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Research Article

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Renal Metabolism of Alanine *

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Summary. In the acidotic dog, alanine is extracted from plasma and utilized as a precursor of ammonia. Simultaneously, it is formed *de novo* within tubular cells and added to renal venous blood. When plasma concentration is within a normal range, production of alanine greatly exceeds utilization. Increasing the plasma concentration reduces production and increases utilization of plasma alanine. The infusion of glutamine increases the renal production of alanine without appreciable change in utilization of plasma alanine. These results are consonant with the view that alanine is metabolized by transamination with α-ketoglutarate to form glutamate, which is subsequently deaminated oxidatively to liberate ammonia. Conversely, alanine is formed by transamination of pyruvate with either glutamate or glutamine and is added to renal venous blood. The balance between production and utilization is dependent, at least in part, on the concentrations of the reactants.

Introduction

The intravenous infusion of alanine in acidotic dogs increases the urinary excretion of ammonia (1, 2), a fact which indicates that alanine is a renal precursor of the base. This concept has been extended by the observation that the infusion of alanine into one renal artery of the dog increases ammonia excretion by the infused kidney relative to the control kidney (3). Thus, the nitrogen of alanine must, at least in part, be converted to ammonia within the kidney. The extra ammonia could not arise solely from the transfer of alanine nitrogen to more immediate renal precursors at extrarenal sites. Subsequent studies on both the acidotic dog and man have shown that the kidney normally produces alanine and adds it in net amounts to renal venous blood (4, 5). Two possibilities are evident: either the kidney produces alanine when plasma concentration is normal and utilizes it as a precursor of ammonia when plasma concentration is elevated, or production and utilization proceed simultaneously, possibly varying in their proportions as plasma level changes.

Our studies with ^{15}N–labeled alanine have shown that this plasma amino acid is the nitrogen source of 2 to 8% of the urinary ammonia when plasma concentration is within a normal range. Under these conditions, renal production of alanine considerably exceeds utilization. Thus, alanine is added in net amounts to renal venous blood. However, when plasma alanine is elevated, utilization of this amino acid as a precursor of ammonia increases, and net production diminishes. Infusion of glutamine increases renal production of alanine but has little effect on utilization of plasma alanine as a source of ammonia. These findings are consonant with the view that alanine is both produced and utilized by transamination, and that the balance favors production when the plasma concentration of alanine is normal, whereas it favors utilization when the plasma concentration is elevated.

Methods

The use of ^{15}N-labeled amino acids as tracers in studies of renal ammonia production has been described in detail in previous communications (6, 7). In summary, the specific activity (atoms per cent excess ^{15}N) of plasma alanine, corrected for recirculation, is calculated from the rate of infusion of ^{15}N as labeled alanine and the perfused renal alanine load. The specific activity of the urinary ammonia, similarly corrected for recirculation, is the

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TABLE I

*Utilization of the nitrogen of plasma alanine for the production of urinary ammonia at normal and at elevated arterial plasma concentrations of alanine**

Period	Elapsed time	Urine		Glomerular filtration rate	Renal plasma flow	Arterial plasma alanine	Specific activity arterial plasma alanine†	Specific activity urinary ammonia†	Ammonia excretion	Origin of urinary ammonia			
		Flow	pH							Alanine		Δ from control	All other sources
	minutes	*ml/minute*		*ml/minute*	*ml/minute*	*μmoles/ml*	*atoms % excess*	*atoms % excess*	*μmoles/minute*	*%*	*μmoles/minute*	*μmoles/minute*	*μmoles/minute*
	0	Infuse: 6.76 μmoles/minute alanine (87.6 atoms % ^{15}N excess) into left renal artery throughout experiment											
1	15–20	3.82	5.51	34.6	174	0.319	9.50	0.648	34.2	6.82	2.33		31.9
2	20–25	4.34	5.61	36.2	182	0.320	9.11	0.693	34.9	7.61	2.66		32.2
3	25–30	4.48	5.69	35.7	175	0.321	9.40	0.707	35.8	7.52	2.69		33.1
									35.0	7.32	2.56		32.4
	31	Infuse: 100 μmoles/minute unlabeled alanine intravenously											
4	45–50	4.50	5.79	33.9	154	0.535	6.64	0.720	37.7	10.8	4.07	1.51	33.6
5	50–55	4.40	5.74	34.9	141	0.524	7.35	0.747	37.7	10.2	3.85	1.29	33.8
6	55–60	4.36	5.72	34.8	140	0.513	7.54	0.789	37.5	10.5	3.94	1.38	33.6
									37.6	10.5	3.95	1.39	33.7
	61	Infuse: 300 μmoles/minute unlabeled alanine intravenously											
7	75–80	4.04	5.57	34.7	139	1.36	3.02	0.563	42.4	18.7	7.94	5.38	34.5
8	80–85	4.02	5.58	35.4	129	1.46	3.04	0.611	44.1	2.01	8.86	6.30	35.2
9	85–90	3.98	5.62	34.7	123	1.57	2.96	0.634	43.6	21.4	9.33	6.77	34.3
									43.4	20.1	8.71	6.15	34.7

