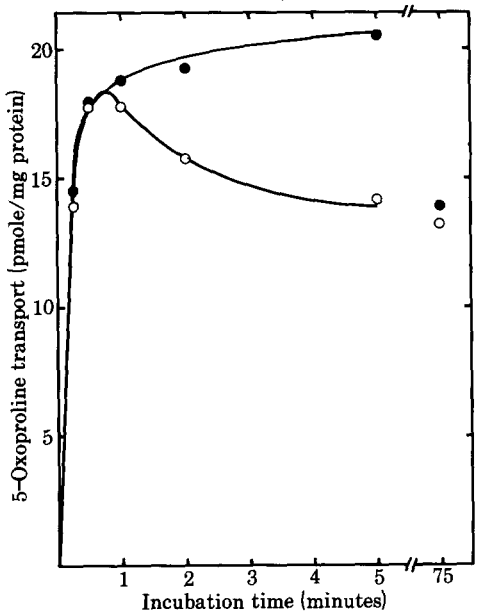

Fig. 3. Effect of valinomycin-induced K^+ diffusion potential (interior-positive) on the Na^+-gradient-dependent 5-oxoproline transport. The membrane vesicles were preloaded with 300 mM mannitol in 1 mM Hepes-Tris buffer, pH 7.5. The transport buffer contained 50 mM Na_2SO_4, 50 mM K_2SO_4 and 30 μM labeled 5-oxoproline in 1 mM Hepes-Tris buffer, pH 7.5. Pretreatment with valinomycin in ethanol (●) or ethanol alone (○) was done as described in Fig. 2.

Effect of potassium ions on 5-oxoproline transport

In order to test the hypothesis that the presence of potassium inside the vesicles stimulates 5-oxoproline transport, the effects of potassium ions on 5-oxoproline transport was systematically analyzed. Our previous studies [2] have shown that an inward Na^+ gradient can energize the uphill transport of 5-oxoproline in renal brush-border membrane vesicles whereas an inward K^+ gradient cannot. In the present paper, we studied the effects of an outwardly directed K^+ gradient on the Na^+ gradient-dependent 5-oxoproline transport. The membrane vesicles were preloaded with either 300 mM mannitol or 100 mM KCl and 100 mM mannitol. The transport buffer contained 100 mM NaCl and 100 mM mannitol. The results in Fig. 4 show that the presence of K^+ inside the vesicles greatly stimulates the Na^+ gradient-dependent transport of 5-oxoproline, without affecting the equilibrium level.

We then studied the necessary conditions for the stimulation of 5-oxoproline transport by the outward K^+ gradient. In the complete absence of sodium, an outward K^+ gradient did not increase 5-oxoproline transport (Fig. 5). The figure also describes an experiment in which the effect of an outward K^+ gradient was studied in the presence of Na^+, but in the absence of a Na^+ gradient ($[Na^+]_i = [Na^+]_o$). In the presence of Na^+, but in

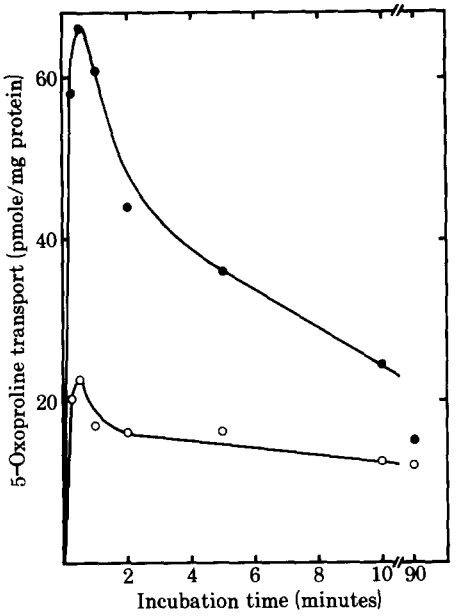

Fig. 4. Effect of an outward K^+ gradient on the Na^+-gradient-dependent transport of 5-oxoproline. The membrane vesicles were preloaded with either 300 mM mannitol (○) or 100 mM KCl and 100 mM mannitol (●) in 1 mM Hepes-Tris ubffer, pH 7.5. The transport buffer contained 100 mM NaCl, 100 mM mannitol and 30 μM labeled 5-oxoproline in 1 mM Hepes-Tris buffer, pH 7.5.

