Studies on the Transport Mechanism of Aminoacids in the Renal Tubules

Part I Studies on the Mechanism of Aminoaciduria from the Analytical Standpoint of Titration Curve

MINORU SUGITA, KO SUGITA

TOSHIYUKI FURUKAWA AND HIROSHI ABE

*The First Department of Internal Medicine, Osaka University Hospital,
Osaka University Medical School, Osaka*

(Director : Prof. H. Abe)

THE IMPORTANCE of transport mechanism of a substance across the membrane in a metabolic process has been increasingly recognized. The presence and the clinical significance of "diseases of an abnormal transport system" as one of diseases of metabolic disorders have recently attracted much attention. Aminoacids are essential for the living body and aminoaciduria due to renal tubular cell abnormalities has interested researchers as a subject of molecular level in relation to congenital metabolic disorders.

The maximal rate of tubules to transport a substance can be estimated by applying the clearance concept introduced by VAN SLYKE and MCINTOSH¹⁾ and a quantitative study of reaction of the transport mechanism is possible by a comparison of the transport maximum with the loading amount of a substance. The transport maximum of glucose or para-aminohippurate, for example, is known to be low in parallel to the severity of renal parenchymatous damage.

A titration curve has been used for the analysis of statistical distribution of the transport maximum per nephron²⁾. The authors paid an attention to the difference in reabsorption ratios between healthy and diseased

kidneys, rather than the transport maximum itself. In the present study the presence of a transfer abnormalities which has been left unexplained by hitherto theories was demonstrated. A new classification of aminoaciduria was made from the standpoint of mechanism with a model following an analysis of titration curve.

METHOD

- (1) Titration curves of aminoacid in healthy person and nephritide
 - a) Reabsorption rates and ratios of several neutral aminoacids under aminoacid-mixture loading : Conventional clearance experiments were performed on a normal adult (27-year-old man) and a patient with chronic nephritis (29-year-old woman). A solution containing 2 per cent sodium-thiosulfate in isotonic saline was administered intravenously at a rate of 3 ml/min. When the urine flow became stable, a 20-minute interval was served as the control period. Then the first infusion was replaced with a mixture of 2 per cent sodium-thiosulfate and 10 per cent aminoacid mixture which contained 1-arginine HCl 0.80, 1-histidine HCl H₂O 0.40, 1-isoleucine 0.55, 1-leucine 1.23, 1-lysine HCl 2H₂O 2.23, 1-methionine 0.71, 1-phenylalanine 0.87, 1-threonine 0.54, 1-tryptophan 0.18, 1-valine 0.61 and glycine 1.00 (W/V per cent). One or two 20-minute

(Received for Publication, October 1, 1966)

experimental clearance periods were then begun. In total the control and nephritid were infused with 60 g and 20 g of an aminoacid mixture, respectively.

b) Reabsorption rates and ratios of histidine under 1-histidine loading :

Conventional clearance experiments were carried out on a normal adult (32 year-old man) and a patient with chronic nephritis (27 year-old man) to whom 0.4 per cent inulin in saline was given during the control period and then 10 per cent 1-histidine was replaced with saline. In total the control and nephritide received 20g and 15g of 1-histidine, respectively.

(2) Reabsorption rates and ratios of endogenous aminoacids in aminoaciduria :

Conventional clearance studies of endogenous aminoacids were carried out in two cases of hepatic coma, two cases of phenylketonuria, chronic nephritis, and a normal adult. 60 ml of 10 per cent sodium-thiosulfate was infused intravenously and reabsorption rates and ratios of individual aminoacid were calculated.

All blood samples were centrifuged immediately and the plasma was collected for the determinations of individual or total α -amino N and sodium-thiosulfate or inulin. Total α -amino N was determined following to COCK and YEMM's method³⁾, sodium-thiosulfate by the iodometry⁴⁾ and inulin

Fig. 1. Analysis of amino acids.

by resorcinothiurea⁵⁾.

Plasma samples for individual aminoacid determination were dealt with as shown in Fig. 1 and paperchromatographical techniques were used after each aminoacid was reacted to form corresponding dinitrophenylamido derivatives. Yellow spots were removed, eluted in 4 per cent sodium-bicarbonate solution and spectrophotometric measurements were carried out at the wave length of 380 m μ for proline and hydroxyproline and at 360 m μ for the others. Determination of histidine in 1-histidine loading experiment was done by a Hitachi aminoacid analyzer using a 50 cm column.