* Experiment performed on an acidotic dog. All data refer to the left experimental kidney only, except that the specific activity of the urinary ammonia has been corrected for recirculation of label by subtracting the atoms per cent ^{15}N excess of right kidney urine from that of left. See methods and previous publications (6, 7) for correction of specific activity of the arterial plasma alanine.

† Specific activities corrected for recirculation of ^{15}N.

atoms per cent excess of ammonia-^{15}N of the urine produced by the infused kidney minus that produced by the control kidney. The ratio of corrected specific activity of the urinary ammonia to corrected specific activity of the plasma alanine × 100 equals the per cent of the urinary ammonia derived from plasma alanine. We have followed this previously described procedure in the present study with one modification to increase precision. Renal plasma flows have been calculated from hematocrits and measured whole blood flows. Whole blood flows have been derived from rates of excretion and arteriovenous whole blood extractions of para-aminohippurate (PAH) with the Wolf equation (8), which corrects for the volume difference of arterial inflow and venous outflow of the kidney, i.e., urine flow. The use of whole blood PAH measurements obviates the possibility of error due to shift of PAH from cells to plasma in renal venous blood after withdrawal of the sample and before centrifugation (9).

Arterial blood was drawn from the femoral artery through a retention needle fitted with a tight stylet. Renal venous blood was drawn through a radiopaque catheter positioned in the left renal vein under fluoroscopic visualization. A slow drip of heparinized saline kept the catheter patent. A curved 25-gauge needle with shank removed was inserted into the left renal artery close to the aorta and connected to a constant infusion pump through a length of polyethylene tubing. The artery was visualized through a flank incision with minimal manipulation. Both ureters were catheterized at their points of entrance into the bladder.

Creatinine, for measurement of glomerular filtration rate; PAH, for measurement of renal blood flow; and unlabeled alanine or glutamine, as noted, were incorporated in two-thirds isotonic sodium sulfate and administered intravenously at a rate of 5 ml per minute. Alanine-^{15}N was infused into the left renal artery at a rate of 6 to 10 μmoles per minute for 20 minutes before and throughout the course of the experiment.

L-Alanine-^{15}N was prepared from labeled L-glutamate (6) and pyruvate with glutamic–pyruvic transaminase.[1] The alanine was purified by chromatography on a Dowex 1 acetate resin eluted with 0.5 M acetic acid and crystallized from alcohol. The alanine was labeled + 88.0 atoms % ^{15}N. Chemical methods utilized in this study, including amino acid analysis, have been described in previous communications (4, 10). Mongrel dogs of both sexes, approximately 20 kg in weight, were made acidotic by the administration of 10 g per day of ammonium chloride mixed with their food for 3 days before an experiment.

Results

Utilization of alanine as a direct renal precursor of ammonia. A typical experiment designed to quantify the proportion of the urinary ammonia derived from plasma alanine at normal and elevated alanine levels is summarized in Table I. Tracer

[1] C. F. Boehringer and Sons, Mannheim, Germany.

