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RELATIONSHIP BETWEEN L-ALANINE AND SODIUM ION TRANSPORT IN ISOLATED RENAL TUBULES

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SUMMARY

1. Rat renal tubules were isolated by incubation with collagenase. The Na^+ concentration in the tubules at 37 °C was increased by additions of g-strophantin and L-alanine. The increase of Na^+ in the presence of both g-strophantin and L-alanine was stronger than with either alone.

2. Radioactive sodium (^{22}Na), which was taken up by the tubules at 0 °C in K^+-free medium, was more slowly washed out in the buffer with added g-strophantin than in the control buffer, but L-alanine had no effect.

3. At 0 °C incubation without K^+, g-strophantin did not affect the ^{22}Na transport of the tubules. But under the same conditions, L-alanine increased Na^+ uptake significantly, and in conjunction with it, L-alanine uptake was also increased.

4. The relationship between L-alanine uptake and intra- extracellular Na^+ concentration gradients was linear. The ratio of L-alanine to Na^+ uptake at 0 °C was about 1 : 2.

5. In the incubation without K^+ at 0 °C, L-alanine could be accumulated in tubules against the chemical concentration gradient (about 1.5-fold).

6. In the incubation without K^+ at 37 °C, the L-alanine concentration in tubules after 5 min was already steady ($C_i/C_e = 2.2$), but with K^+ it was not stabilized after 10 min. The ratio C_i/C_e with K^+ was higher than without K^+.

7. g-Strophantin, *p*-hydroxymercuribenzoate, amiloride, and 2,4-dinitrophenol inhibited L-alanine uptake in the tubules and at the same time increased Na^+ concentration. The relationship between the L-alanine uptakes inhibited by g-strophantin, amiloride and dinitrophenol, and the respective intra- extracellular Na^+ concentration gradients was strikingly linear. But in the case of *p*-hydroxymercuribenzoate there was no correlation.

8. The results indicate that L-alanine transport into the renal tubules might be regulated mainly by the intra- extracellular Na^+ concentration gradient and that inhibitors such as g-strophantin, amiloride, and dinitrophenol could have a secondary effect on the L-alanine transport which follows the change of Na^+ concentration in cells. *p*-Hydroxymercuribenzoate might have an inhibiting effect on the binding of carrier with Na^+ and/or L-alanine.

INTRODUCTION

It is now well known that various organic solutes, particularly amino acids and sugars, are accumulated in certain cells and tissues against their electrochemical potentials. Many of these active transport systems are stimulated by extracellular Na^+ and are inhibited by Na^+-free medium. The mechanism of cation-dependent organic solute transport was first reported by Christensen and Riggs [1]. They showed that when mouse ascites tumour cells absorbed various neutral amino acids, the cellular Na^+ content increased and K^+ decreased. With respect to the role of these cations in amino acid transport, there are two hypotheses. The first one was mainly presented by Crane [2, 3] and important aspects for this hypothesis were contributed by Riggs et al. [4], Vidaver [5], and Eddy [6, 7]. According to this hypothesis, Na^+ is thought to be involved in forming a ternary complex with the organic solute and the carrier, which leads to transport of both molecules. Series of investigations with rabbit intestine by Schultz et al. [8, 9] support this opinion. The second hypothesis is that of Csaky [10], which attributes the Na^+-amino acid relationship to an intracellular Na^+ requirement. In this case the intracellular Na^+ concentration, which is influenced by the extracellular one, is necessary for active amino acid transport.

On the other hand, it is also shown that cardiac glycosides, e.g. g-strophantin, and metabolic inhibitors like 2,4-dinitrophenol inhibit strongly the active transport system of amino acid and sugars [11]. However, it has not been clarified whether these inhibitors act directly on this transport.

Most studies on amino acid transport have been carried out on isolated cells, namely tumour cells or red cells, and tissues such as small intestine and muscle. Due to technical difficulties with kidney, in which many sorts of active transport can be observed, this tissue is not often employed. In 1962, Burg and Orloff [12] prepared suspensions of isolated renal tubules by digestion of minced rabbit renal cortex with collagenase. This tissue preparation concentrated *p*-aminohippurate, consumed oxygen and maintained normal concentration gradients for Na^+ and K^+ for several hours. In this paper isolated renal tubules from rats were used for the experiments. We describe the direct measurements of Na^+ and L-alanine movements in the tubules by using a ^{14}C-^{22}Na double isotope method. The evidence obtained indicates that L-alanine uptake into the tubules depends on the sodium concentration gradient between intra- and extracellular spaces. This finding is consistent with the Na^+ gradient hypothesis. g-Strophantin and dinitrophenol seem to have a secondary effect on the L-alanine transport.

METHODS

Preparation of isolated tubules

Male and female Wistar rats of 150–250 g body weight were used, which had been maintained on normal food intake and water ad libitum. The methods of tissue preparation which were described by Guder and Wieland [13] and by Nagata and Rasmussen [14] were used with modifications.