TABLE I AMINO ACIDS MIXTURE INFUSION (S. M. 27 Y. O. ♂ NORMAL)

		P	GFR	P·GFR	U	V	U·V	CAA	P·GFR-UV	P·GFR-UV	T
		$\mu\text{M/dl}$	ml/min	$\mu\text{M/min}$	$\mu\text{M/dl}$	ml/min	$\mu\text{M/min}$	ml/min	$\mu\text{M/min}$	$\frac{\text{P·GFR}}{\text{U·V}} \times 100 \%$	$\frac{\text{GFR}}{\text{U·V}} \mu\text{M/dl}$
gly	1	43.0	184	79.2	45.2	1.49	0.67	1.57	78.5	99.2	42.6
	2	95.2	143	136	422	2.05	8.65	9.08	128	95.2	89.7
	3	191	151	288	1070	2.22	23.8	12.4	250	86.8	166
ala	1	39.8	184	73.3	36.8	1.49	0.54	1.38	72.8	99.4	39.6
	2	61.4	143	87.8	55.4	2.05	1.13	1.85	86.7	98.9	60.6
	3	72.3	151	109	59.0	2.22	1.31	1.81	108	99.0	71.6
thr	1	13.4	184	24.6	7.36	1.49	0.11	0.82	24.5	99.5	13.3
	2	31.5	143	45.1	155	2.05	3.18	1.01	41.9	93.0	29.3
	3	49.5	151	74.7	257	2.22	5.71	1.15	69.0	92.3	45.7
phe	1	6.21	184	11.4	5.17	1.49	0.07	1.24	11.3	99.4	6.15
	2	31.0	143	44.4	21.0	2.05	0.43	1.39	44.0	99.2	30.8
	3	95.7	151	145	125	2.22	2.78	2.90	142	97.9	94.3

TABLE II AMINO ACIDS MIXTURE INFUSION (S. H. 29 Y. O. ♀ UREMIA)

		P	GFR	P·GFR	U	V	U·V	CAA	P·GFR-UV	P·GFR-UV	T
		$\mu\text{M/dl}$	ml/min	$\mu\text{M/min}$	$\mu\text{M/ml}$	ml/min	$\mu\text{M/ml}$	ml/min	$\mu\text{M/min}$	$\frac{\text{P·GFR}}{\text{U·V}} \times 100 \%$	$\frac{\text{GFR}}{\text{U·V}} \mu\text{M/dl}$
gly	1	24.2	8.65	2.09	20.8	3.26	0.682	2.81	1.41	67.5	16.3
	2	62.6	1.99	1.25	46.7	0.935	0.436	0.70	0.81	64.8	40.6
ala	1	19.6	8.65	1.69	14.2	3.26	0.463	2.40	1.23	72.8	14.2
	2	34.8	1.99	0.693	21.7	0.935	0.203	0.58	0.490	70.8	24.6
thr	1	7.75	8.65	0.671	7.39	3.26	0.241	3.11	0.430	64.1	4.97
	2	20.8	1.99	0.414	16.4	0.935	0.153	0.74	0.261	63.0	13.1
phe	1	3.25	8.65	0.282	4.28	3.26	0.139	4.28	0.143	50.7	1.65
	2	21.8	1.99	0.434	22.6	0.935	0.212	0.97	0.222	51.0	11.1

Fig. 2. Histidine titration curve.

RESULTS

(1) a) Reabsorption rates and ratios of several neutral aminoacids under aminoacid-mixture loading:

The result is presented in Table I. The period 1 indicates control observations and 2 and 3 show observations made during infusion of an aminoacid-mixture.

Reabsorption ratios of endogenous aminoacids were about 99 per cent and those alanine or phenylalanine did not change until the filtered load reached $110 \mu\text{M}/\text{min}$ or $150 \mu\text{M}/\text{min}$, respectively. As for glycine, reabsorption ratio was found to be decreased and at a level of filtered load of $290 \mu\text{M}/\text{min}$, the reabsorption ratio decreased to 86.8 per cent. Threonine also showed a reduction in reabsorption ratio when the filtered load was only $45 \mu\text{M}/\text{min}$.

In chronic glomerulonephritis, tubular load

was small and a reabsorption rate was low but reabsorption ratios of endogenous individual aminoacid ranged from 50 to 70 per cent suggesting the presence of tubular damage of the aminoacid transport mechanism. (Table II)

To compare the transport ability of a single nephron, relations between filtered load per GFR $\left(\frac{P \cdot \text{GFR}}{\text{GFR}} = P\right)$ and tubular reabsorption rate per GFR $\left(\frac{T}{\text{GFR}}\right)$ were investigated. The relations represent a tubular load to transport rate per unit nephron. In nephritis, $\frac{T}{\text{GFR}}$ value at the same level of P is smaller than normal, suggesting a reduced ability of a single nephron to transport aminoacids. Reabsorption ratios of glycine, alanine, threonine or phenylalanine do not change until the tubular loads reach $2 \mu\text{M}/\text{min}$, $2 \mu\text{M}/\text{min}$, 0.7

Fig. 3. Relation between filtered load and urinary excretion.