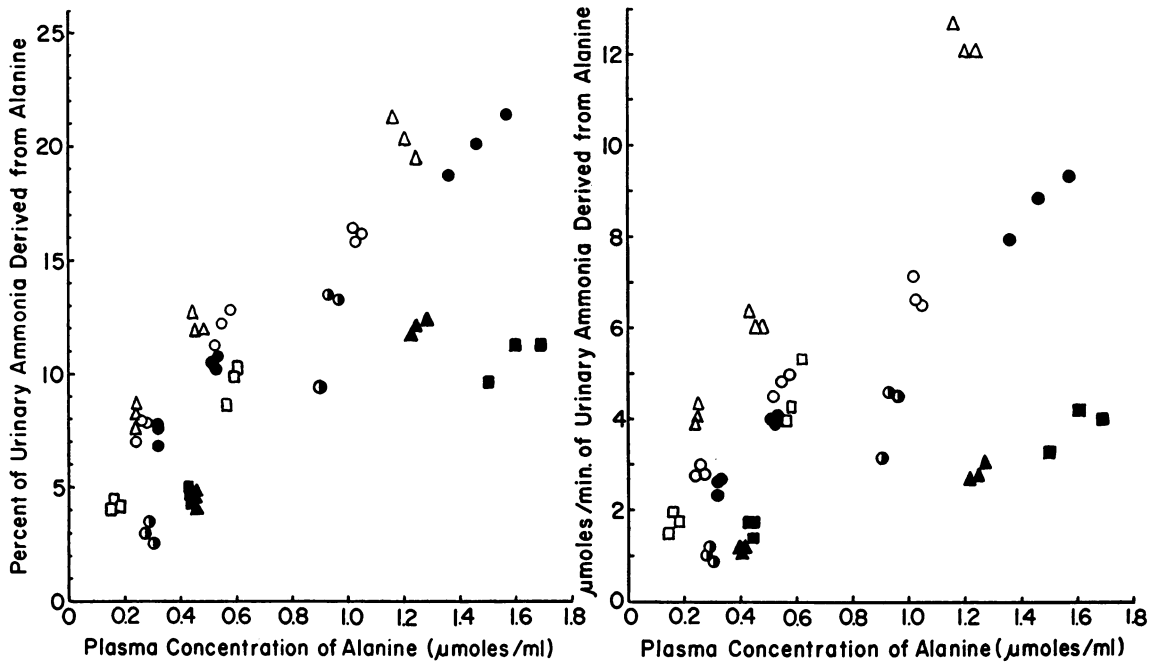

FIG. 1. LEFT: RELATIONSHIP BETWEEN ARTERIAL PLASMA CONCENTRATION OF ALANINE AND THE PER CENT OF THE URINARY AMMONIA DERIVED FROM PLASMA ALANINE. RIGHT: RELATIONSHIP BETWEEN ARTERIAL PLASMA CONCENTRATION OF ALANINE AND THE QUANTITY OF URINARY AMMONIA DERIVED FROM PLASMA ALANINE. Each of seven experiments is represented by a different symbol. Arterial plasma alanine concentration was increased above control value of 0.2 to 0.4 μmole per ml by the intravenous infusion of unlabeled alanine.

amounts of labeled alanine were infused at a constant rate into the left renal artery throughout the entire experiment. During the first three clearance periods, while plasma alanine was normal, 7.32% of the 35 μmoles of ammonia excreted per minute by the left kidney was derived from circulating plasma alanine. The intravenous infusion of 100 μmoles of unlabeled alanine per minute, to raise plasma concentration modestly from 0.320 to 0.524 μmoles per ml, resulted in increased total production of ammonia and increased percentage contribution of plasma alanine. Further elevation of plasma concentration by the infusion of 300 μmoles per minute of unlabeled alanine resulted in even more pronounced increase in excretion of ammonia and further increase in the contribution of plasma alanine to 20% of the total. Although in this experiment the ammonia derived from all sources other than plasma alanine increased from 32.4 to 34.7 μmoles per minute, this was not a constant finding. In other experiments, a decrease or no change was observed. Accordingly, we believe that in the severely acidotic dog, provision of increased amounts of exogenous alanine does not significantly alter the utilization of other endogenous substrates as precursors of ammonia. This statement obviously applies only to the limited range of plasma concentration included in our studies.