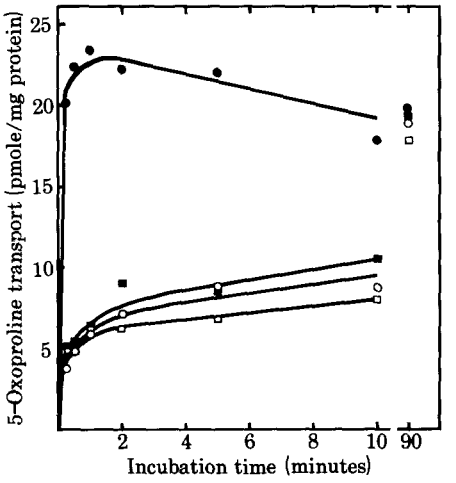

Fig. 5. Effect of an outward K^+ gradient on the Na^+-dependent (in the absence of a Na^+ gradient) and Na^+-independent transport of 5-oxoproline. In one set of experiments, the membrane vesicles were preloaded with 50 mM NaCl and 200 mM mannitol (○) or 50 mM NaCl and 100 mM KCl (●) in 1 mM Hepes-Tris buffer, pH 7.5. The transport buffer contained 50 mM NaCl, 200 mM mannitol and 30 μM labeled 5-oxoproline in 1 mM Hepes-Tris buffer, pH 7.5. In another set of experiments, the membrane vesicles were preloaded with 300 mM mannitol (□) or 100 mM KCl and 100 mM mannitol (■) in 1 mM Hepes-Tris buffer, pH 7.5. The transport buffer contained 300 mM mannitol and 30 μM labeled 5-oxoproline in 1 mM Hepes-Tris buffer, pH 7.5.

the absence of a Na^+ gradient, an outwardly directed K^+ gradient markedly stimulated 5-oxoproline transport, but there was no overshoot. Thus, the stimulation of 5-oxoproline transport by an outward K^+ gradient has an absolute requirement for Na^+, but not for a Na^+ gradient.

The effects of the ratio of intravesicular concentration of K^+ to the extravesicular concentration of K^+ on Na^+-gradient-dependent 5-oxoproline transport was then studied. The membrane vesicles were preloaded with 75 mM KCl and 150 mM mannitol. The transport buffer contained 75 mM NaCl and different concentrations of KCl and mannitol so as to vary the ratio $[K^+]_i/[K^+]_o$ from 1 to 6. The transport assay was done using 15 s incubation. Lowering the $[K^+]_i/[K^+]_o$ from 6 to 1 did not affect the initial rates of 5-oxoproline transport (data not shown), thus indicating that the stimulation is the result of the intravesicular K^+ rather than a K^+ gradient.

That the potassium effect was an intravesicular event is clearly shown from the results in Table I. The data describe the potassium effects on 5-oxoproline transport in the presence ($[Na^+]_o > [Na^+]_i$) and absence ($[Na^+]_o = [Na^+]_i$) of a Na^+ gradient. In both these cases, when KCl was added to the transport medium (extravesicular medium) alone, it did not affect the 5-oxoproline transport into the vesicles preloaded with mannitol. However, when vesicles were preloaded with KCl, a 2- to 3-fold increase in 5-oxoproline transport was observed, and the stimulation was not affected by the presence or absence of KCl in the extravesicular medium. Therefore, potassium must be present inside the vesicles in order to elicit the stimulation. That the stimulatory effect was specific for K^+ is evident, because membrane vesicles preloaded with tetraethylammonium chloride instead of KCl did not show enhanced transport.

Fig. 6 illustrates the effect of potassium concentration inside the vesicles on the initial rates of 5-oxoproline transport in the presence of an in-

TABLE I

EFFECTS OF INTRAVESICULAR AND EXTRAVESICULAR MEDIA COMPOSITION ON THE Na^+-DEPENDENT 5-OXOPROLINE TRANSPORT, IN THE PRESENCE AND ABSENCE OF A Na^+-GRADIENT

The membrane vesicles were preloaded with the desired medium and the initial rates of transport (15-s incubation) were measured in different media. The concentration of 5-oxoproline in the incubation medium was 25 μM. TEA Cl, tetraethyl ammonium chloride.