Rats were anaesthetized with ether and the abdomen was opened. The abdominal, mesenterial and femoral arteries and veins were clamped. Through the abdom-

inal aorta 2 ml ice-cold collagenase solution (0.2 % in Krebs/Henseleit/HCO_3 buffer, in which 10 mM NaCl were replaced by sodium acetate) was infused and the kidneys were removed. The medulla was cut out and the cortex was minced with a razor. From 20 to 24 kidneys were used for one experiment. The minced tissue was incubated at 37 °C with 40 ml collagenase solution (above) in a 100 ml round bottom flask which was placed in a water bath with a magnetic stirrer. The tissue was stirred at low speed. The suspension was continuously gassed by a fine stream of humidified 95 % O_2/5 % CO_2. After 45 min the suspension was placed on ice and then filtered with nylon cloth. The filtrate was centrifuged at 40 $\times g$ for 30 sec in a refrigerated centrifuge and washed 3 times with 80 ml cold buffer solution. The tubules were suspended in 20–40 ml buffer, and the protein concentration was measured by the biuret method. Usually the concentration of tubule protein was 7–10 mg/ml.

Experiments with 37 °C incubation

1 ml bicarbonate buffer with or without metabolic inhibitors and 1 ml tubule suspension were pipetted into 25 ml erlenmeyer flasks, gassed with 95 % O_2/5 % CO_2, and incubated in a shaking water bath at 37 °C. After a 5 min period of temperature equilibration, 0.2 ml mixed solution of 0.8 μCi ^{22}Na and 0.5 μCi L-[^{14}C]alanine with the desired concentration of unlabelled L-alanine was added to the experimental group. To the controls 0.2 ml of 0.8 μCi ^{22}Na and 0.5 μCi [^{14}C]inulin (see below) with the identical concentration of mannitol instead of L-alanine was added. The incubation time lasted from 1 to 30 min. The reaction was terminated by pipetting 20 ml ice-cold 0.9 % NaCl into the flask. Then the mixture was quickly poured into tubes and centrifuged at 600 $\times g$ for 30 sec. 1 ml supernatant was pipetted for counting and the remainder was removed as quickly as possible. In order to correct the pellets for extracellular space, control and experimental groups should always be incubated simultaneously. If care was taken to remove the supernatant equally, the inulin space of the pellets was almost the same. The pellets were dissolved in Soluene-350.

When K^+-free medium was required, the potassium salts in buffer were replaced by sodium salts. In Na^+- and K^+-free experiments the medium consisted of isotonic cholinchloride and Tris · HCl.

Experiments at 0 °C incubation

The tubule suspension was placed in 100 ml round bottom flasks which were set in ice-cold water on a magnetic stirrer and permitted continuous gentle stirring and oxygenation by bubbling 95 % O_2/5 % CO_2. Only K^+-free buffer was used. At the beginning of the experiment 10 μCi ^{22}Na and 5 μCi L-[^{14}C]alanine with unlabelled L-alanine were added to 25 ml medium of the experimental group. Instead of labelled and unlabelled L-alanine, the control group contained 5 μCi [^{14}C]inulin and mannitol, respectively. The experiments were terminated by pipetting duplicate 1 ml samples into tubes with 20 ml ice-cold 0.9 % NaCl. Further treatment was identical to the 37 °C experiment.

^{22}Na washout experiments

The tubule suspension was washed twice with potassium free buffer and incubated with ^{22}Na (10 μCi/20 ml suspension) at 0 °C (see above). After 1 h the tubules

were washed twice with ice-cold K^+-containing buffer. The sample was divided into a control group and an experimental group with 10^{-3} M g-strophantin or 10^{-2} M L-alanine. Before the round bottom flasks were incubated at 37 °C, [^{14}C]inulin (5 μCi/20 ml) was added and duplicate 1 ml samples were pipetted as zero controls into 20 ml ice-cold 0.9 % NaCl.

Measurement of intracellular water and extracellular inulin space

In order to calculate the uptakes of L-[^{14}C]alanine and ^{22}Na by the tissue it is necessary to correct for extracellular space. The behaviour of [^{14}C]inulin, [^{3}H]inulin, and [^{14}C]sucrose was checked for this purpose. In all cases the dose of 1 μCi was added to the 2 ml incubating medium. After incubation, the medium was transferred into 20 ml ice-cold 0.9 % NaCl and centrifuged at $600 \times g$ for 30 sec. The pellets were washed twice with the same solution, dissolved, and counted. With the correction of [^{3}H]inulin the Na^+ concentration in the tubules paradoxically yielded negative values. [^{14}C]Sucrose showed the same tendency as did [^{3}H]inulin. We, therefore, used only [^{14}C]inulin throughout the experiments to correct for the extracellular space.

By using [^{14}C]inulin the intracellular water was found to be 2.63 ± 0.12 ($N = 6$) μl/mg tubule protein, i.e. 65.0 ± 1.2 ($N = 6$) percent water content. These values were also used for the calculations.

Counting

The ^{14}C and ^{22}Na activities in supernatants and pellets dissolved by Soluene-350 were determined by using plastic counting vials containing 10 ml of Dimilume. All counting was performed by a Packard liquid scintillation spectrometer (Model 3380) and analysed automatically (Model 544).