Figure 1 summarizes data obtained in a series of seven experiments similar to the one presented in Table I. In all experiments, elevation of plasma level resulted in an increase in the percentage contribution of plasma alanine to urinary ammonia and in the total quantity of plasma alanine utilized as a precursor of urinary ammonia. Although the scatter of the massed data is great, in each experiment, which is designated by a different symbol, the relationship is essentially a linear one.

Simultaneous renal production and utilization of alanine. In a previous study (6), we observed that the kidney of the acidotic dog invariably extracts glutamine and glycine from the plasma and adds alanine and serine to the plasma in net amounts. On the average, the kidney neither extracts from nor adds to the plasma significant

TABLE II

*Simultaneous utilization and production of alanine at normal and elevated arterial plasma concentrations of alanine**

Period	Elapsed time	Urine		Glomerular filtration rate	Renal plasma flow	Urinary ammonia excretion	Amino acid	Arterial plasma concentration	Renal venous plasma concentration	Net extraction	Ammonia from alanine		Minimal net alanine production
		Flow	pH										
	minutes	*ml/minute*		*ml/minute*	*ml/minute*	*μmoles/minute*		*μmoles/ml*	*μmoles/ml*	*μmoles/minute*	*%*	*μmoles/minute*	*μmoles/minute*
	0	Infuse: 6.49 μmoles per minute alanine (90.3 atoms % ^{15}N excess) into left renal artery throughout experiment											
1	20–25	1.86	5.43	37.4	176	32.9	Threonine	0.211	0.217	−0.9			
							Serine	0.091	0.129	−6.6			
							Glutamine	0.368	0.254	+20.3			
							Glycine	0.146	0.133	+2.4			
							Alanine	0.300	0.403	−11.3	2.58	0.85	12.2
2	25–30	1.84	5.42	38.0	183	33.9	Threonine	0.206	0.209	−0.4			
							Serine	0.091	0.126	−5.1			
							Glutamine	0.345	0.237	+20.0			
							Glycine	0.141	0.128	+2.5			
							Alanine	0.286	0.385	−11.3	3.53	1.20	12.5
3	30–35	2.00	5.45	37.5	184	34.0	Threonine	0.200	0.201	0.0			
							Serine	0.090	0.123	−5.9			
							Glutamine	0.322	0.219	+19.2			
							Glycine	0.135	0.123	+2.3			
							Alanine	0.271	0.367	−10.9	3.00	1.02	11.9
	37	Infuse: 300 μmoles per minute unlabeled alanine intravenously											
4	55–60	2.52	5.51	37.9	183	33.0	Threonine	0.207	0.217	−1.5			
							Serine	0.104	0.140	−6.4			
							Glutamine	0.325	0.233	+17.2			
							Glycine	0.131	0.127	+1.0			
							Alanine	0.907	0.980	−5.4	9.44	3.12	8.5
5	60–65	2.84	5.53	39.7	184	34.1	Threonine	0.202	0.211	−1.2			
							Serine	0.103	0.139	−6.3			
							Glutamine	0.322	0.227	+18.0			
							Glycine	0.128	0.118	+2.1			
							Alanine	0.933	1.008	−5.5	13.5	4.60	10.1
6	65–70	2.96	5.57	38.0	173	34.3	Threonine	0.196	0.205	−1.1			
							Serine	0.101	0.138	−6.1			
							Glutamine	0.318	0.220	+17.4			
							Glycine	0.124	0.108	+2.9			
							Alanine	0.959	1.035	−4.5	13.3	4.56	9.1

* Concurrent studies of net extraction (+) or net production (−extraction) of glutamine, glycine, serine, and threonine. Experiment performed on an acidotic dog. All data refer to left experimental kidney only, except as noted in first footnote to Table I.