Expt.	Intravesicular medium	Extravesicular medium	Transport	
			pmol/mg protein	%
$[Na^+]_o > [Na^+]_i$				
1	300 mM mannitol	75 mM NaCl		
		150 mM mannitol	17.0 ± 0.7	100
2	300 mM mannitol	75 mM NaCl		
		75 mM KCl	20.8 ± 0.5	122
3	75 mM KCl	75 mM NaCl		
	150 mM mannitol	150 mM mannitol	38.7 ± 0.6	228
4	75 mM KCl	75 mM NaCl		
	150 mM mannitol	75 mM KCl	41.5 + 0.7	244
5	75 mM TEA Cl	75 mM NaCl		
	150 mM mannitol	150 mM mannitol	21.8 ± 0.2	128
$[Na^+]_o = [Na^+]_i$				
1	50 mM NaCl	50 mM NaCl		
	200 mM mannitol	200 mM mannitol	4.6 ± 1.0	100
2	50 mM NaCl	50 mM NaCl		
	200 mM mannitol	100 mM KCl	6.8 ± 0.1	148
3	50 mM NaCl	50 mM NaCl		
	100 mM KCl	200 mM mannitol	13.3 ± 0.8	289
4	50 mM NaCl	50 mM NaCl		
	100 mM KCl	100 mM KCl	16.0 ± 0.7	348
5	50 mM NaCl	50 mM NaCl		
	100 mM TEA Cl	200 mM mannitol	5.5 ± 0.9	120

ward Na^+ gradient. In this experiment, the membrane vesicles were preloaded with varying concentrations of KCl and the transport was assayed using 15-s incubation in the presence of a Na^+ gradient. Increasing concentrations of potassium inside the vesicles result in increasing stimulation of 5-oxoproline transport. Significant enhancement of transport was seen at an intravesicular potassium concentration as low as 0.5 mM. However, the stimulatory effect was a saturable process and the system appeared to saturate at about 150 mM K^+. An intravesicular concentration of KCl as low as 5 mM resulted in half-maximal stimulation.

Effect of extravesicular pH on 5-oxoproline transport

The effect of extravesicular pH on Na^+-gradient-dependent transport of 5-oxoproline was studied in the presence of intravesicular K^+ (100 mM). The membrane vesicles were preloaded with 100 mM KCl and 100 mM mannitol in 1 mM Hepes-Tris buffer, pH 7.5. Transport buffer contained 100 mM NaCl and 90 mM mannitol in either 10 mM acetate or 10 mM Hepes-Tris buffer of varying pH. The extravesicular pH was thus varied from 3.5 to 8.0 and the transport assay was performed using a 15-s incubation. Fig. 7 shows that the initial rate of 5-oxoproline transport increased with increasing extravesicular pH and reached a maximum value at pH 6.0 which remained almost constant between pH 6.0 and 8.0.

Effect of glutamate on 5-oxoproline transport

Our earlier studies have shown that glutamate does not inhibit 5-oxoproline transport in the presence of an inward Na^+ gradient [2]. However, since an outward K^+ gradient stimulates Na^+-de-

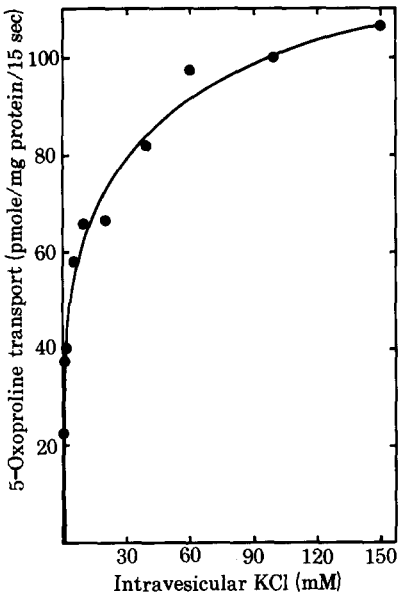

Fig. 6. Effect of intravesicular K^+ concentration on the Na^+-gradient-dependent transport of 5-oxoproline. The membrane vesicles were preloaded with varying concentrations of KCl (and mannitol to adjust the osmolality) in 1 mM Hepes-Tris buffer, 7.5. The assay was done with 15-s incubations.