Chemicals and isotopes

Collagenase type II was obtained from Sigma Chemical Co., St. Louis, Mo., L-alanine, g-strophantin, 2,4-dinitrophenol, and parahydroxymercuribenzoate sodium salt were of highest available purity from commercial sources. Dimilume and Soluene-350 were purchased from Packard Instrument GmbH, Frankfurt. ^{22}Na (specific activity 5.2 Ci/mmol), [^{14}C]inulin (specific activity 7.7 Ci/mole), L-[^{14}C]alanine (specific activity 159 Ci/mole, carrier free), [^{3}H]inulin (specific activity 300 Ci/mole), [^{14}C]sucrose (specific activity 600 Ci/mole) were purchased from Amersham Buchler, Braunschweig. Amiloride was a gift of Sharp and Dohme GmbH., München. All other chemicals were of analytical grade.

RESULTS

Effects of L-alaline and g-strophantin on sodium uptake into tubules

If 10^{-2}M L-alanine was added to the medium, sodium uptake was accelerated (Fig. 1). This finding is the same as the results by Schultz et al. [9], who used rabbit intestine with L-alanine. Schaefer and Jacquez [15] also studied the same with α-aminoisobutyrate in Ehrlich ascites cells. g-Strophantin, an inhibitor of (Na^+-K^+)-activated ATPase, increased Na^+ concentration similar to L-alanine and inhibited L-alanine uptake (Fig. 2). When both L-alanine and g-strophantin were added as

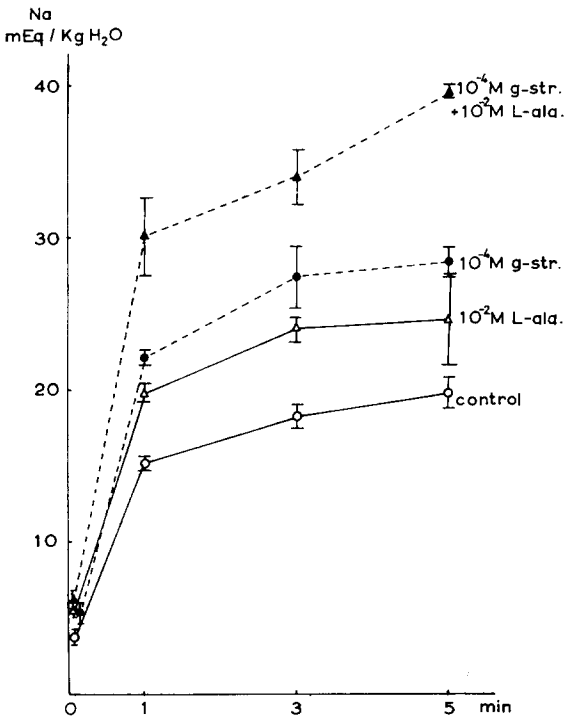

Fig. 1. The effects of L-alanine and g-strophantin on the intracellular Na^+ concentration of the isolated renal tubules. Each value represents the mean ±S.E.M. of 3 independent determinations.

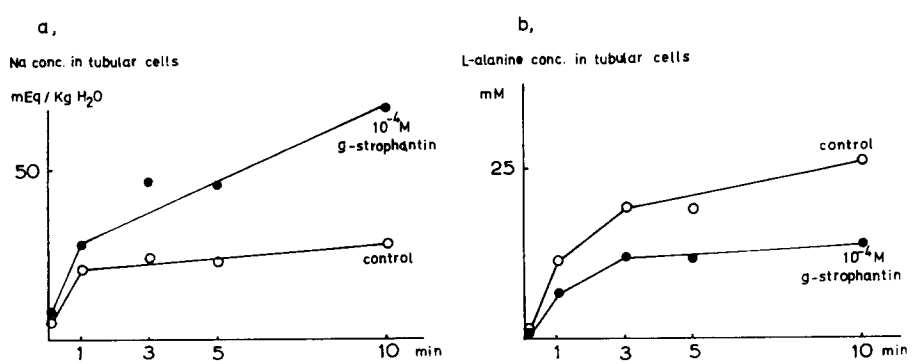

Fig. 2. Effects of 10^{-4} M g-strophantin on the Na^+ and L-alanine concentrations in the isolated renal tubules. The mean of either 2 or 4 experimental data are shown.

described in Fig. 1, the sodium uptake became higher than with L-alanine or g-strophantin alone. In order to ascertain whether L-alanine might play a role similar to that of g-strophantin, two different types of experiments were carried out. Firstly, if tubule suspension is incubated in K^+-free medium, the Na^+ concentration will increase, because the ATP-dependent (Na^+-K^+) pump is inhibited by lack of extracellular potassium. Since at 37 °C another pump might act which extrudes Na^+

accompanied by chloride [16], and because at 0 °C the effect of g-strophantin on Na^+ transport would be impossible to observe, the uptake experiments were carried out in ice-cold K^+-free medium. The concentration of 10^{-3} M g-strophantin could not increase the Na^+ uptake into the tubules, whereas L-alanine, as shown in Fig. 3, stimulated the uptake significantly under the same conditions.