μM/min and 0.4 μM/min respectively. (Table II)

b) Reabsorption rates and ratios of histidine under l-histidine loading :

Reabsorption ratio of endogenous histidine in healthy person was about 96 per cent which

tended to decrease as the tubular load was increased to 40 μM/min, being 66 per cent with the load of 77 μM/min. The reabsorption rate gradually approached the transport maximum.

In spite of a small tubular load due to

decreased GFR, the reabsorption ratio was only 52 per cent at the endogenous plasma histidine level in chronic nephritide. The ratio P to $\frac{T}{GFR}$ of the nephritide was also decreased in this case too. A histidine titration curve in chronic nephritide was similar to that of a healthy subject. As the tubular load increased, the reabsorption ratio was decreased and the reabsorption rate approached the transport maximum. (Fig. 2)

(2) Reabsorption rates and ratios of endogenous aminoacids in aminoaciduria :

Relations between filtered load and urinary excretion of endogenous individual aminoacid in normal adult (GFR 184 ml/min), hepatic coma (L-1, GFR 1.1 ml/min) and (L-2, GFR 1.3 ml/min), phenylketonuria (P-1, GFR 112 ml/min) and her young brother (P-2, GFR 84 ml/min), Fanconi syndrome (F-1, GFR 3.7 ml/min) and (F-2, GFR 39 ml/min), and chronic nephritis (GFR 6.8 ml/min) are presented in Fig. 3. In Fanconi syndrome and chronic nephritis, a reduction in the filtered load and increased urinary excretion of almost all sorts of aminoacids, especially those of glycine, threonine and cystine were observed. These findings implied reduced reabsorption rates and ratios in those patients. Hepatic coma showed a similar trend.

Plasma concentrations of phenylalanine in phenylketonuric patients were markedly increased, being 138, and 178 μM /dl and filtered loads reached several times higher than that of normal persons. In those cases, reabsorption ratios at an endogenous plasma level, however, were 98.0 and 99.4 per cent which were almost the same as those of a normal person with phenylalanine loading. The plasma phenylalanine concentration of (P-2) was reduced to 53 μM /dl after feeding a low phenylalanine milk for one week. The reabsorption ratio, however, remained unchanged.

DISCUSSION

For a quantitative analysis of the tubular transport mechanism estimation of transport maximum (T_m) has been widely used. In dogs T_m values of glycine, 1-lysine, and 1-arginine are known⁶⁻⁸⁾ but not of 1-glutamic acid, 1-

leucine, 1-isoleucine, d, 1-alanine, d, 1-methionine, 1-valine, d, 1-threonine, d, 1-phenylalanine, 1-tryptophan, and 1-histidine⁶⁻¹¹⁾. Little has been known about T_m value of aminoacid in humans. Those of glycine, serine and histidine have been reported only¹²⁾¹³⁾. Namely T_m of aminoacids is individually different and many aminoacids remain unknown as to their possession of T_m .

Assuming that a transported substance has T_m value and the kidney consists of nephrons with equal or uniform transport ability for the substance, one can postulate a titration curve as shown in Fig. 4. In the figure, the abscissa shows a loaded amount of the transported substance ($P \cdot GFR$) and the ordinate indicates reabsorbed amount ($P \cdot GFR - UV$). The curve can be expressed in an equation, $Y = X$, if the X (loaded amount) is below T_m . This means that the loaded material is completely reabsorbed from the tubules until the material increases up to a level (X_m), where the carrier is saturated with the material. The ordinate value corresponding to X_m on the abscissa shows T_m for the material ; $Y = T_m$.

This shifting point on the curve is designated as "shoulder". The reabsorption ratio is 100 per cent and it remains constant as far as the filtered load is less than X_m , and is decreased along the equation line : reabsorption ratio is T_m/X beyond X_m .

In some aminoacids, the reabsorbed amount never reaches a plateau level when the loaded amount is increased. In such a case "shoulder" is not clearly observed. This phenomenon is seen under two conditions as follows :

- (1) Equilibrium constant of active transport reaction between the transported material and transport carrier is low.
- (2) Distribution of a nephron population for the aminoacid reabsorption ability is wide.