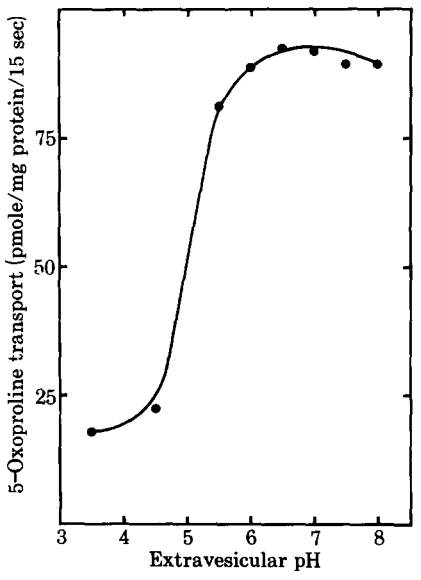

Fig. 7. Effect of extravesicular pH on the Na^+-gradient-dependent transport of 5-oxoproline in the presence of intravesicular K^+. The membrane vesicles were preloaded with 100 mM KCl and 100 mM mannitol in 1 mM Hepes-Tris buffer, pH 7.5. The transport buffer contained 30 μM labeled 5-oxoproline, 100 mM NaCl and 90 mM mannitol in 10 mM acetate buffer (pH 3.5–5.5) or 10 mM Hepes-Tris buffer (pH 5.5–8.0). The assay was done with 15-s incubations.

pendent transport of glutamate [9,10] as well as 5-oxoproline, we studied the effect of glutamate on Na^+-dependent 5-oxoproline transport in the presence of an outward K^+ gradient. A 100-fold excess of unlabeled glutamate did not have an inhibitory effect on 5-oxoproline transport under these conditions. The initial rate (15-s incubation) of 5-oxoproline transport was 50.5 ± 3.3 pmol/mg protein in the absence of glutamate and 48.1 ± 1.4 pmol/mg protein in the presence of glutamate.

Discussion

Maximum transport of 5-oxoproline in the presence of an inward Na^+ gradient occurs in renal brush-border membrane vesicles at an extravesicular pH of 6.0 to 8.0. Over that pH range, 5-oxoproline exists completely as an anion with a net negative charge. Under these conditions, 5-oxoproline/Na^+ cotransport is mediated by an electrogenic mechanism. Since an interior-negative membrane potential produced by anions or by valinomycin stimulated Na^+-dependent 5-oxoproline transport, 5-oxoproline/Na^+ cotransport was apparently associated with a net transfer of positive charge. This is in contrast to the Na^+-dependent transport of glutamate which also exists as an anion at a similar pH range but whose transport is electroneutral [8]. The simplest stoichiometry consistent with the electrogenic transport of 5-oxoproline would be the cotransport of two sodium ions with one 5-oxoproline anion. Neutral amino acids which exist as zwitterions are also transported by an electrogenic process and the simplest stoichiometry in this case is the cotransport of one zwitterion with one Na^+. This being the case, the interaction between 5-oxoproline and neutral amino acids during transport in renal brush-border membrane vesicles [2] can be explained if one assumes that a single transport system is involved in the transport of these amino acids. The 5-oxoproline anion and one Na^+ may satisfy the requirements for binding with the same site of the neutral amino acid carrier which binds the zwitterions of the neutral amino acids. However, the possibility that the mutual inhibition is the result of competition between 5-oxoproline and the neutral amino acids for a Na^+ gradient or membrane potential or both cannot be ruled out. Interest-

ingly, it has been shown that more than one Na^+ ion is cotransported with each succinate, citrate [16,17] or lactate [18] anion.