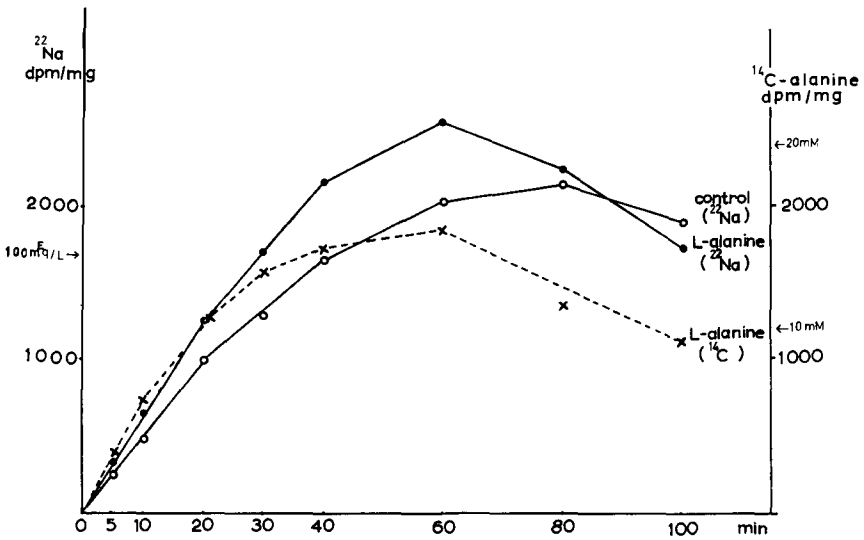

Fig. 3. Time curves of ^{22}Na and L-[^{14}C]alanine concentrations at 0 °C incubation without K^+ in the isolated renal tubules. All points represent mean of at least 2 determinations.

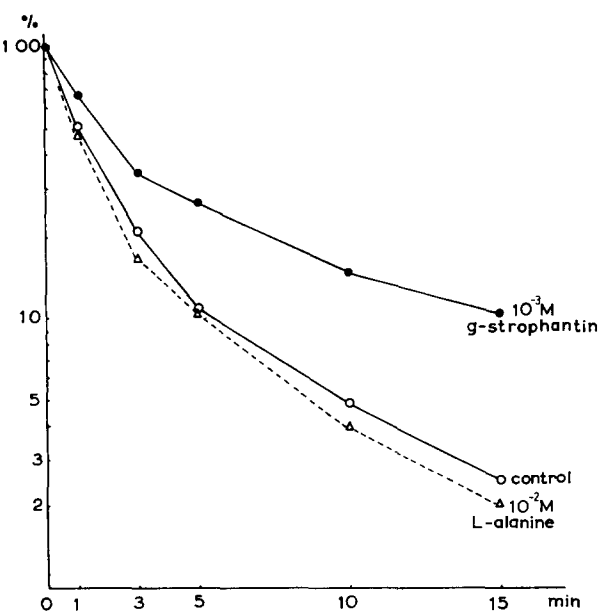

Fig. 4. Washout curves of ^{22}Na in rat renal isolated tubules with 10^{-3} M g-strophantin and 10^{-2} M L-alanine. All points represent mean of duplicate observations in a single representative experiment.

Secondly, washout experiments were carried out (Fig. 4). After loading the tubules with ^{22}Na for 1 h at 0 °C in the K^+-free medium, rewarming to 37 °C in fluid with K^+ was begun at time 0. A considerable suppression of washout effect by g-strophantin was observed. However, L-alanine showed a slight accelerating effect. These results indicate that L-alanine stimulated Na^+ uptake into the tubules but had no effect on Na^+ washout, and that g-strophantin inhibited sodium washout but had no effect on Na^+ uptake. In the presence of L-alanine and g-strophantin together (Fig. 1), therefore, the two substances played two different roles.

Relationship between L-alanine and sodium movements

As seen in Fig. 3, it is surprising that L-alanine was accumulated against a concentration gradient although the incubation was carried out at 0 °C in K^+-free buffer. This finding may cause us to question what was the energy source for the L-alanine accumulation. We measured the sodium uptake simultaneously. The relationship between L-alanine uptake and the intra- extracellular Na^+ concentration gradient was strikingly linear (Fig. 5). As shown in Table I, intracellular L-alanine and

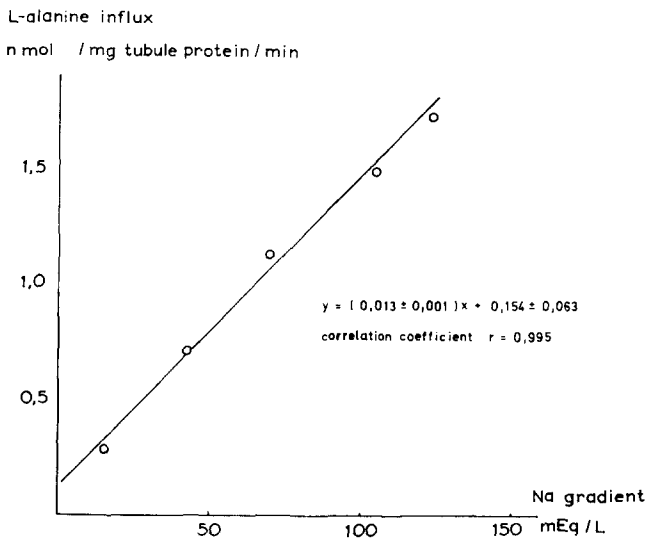

Fig. 5. Relationship between Na^+ concentration gradients and L-alanine influx in isolated renal tubules at 0 °C incubation. The values are calculated from the data of Fig. 3.

alanine-dependent Na^+ uptake were correlated, and the ratio of Na^+ to alanine in equilibrium was about 2 : 1, which is consistent with the result of unidirectional alanine and sodium influxes in mucosal border of rabbit ileum [9]. From the constant Na^+/alanine ratio, the transport of these two molecules seemed to be coupled. Thus the mechanism of L-alanine accumulation at 0 °C could be understood. The driving force of the L-alanine transport might be the sodium concentration gradient since, when sodium concentration in the tubules reached its peak (151 mequiv/l, see Fig. 3) at 60 min, the alanine accumulation was blocked.