Regarding the first condition, if one considers the effect of the above mentioned equilibrium constant on the titration curve, transport reaction can be expressed as follows :

where A is the transported material, B being carrier and AB their complex. The presence of T_m is considered to be due to a limited range of the number of the carriers. In addition,

Fig. 4. Relation between filtered load and reabsorption rates and ratios of phenylalanine.

transport velocity of the material should be taken into consideration. For the convenience' sake the above equation can be simplified as follows:

where AB corresponds to the transported amount and is designated as y and also B equals $(c-y)$ where c is the number of carriers and also A corresponds to $(x-y)$ where x is the loaded amount of a substance. Thus the equation is transformed into $K(x-y)(c-y) = y$, where K shows a constant value of each aminoacid and c is a constant value for each aminoacid group having the same transport system. This is illustrated in Fig. 6(a). Fig. 6(b) shows the curve in a condition where K is constant and c is variable.

In relation to the second condition, the concept of nephron population is originally derived from an analysis of the glucose

titration curve. As is well known, glucose is not recovered from the urine under a threshold level of plasma concentration and the equilibrium constant (K) in the tubule is considered to be greatly deviated. In other words, it should theoretically afford a titration curve under the condition where K approaches infinity. The titration curve of glucose in general, however, shows a gentle slope in the vicinity of X_m . To explain the gentle slope, SMITH et al.^{2,14}, introduced the concept of a nephron population on the basis that glucose reabsorption ability of the nephron shows a standard distribution.

When a mixture of multiaminoacids is infused to healthy subjects, "shoulder" of the titration curve is not clearly shown in glycine and threonine. However, the presence of T_m is suggested by findings that the reabsorption ratio is decreased as the loaded amount is in-

Fig. 5. Model of titration curve.

Fig. 6. Model of titration curve.

creased. In alanine and phenylalanine, T_m can not be estimated in a range of 100–140 $\mu\text{M}/\text{min}$ of the loading amount because a reabsorption ratio remains unchanged. In these four aminoacids, a reabsorption ratio fails to reach 100 percent below the level of T_m and their equilibrium constants do not seem to be so much deviated as glucose.

A reabsorption ratio of endogenous plasma aminoacid decreases in diseased kidneys with impaired GFR, and the decreased reabsorption ratio is constant in a certain range of the loading amount.

It has been known that there is a transport competition phenomenon between certain groups of aminoacids. In cases of an aminoacid-mixture loading, an increase of urinary excretion of these four aminoacids may occur due to the competition phenomenon, although the kinetics in this phenomenon is still difficult to treat theoretically at the present time. Therefore, in the present study, the titration curve

was analyzed by a single aminoacid loading for the purpose of eliminating the competition phenomenon.

On an 1-histidine loading to a healthy subject, the presence of T_m was suggested from a decline of reabsorption ratio by an increase of the loading amount, although the reabsorption ratio did not reach 100 per cent even in low plasma concentration. In cases of renal diseases, a reabsorption ratio of 1-histidine was reduced to 52 per cent even at an endogenous plasma level. The above data with similar phenomena in experiments of a multiaminoacid loading suggest the existence of a factor or factors which do not refer to the impairment of the transport reaction system in the pathogenesis of low reabsorption ratio of aminoacids in diseased kidneys. Presently it was also shown that T_m value of aminoacid is decreased in diseased kidneys as T_m of glucose decreases.

From these results, an analysis of the titration curve of aminoacid was attempted by intro-

Fig. 7. Mechanism of Aminoaciduria

Fig. 8. Relation between filtered load and urinary excretion.

ducing a single nephron model. Urinary aminoacids are generally detected in a various amount at an endogenous plasma level, being reflected in an equation $y=ax$ ($0 \leq a \leq 1$) where "a" differs in each aminoacid and is zero in an aminoacid without a threshold. Urinary excretion (UV) of an aminoacid can be calculated by the following equation; $UV=(1-a)x$. In comparison with a healthy kidney, the titration curve of diseased kidney shows a decrease of reabsorption ratio even at an endogenous plasma level of aminoacids. This finding has never been pointed out previously. One of the explanations for changes of the titration curve in chronic nephritide is a decrease of equilibrium constant. However, such decrease seldom

happens, in non-specific renal disease.

A normal distribution curve is obtained by the second differential treatment of the glucose titration curve and this is considered to be due to the presence of the nephron population. On the other hand, the second differential treatment of the shoulder of the histidine titration curve in a case of the renal disease shows no peak suggesting a splay of transport ability of a single nephron until the curve reaches "shoulder".