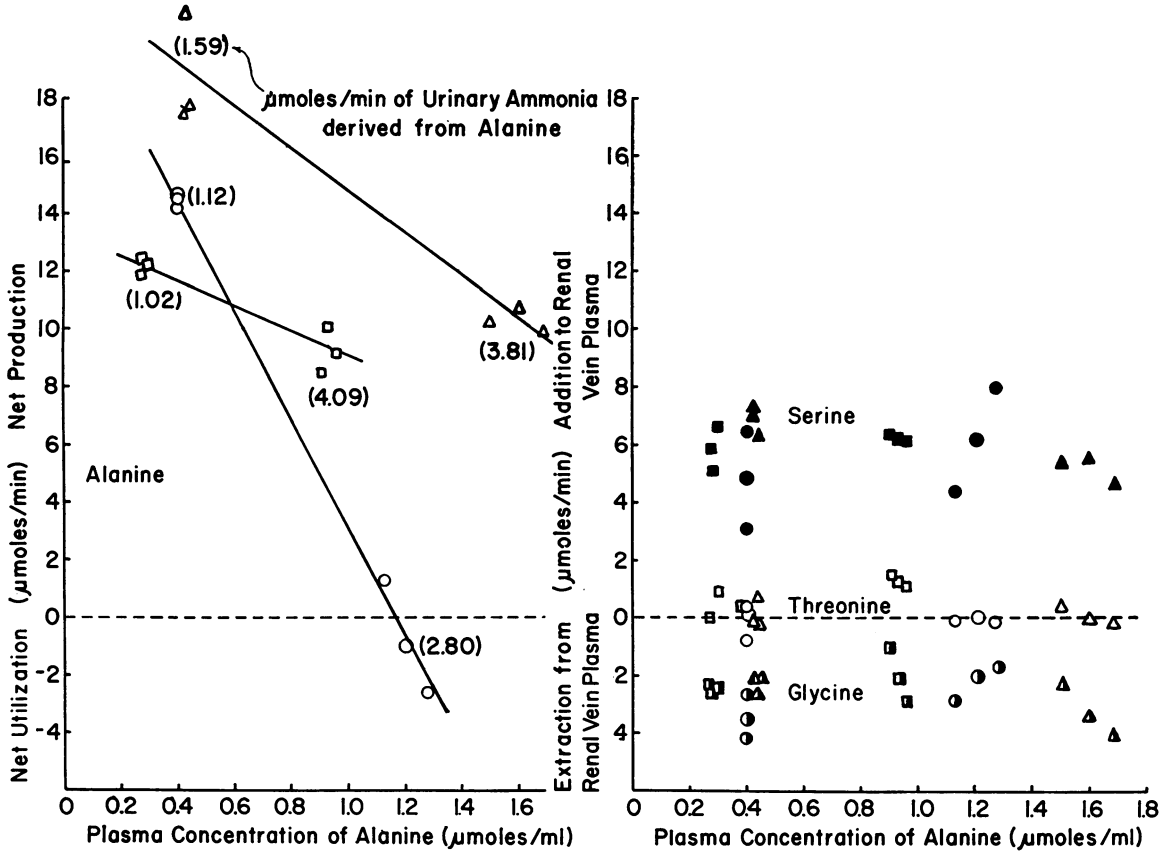

FIG. 2. LEFT: RELATIONSHIP BETWEEN PLASMA CONCENTRATION OF ALANINE AND MINIMAL NET RENAL PRODUCTION OF ALANINE. Figures in parentheses are rates of production of urinary ammonia from plasma alanine. Arterial plasma alanine was increased above control values of 0.2 to 0.4 μmole per ml by the intravenous infusion of unlabeled alanine.

RIGHT: SIMULTANEOUSLY OBSERVED RATES OF ADDITION OF SERINE TO RENAL VENOUS PLASMA, EXTRACTION OF GLYCINE FROM RENAL VENOUS PLASMA, AND EQUIVALENCE OF RENAL INFLOW AND OUTFLOW OF THREONINE AS FUNCTIONS OF ARTERIAL PLASMA ALANINE CONCENTRATION.

amounts of threonine. Since these observations had been made only under conditions of normal plasma concentrations of alanine, they were repeated in three experiments in which plasma alanine was within a normal range during the initial three clearance periods and elevated by the infusion of unlabeled alanine during the final three periods.