TABLE I

Relationship between Na^+ dependent L-alanine uptakes and L-alanine dependent Na^+ uptakes in isolated rat kidney tubules at 0 °C incubation in K^+-free medium with and without Na^+. Na^+-dependent L-alanine uptakes were calculated from Fig. 3 and the Na^+-free experiment (see Methods). All values represent mean of duplicate observations in a single representative experiment.

Incubation time (min):	5	10	20	30
Na^+ uptake (nmol/mg tubule protein)				
10 mM L-alanine	82.6	99.2	192.9	262.5
Without L-alanine	69.7	72.1	152.9	197.8
L-Alanine dependent sodium uptake	12.9	27.1	40.0	64.7
L-Alanine uptake (nmol/mg tubule protein)				
149 mequiv./l Na^+	8.4	15.8	27.1	34.2
Without Na^+	0.1	4.1	6.7	7.8
Na^+-dependent L-alanine uptake	8.3	11.7	20.4	26.4
Na^+/alanine transport ratio	1.56	2.32	1.96	2.45

As to the effect of different concentrations of L-alanine in medium on the Na^+ and L-alanine movements, another experiment was carried out. When tubules were incubated at 37 °C for 5 min in different L-alanine concentrations in media adjusted to iso-osmolality with mannitol, the uptake of L-alanine depended on the extracellular alanine concentration (Fig. 6). As shown in 37 °C incubation experiments, the relationship between alanine and Na^+ concentrations in tubules was linear (Fig. 7). Thus, with respect to the movements of L-alanine and Na^+, there could be no qualitative difference between experiments at 0 °C and at 37 °C (Figs 5 and 7).

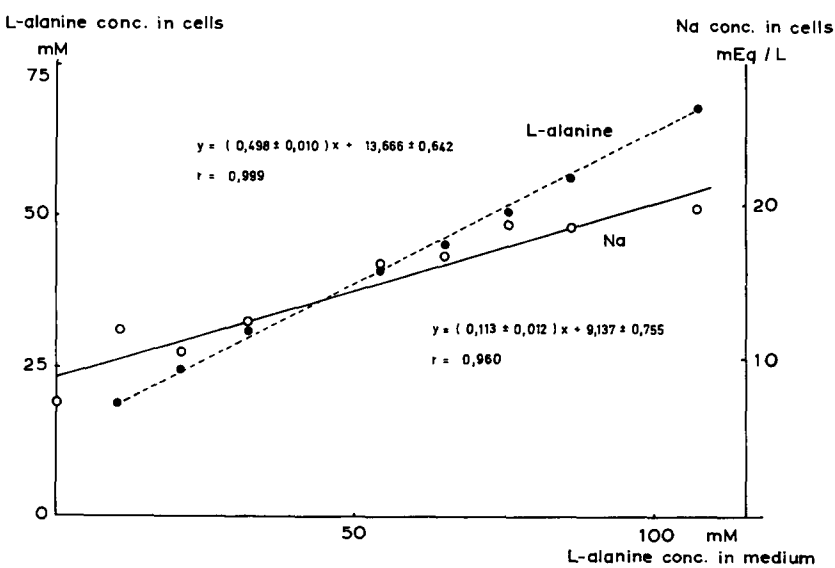

Fig. 6. Correlation of L-alanine concentrations in the incubation medium and the intracellular concentrations of L-alanine and Na^+ at 37 °C incubation for 5 min.

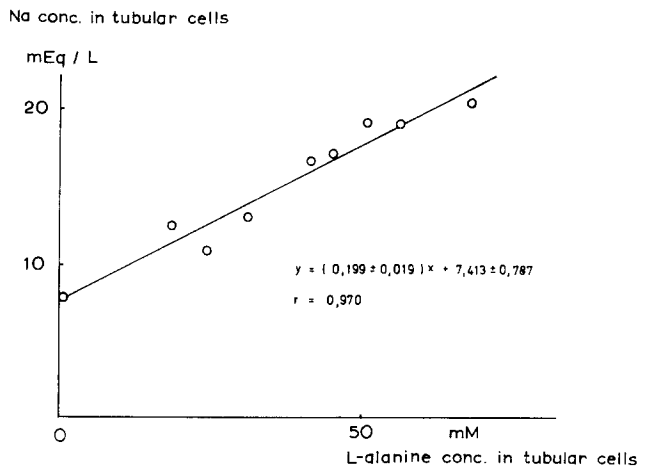

Fig. 7. Correlation of Na^+ and L-alanine concentrations in the isolated renal tubules. Values are calculated from the data of Fig. 6.