In cases of the renal diseases with decreased GFR, a considerable decrease in nephrons is reasonably considered. Therefore, the loaded amount per nephron of the diseased kidney should be larger than that of the healthy kidney

if a total amount loading is equal, so that correction is needed of the urinary output of aminoacids before making a comparison. According to the BRIKER's "intact nephron theory"¹⁵⁾, that the function of remaining nephrons is within a normal range and GFR is proportional to the number of functioning nephrons, the loaded amount per nephron is calculated by dividing the whole loaded amount ($P \cdot GFR$) by GFR.

This quotient should be proportional to plasma concentration. Also the urinary output per nephron can be expressed by UV/GFR . In cases of 1-histidine loading the results were reevaluated following this correction. It was found that the urinary output of aminoacids was still greater in a nephritide than that in a healthy subject. (Fig. 8) This will be discussed in the subsequent paper.

From the model mentioned above, aminoacidurias can be classified into two groups from the standpoint of their pathogenesis:

1) Renal aminoaciduria:

An increased urinary output of aminoacid with a normal loading amount is considered to be due to the impairment of transfer reaction that may be caused by a decrease of equilibrium constant (K) of the reaction. This is a molecular level definition of the so-called renal aminoaciduria in a narrow sense.

2) Extrarenal aminoaciduria:

Urinary output of aminoacids is increased due to an excess load, in spite of an intact tubular aminoacid transfer ability.

A reabsorption ratio of aminoacids, unlike the case with glucose, never reaches 100 per cent although a loading amount is less than T_m and aminoacidurias can occur even if the plasma concentration is at a normal level. (Fig. 7) Aminoaciduria seen only in excess load above T_m has been called overflow aminoaciduria. The authors would like to propose a term "overload aminoaciduria" for a case in which abnormal amounts of aminoacids appear in the urine even the load is less than T_m . The authors believe that the conventional term "overflow" aminoaciduria should be used only in a case in which aminoaciduria occurs when the load is more than T_m . As shown in the results of the present study, in uremia and

Fanconi syndrome a marked decrease of reabsorption ratio is shown at the endogenous aminoacid level, which are typical renal aminoacidurias. The aminoaciduria observed in two cases of hepatic coma has been considered to be an overflow type since their plasma aminoacid level is significantly elevated. However a decrease of their GFR is more marked than an increase of plasma aminoacid and the loaded amounts are far below a normal range, in addition to the decrease of reabsorption ratio. Thus the aminoaciduria in hepatic coma should be classified as renal aminoaciduria.

Since in phenylketonuria, the plasma concentration of phenylalanine is much higher than normal and the reabsorption ratio is within normal, this disease should be classified as overload aminoaciduria. In addition, T_m of phenylalanine has not been obtained, so that the term overflow aminoaciduria does not seem applicable to this disease.

SUMMARY

1) It has been revealed that glycine, threonine, and histidine have their own T_m . Experimentally an analysis is made of the titration curve of the aminoacids after loading of aminoacid-mixture and 1-histidine loading to patients with renal diseases and to normal subjects. A reabsorption ratio is decreased in renal disease cases in a range up to the load area of T_m .

2) An increase of loaded amounts of aminoacids resulted in a gentle continuous slope but never "shoulder" on the titration curve. This "shoulder" phenomenon observed on the glucose titration curve is considered to be due to the presence of the nephron population. It is therefore, not referable to aminoacids.

Assuming that the transfer reaction proceeds as follows: $A + B \rightleftharpoons AB \rightarrow B + A$ where A is transferred material B is carrier AB is complex, then, equation, $K(x-y)(c-y)=y$, can be introduced where x corresponds to loaded amount, c the number of carriers, y a transferred amount and K equilibrium constant. If K is of a finite value in the reaction of the first degree, one can not expect "shoulder" phenomenon in the titration curve.

3) A deformed titration curve in cases

of diseased kidney suggests the presence of a decrease of *c* (carrier) and also of an apparent decrease of *K*. Although the decrease of *K* can be expected from transfer enzyme abnormalities in cases of congenital aminoaciduria, it is hard to expect its occurrence in non-specific renal lesions.

4) On the basis of the present data, a new classification is proposed of aminoaciduria. Renal aminoaciduria in a narrow sense is due to abnormalities of the transfer reaction system, manifested by a decrease of *c* (carrier) and *K* (equilibrium constant), characterized by a decrease of *T_m* and reabsorption ratio. In aminoaciduria of hepatic coma cases which has been, formerly grouped in overflow type, abnormalities are pointed out in the transfer reaction system as well as increase of plasma aminoacids. A new term of "overload aminoaciduria" is proposed for cases with a filtered load below *T_m*. Phenylketonuria is considered to be a typical overload aminoaciduria.

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