The present investigation also demonstrated that the Na^+-dependent transport of 5-oxoproline was stimulated by intravesicular potassium. This stimulatory effect was specific for K^+ and requires the presence of Na^+. The presence of a Na^+ gradient was not mandatory for the potassium action. In this regard, the potassium effect on the Na^+-dependent 5-oxoproline transport is similar to that on the Na^+-dependent glutamate transport [9]. However, the two systems differ in one important aspect. In the case of Na^+-dependent glutamate transport, the stimulation was observed only in the presence of an outwardly directed K^+ gradient. The intravesicular potassium ions had no effect in the absence of a K^+ gradient ($[K^+]_i = [K^+]_o$). In the case of 5-oxoproline, the stimulation by the intravesicular potassium was seen in the presence as well as in the absence of a K^+ gradient. In addition, an outward K^+ gradient could energize the uphill transport of glutamate in the presence of Na^+, but in the absence of a Na^+ gradient. However, an outward K^+ gradient cannot provide the driving force for the active transport of 5-oxoproline under similar conditions. As was evident from Fig. 5, an outward K^+ gradient stimulated the Na^+-dependent 5-oxoproline transport in the absence of a Na^+ gradient. However, the transport did not occur against a concentration gradient as evidenced by the lack of overshoot. These data clearly demonstrate that the mere presence of K^+ inside the vesicles caused stimulation of Na^+-dependent 5-oxoproline transport. These data also explain why an induction of an interior-positive K^+ diffusion potential by valinomycin did not reduce the Na^+-dependent transport of 5-oxoproline. Increased influx of K^+ in the presence of valinomycin increased the intravesicular concentration of K^+ which stimulated 5-oxoproline transport, and this stimulation nullified the inhibitory effect of the interior-positive membrane potential. This argument can be appreciated if one realizes that an intravesicular K^+ concentration as low as 0.5 mM can cause 60% enhancement of 5-oxoproline transport over the control value.

The role of K^+ in amino acid transport is not well understood. Before the Na^+ gradient hypothesis for the active transport of amino acids and sugars was postulated by Crane [19,20], an outward K^+ gradient was favored as the driving force for accumulation of amino acids inside the cells against a concentration gradient [21]. Even though, now after two decades, the amino acid/Na^+ cotransport has been firmly secured with experimental evidence, the K^+ effects on amino acid transport have not been completely resolved. This subject has been reviewed in detail by Crane [22].

There are many reports on the effect of an outward K^+ gradient on the Na^+-dependent transport of glutamate [9,10,23–25]. In bacterial systems [23,24], the cotransport of Na^+ and glutamate was shown to be electroneutral and the presence of an outward K^+ gradient stimulated this transport. In rat brain synaptosomes [25], the glutamate/Na^+ cotransport was electrogenic and an outward K^+ gradient was an absolute requirement for this transport. In renal brush border membrane vesicles [9], Na^+-dependent transport of glutamate was an electroneutral process and was stimulated by an outward K^+ gradient. The stimulation had an absolute requirement for a K^+ gradient. It was therefore hypothesized that the glutamate/Na^+ cotransport was coupled to the efflux of K^+.

In the case of 5-oxoproline, there was no absolute requirement for the K^+ gradient to cause the stimulation. Therefore, 5-oxoproline/Na^+ cotransport is not associated with the efflux of K^+. These data show that the intravesicular K^+ accelerates the movement of the 5-oxoproline carrier in the membrane, thus increasing the overall flux of the carrier. As an alternate view, we may consider that the presence of K^+ inside the vesicles reduces the efflux of 5-oxoproline by diminishing the rate of recombination of 5-oxoproline and the carrier at the inside of the membrane. However, the extravesicular K^+ is not able to affect the rate of binding at 5-oxoproline to the carrier at the outside of the membrane as is evident from Table I. We may, therefore, have to assume that the binding sites of the carrier are different at the inside and at the outside of the membrane. This is possible in a nonmobile model of solute/Na^+ cotransport. According to this recently proposed model [26–28], the transport protein(s) spans the membrane bilayer and conformational changes, not the

actual movement of the carrier as suggested by Crane's model [20], result in the translocation of the solute across the membrane. The binding of K^+ to the carrier may also bring about changes in the association and dissociation characteristics of the carrier, thereby increasing the transport of the solute. The mechanisms suggested above remain hypothetical at present, and elucidation of the actual mechanism at the molecular level must await further experiments.

Acknowledgements

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