Role of K^+ in the movement of L-*alanine and Na^+*

When the tubules were incubated at 37 °C in K^+-free medium, the alanine and Na^+ concentrations reached a steady state within 5 min (Fig. 8). In the absence of K^+, L-alanine could be accumulated against a concentration gradient, but the intra-extracellular alanine concentration ratio was not as high as in the presence of K^+

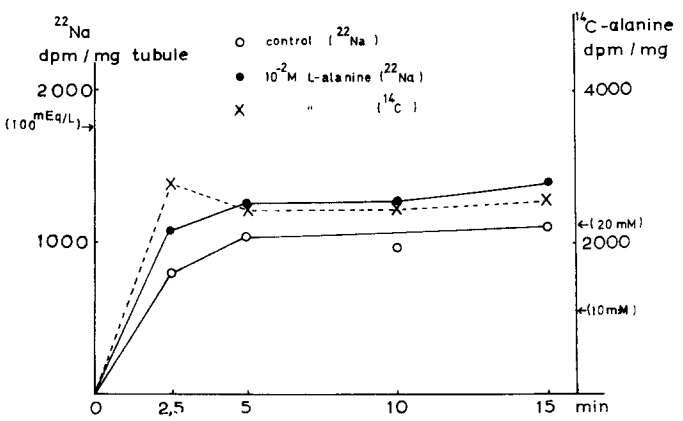

Fig. 8. Time courses of ^{22}Na and L-[^{14}C]alanine concentrations at 37 °C incubation without K^+ in the isolated renal tubules. Each point represents the mean of 3 or 4 independent determinations.

(Fig. 2b). Although there was a time lag in the g-strophantin effect in Fig. 2a, the Na^+ concentrations were almost identical after 10 min in both the K^+-free group and the g-strophantin group with K^+.

This time lag might be caused either by a short duration of action (pre-incubation 5 min and post 1–10 min, see Methods) or by a low concentration (10^{-4} M)

of g-strophantin. At any rate it could be supposed that K^+ might not play a direct part in alanine transport, but an indirect one which seems to act by inhibition of (Na^+-K^+)-activated ATPase.

As shown by comparing Table II with Table I, L-alanine and Na^+ could enter the tubules faster at 37 °C than at 0 °C. The Na^+/alanine transport ratio at 37 °C

TABLE II

Relationship between Na^+-dependent L-alanine uptakes and L-alanine dependent Na^+ uptakes in isolated rat kidney tubules at 37 °C incubation in K^+-free medium with and without Na^+. Na^+-dependent L-alanine uptakes were calculated from Fig. 8 and the Na^+-free experiment. All values represent mean of at least duplicate observations in a single representative experiment.

Incubation time (min):	2.5	5	10	15
Na^+ uptake (nmol/mg tubule protein)				
10 mM L-alanine	154.3	179.0	180.7	202.4
Without L-alanine	109.9	143.2	129.7	152.3
L-Alanine-dependent Na^+ uptake	44.4	35.8	51.0	50.1
L-Alanine uptake (nmol/mg tubule protein)				
149 mequiv./l Na^+	64.7	56.8	57.1	60.0
Without Na^+	24.7	24.1	26.9	28.8
Na^+-dependent L-alanine uptake	40.0	32.7	30.2	31.2
Na^+/alanine transport ratio	1.11	1.09	1.69	1.60

was 1.1–1.7, and these values were lower than those at 0 °C. These differences could be hypothetically explained by the presence of a "chloride pump" [16], by which a part of Na^+ could be transported at 37 °C, but not at 0 °C.

Effects of metabolic inhibitors on L-alanine transport

Table III summarizes the influences of g-strophantin, dinitrophenol, amiloride, and *p*-hydroxymercuribenzoate on the Na^+ and L-alanine concentrations in tubules. All substances tested here were inhibitory to Na^+ transport as many investigators have observed. According to the behaviour of L-alanine it can be said that if the concentration of Na^+ in the tubules increased that of L-alanine decreased.

TABLE III

Effects of metabolic inhibitors on the Na^+ and L-alanine concentration in the tubules. The isolated tubules were pre-incubated in the indicated concentration medium with various metabolic inhibitors at 37 °C for 5 min to equilibrate the temperature and then incubated for 5 min with ^{22}Na and L-[^{14}C]alanine. All values represent mean of at least two determinations.

	Na^+		L-Alanine	
Added substances	Concn (mequiv/l)	%	Concn (mM)	%
Control	23.6	100	16.8	100
10^{-4} M g-strophantin	44.3	188	11.7	70
$2 \cdot 10^{-4}$ M *p*-hydroxymercuribenzoate	27.6	117	8.3	49
$5 \cdot 10^{-4}$ M amiloride	33.0	140	13.1	79
$2.5 \cdot 10^{-4}$ M dinitrophenol	67.6	286	6.2	37

In order to find whether there were any correlations between L-alanine and Na^+ transport, Fig. 9 was plotted from Table III. The relationship between L-alanine uptake and Na^+ concentration gradients was strikingly linear in the case of g-strophantin, amiloride, and dinitrophenol, but not in the case of *p*-hydroxymercuribenzoate.

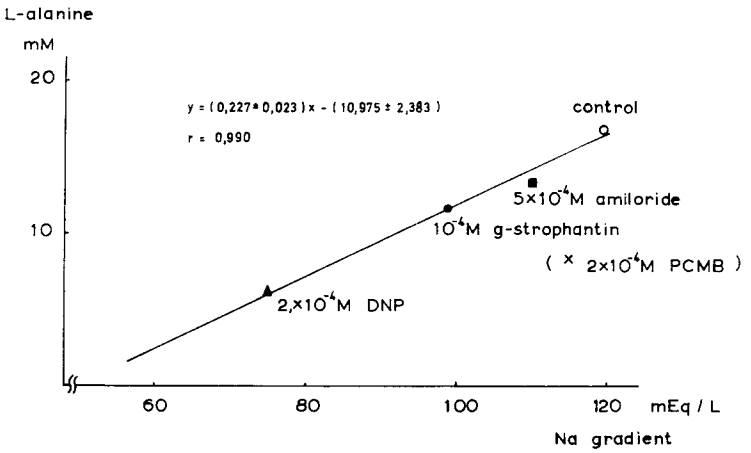

Fig. 9. Correlation of L-alanine concentrations in the renal tubules and the Na^+ concentrations gradients at 37 °C incubation for 5 min with different substances.

The intercept with the abscissa in Fig. 9 was about 50 mequiv/l. This theoretically seems to indicate an ineffective Na^+ concentration gradient on L-alanine transport which is almost identical with the control value (59 mequiv/l) after 5-min incubation without K^+ at 37 °C as shown in Fig. 8.

DISCUSSION

Isolated tubules of rat kidney used here have recently been studied for biochemical purposes, e.g. for gluconeogenesis in kidney cortex [13, 14]. These materials are more useful than kidney slices for short-time incubation experiments because of the lack of intervening tissue between the tubule cells and the bathing medium [17]. As to the condition of the prepared tubules, there is, however, a critical opinion saying that the lumina of the tubules might be closed and only the peritubular side of the membrane could function [18]. Fig. 10 shows that the tubules prepared for the presented experiments increased the so-called inulin space gradually at 37 °C but not at 0 °C. The result indicates that the tubule lumina might be open and inulin could be concentrated in the tubule lumina. This is in opposition to the above mentioned opinion.

The data reported in this paper imply that L-alanine is transported together with Na^+. As shown in Table I, 2 Na^+ and one L-alanine are coupled and transported into the isolated tubules. This supports the observations which were made in the mucosal border of rabbit small intestine [8, 9, 11] and in pigeon-red cells [19]. Concerning the coupling mechanism between Na^+ and Na^+-dependent organic

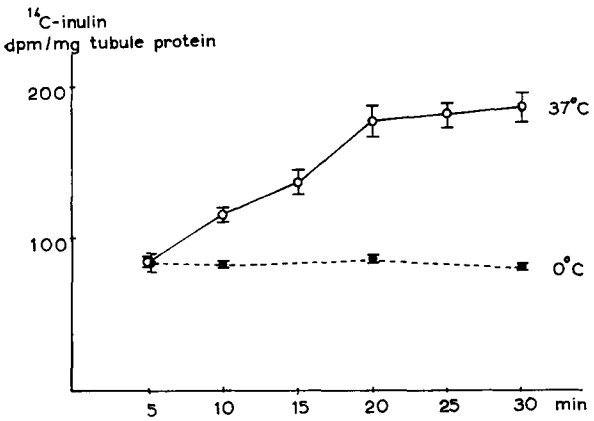

Fig. 10. Time course of inulin space with [^{14}C]inulin in isolated renal tubules from rat. Each value represents the mean ±S.E.M. of 8 determinations.

solutes, Armstrong et al. [20] have recently reported that, in epithelial cells of bullfrog small intestine, the transmucosal Na^+ chemical gradient alone cannot, thermodynamically, account for D-galactose accumulation, unless the Na^+/galactose coupling ratio is significantly greater than 1. This finding is consistent with the data in this paper.

It is possible that Na^+ and L-alanine penetrate through the peritubular membrane from medium into tubule cells, as has been demonstrated for L-arginine and L-leucine, using microinjection technique in rat peritubular capillary [21, 22]. However, it seems to be reasonable that most L-alanine is transported from the tubular lumen into tubule cells because many studies have shown that amino acids are principally reabsorbed in the proximal tubule in vivo [23]. The site of L-alanine transport in the isolated renal tubules cannot be directly demonstrated. According to the experiments at 0 °C, L-alanine can be accumulated against concentration gradient, although all production of metabolic energy is stopped. It can be presumed that Na^+ dependent L-alanine uptake occurs in luminal side membrane, because the peritubular membrane has more energetic character [24], e.g. 100 times greater (Na^+-K^+)-ATPase than the luminal one [25], and in vivo g-strophantin inhibits glycine re-absorption when peritubular capillaries are perfused in rat [26]. On the one hand, at 0 °C L-alanine increases sodium uptake into cells (Fig. 3) and g-strophantin does not affect Na^+ transport. On the other hand, g-strophantin inhibits Na^+ washout at 37 °C (Fig. 4) and decreases L-alanine uptake (Fig. 2). These findings and the linear correlation between Na^+ concentration gradient and L-alanine uptake (Fig. 5) are taken to conclude that the energy source of L-alanine transport is the Na^+ concentration gradient, which depends on active Na^+ pump.

As to Na^+ extrusion, at least two different pumps are postulated in kidney. One is pump A, which transports Na^+ accompanied by net Cl^- efflux and is inhibited by 2 mM ethacrynic acid but not by 1–10 mM g-strophantin. The other is pump B, by which one K^+ is taken up for each Na^+ extruded and which is inhibited by 1 mM g-strophantin [16]. As shown in Figs 2 and 8, L-alanine can be accumulated in the

tubules more than two-fold in buffer with g-strophantin and in K^+ free medium, respectively. Under these conditions, pump B should be inhibited and pump A might function. Concerning the role of Cl^- in amino acid transport, Vidaver [27] has reported that the replacement of Cl^- by mucate causes a nearly complete inhibition of the Na^+ dependent component of glycine entry into pigeon red cells. Gilles-Baillien and Schoffeniels [28] also found that L-alanine transport is linked to Cl^- in Greek tortoise intestine. According to the two-pump theory, Cl^- does not seem to act directly on amino acid transport, but secondarily through changes of the Na^+ concentration gradient, as could be demonstrated by inhibition of (Na^+-K^+)-pump.

The action of metabolic inhibitors on L-alanine transport seems also to be indirect, because they primarily increase sodium concentration in cells (Table III, Fig. 1), and because there is a striking linear relationship between Na^+ concentration gradient and L-alanine uptake (Fig. 9). *p*-Hydroxymercubenzoate, an SH binding inhibitor, decreases L-alanine transport into cells (Table III), but in this case there is no correlation between Na^+ gradient and L-alanine uptake. As chloromercuribenzoate completely inhibits the binding of L-proline to brush border-rich membrane isolated from rabbit kidney cortex [29], it can be concluded that L-alanine, Na^+ and carrier are coupled in luminal side membrane and that they are transported into cells as ternary complex.

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REFERENCES

1 Christensen, H. N. and Riggs, T. R. (1952) J. Biol. Chem. 194, 57–68
2 Crane, R. K. (1962) Fed. Proc. 21, 891–895
3 Crane, R. K. (1965) Fed. Proc. 24, 1000–1006
4 Riggs, T. R., Walker, L. M. and Christensen, H. N. (1958) J. Biol. Chem. 233, 1479–1484
5 Vidaver, G. A. (1964) Biochemistry 3, 803–808
6 Eddy, A. A. (1968) Biochem. J. 108, 195–206
7 Eddy, A. A. (1968) Biochem. J. 108, 489–498
8 Schultz, S. G., Fuisz, R. E. and Curran, P. F. (1966) J. Gen. Physiol. 49, 849–866
9 Schultz, S. G., Curran, P. F., Chez, R. A. and Fuisz, R. E. (1967) J. Gen. Physiol. 50, 1241–1260
10 Csaky, T. Z. (1963) Fed. Proc. 22, 3–7
11 Schultz, S. G. and Curran, P. F. (1970) Physiol. Rev. 50, 637–718
12 Burg, M. B. and Orloff, J. (1962) Am. J. Physiol. 203, 327–330
13 Guder, W. and Wieland, O. (1971) Regulation of Gluconeogenesis (Söling, H. D. and Willms, B., eds), pp. 226–237, Thieme-Verlag, Stuttgart, Academic Press, New York-London
14 Nagata, N. and Rasmussen, H. (1970) Biochim. Biophys. Acta 215, 1–16
15 Schafer, J. A. and Jacquez, J. A. (1967) Biochim. Biophys. Acta 135, 1081–1083
16 Whittembury, G. and Proverbio, F. (1970) Pflügers Arch. 316, 1–25
17 Hillman, R. E., Albrecht, I. and Rosenberg, L. E. (1968) J. Biol. Chem. 243, 5566–5571
18 Morel, E. and De Rouffignac, C. (1973) Annu. Rev. Physiol. 35, 17–54
19 Vidaver, G. A. (1964) Biochemistry 3, 662–667
20 Armstrong, W. McD., Byrd, B. J. and Hamang, P. M. (1973) Biochim. Biophys. Acta 330, 237–241
21 Bergeron, M. and Vadeboncoeur, M. (1971) Nephron 8, 355–366

22 Bergeron, M. and Vadeboncoeur, M. (1971) Nephron 8, 367–374
23 Young, J. A. and Freedman, B. S. (1971) Clin. Chem. 17, 245–266
24 Kinne, R., Schmitz, J. E. and Kinne-Suffran, E. (1971) Pflügers Arch. 329, 191–206
25 Schmidt, U. and Dubach, U. C. (1971) Pflügers Arch. 330, 265–370
26 Silbernagl, S. and Deetjen, P. (1971) Pflügers Arch. 323, 342–350
27 Vidaver, G. A. (1964) Biochemistry 3, 799–803
28 Gilles-Baillien, M. and Schoffeniels, E. (1967) Life Sci. 6, 1257–1262
29 Hillman, R. E. and Rosenberg, L. E. (1970) Biochim. Biophys. Acta 211, 318–326