Table II summarizes the data obtained in one such experiment. ^{15}N–labeled alanine (90.3 atoms % excess) was infused at a rate of 6.49 μmoles per minute into the left renal artery throughout the experiment. During periods 1 to 3, the plasma concentration of alanine was normal. During periods 4 to 6, it was elevated by the intravenous infusion of unlabeled alanine at a rate of 300 μmoles per minute. It is evident that some 3% of the urinary ammonia, approximately 1.0 μmole per minute, was derived from plasma alanine nitrogen when plasma concentration was within a normal range. Simultaneously, some 11 μmoles per minute of alanine was added to renal venous plasma. The minimal rate of production of alanine was therefore 12 μmoles per minute. When plasma concentration was increased threefold by the infusion of alanine, some 13% of urinary ammonia, 4.5 μmoles per minute, was derived from plasma alanine nitrogen. Only about 5 μmoles per minute of alanine was added to renal venous plasma under these circumstances. The minimal rate of

production [2] of alanine, accordingly, diminished modestly from 12 to 9 μmoles per minute. This was the least change in production of alanine observed in any of the three experiments.

It is evident that serine was produced in net amounts (negative extraction) and that negative extraction [3] was unaffected by increasing plasma alanine. The same may be said of the positive extraction of glycine. The small reduction in net extraction of glutamine in consequence of increased plasma alanine was not a constant finding in the other experiments. On the average, no change occurred. Although in this experiment very small quantities of threonine were apparently produced by the kidney, this finding also was not a constant one. Accordingly, we believe that no significant quantities of threonine are either extracted from or added to plasma by the kidney, either at normal or elevated plasma concentrations of alanine.

Figure 2 summarizes data obtained in experiments similar to the one presented in Table II. On the left, net production of alanine is plotted as a function of plasma concentration of alanine. The figures in parentheses are rates of utilization of plasma alanine nitrogen in the production of urinary ammonia, expressed in micromoles per minute. It is evident that an increase in plasma alanine concentration is routinely associated with a decrease in renal alanine production and an increase in the utilization of plasma alanine nitrogen as a source of ammonia. These facts are rendered more significant by data presented in Figure 2, right, derived from these same experiments. Thus, the production of serine, the extraction of glycine, and the equivalence of renal inflow and outflow of threonine are unaltered by an increase in plasma concentration of alanine.

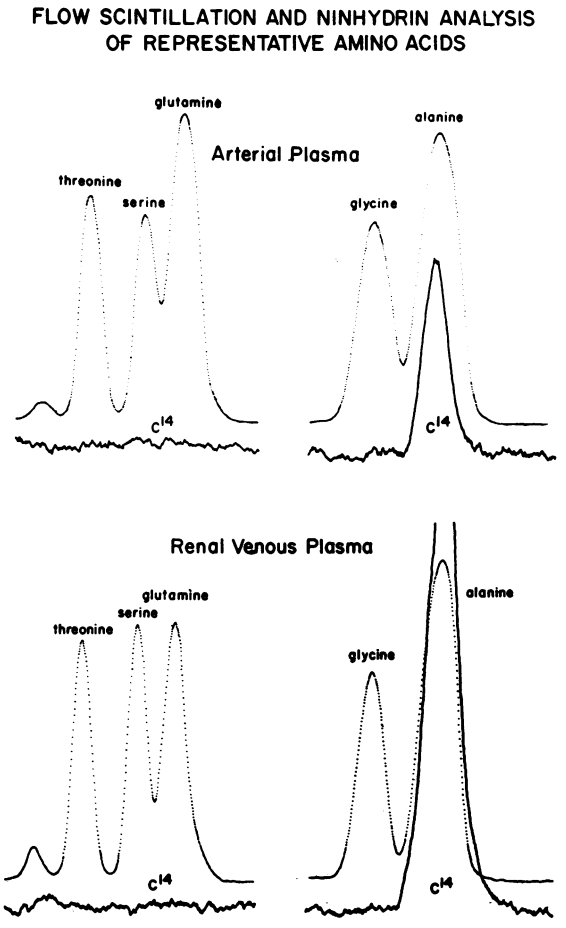

FIG. 3. NINHYDRIN COLORIMETRIC AND ^{14}C ACTIVITY CHROMATOGRAMS OF ARTERIAL AND RENAL VENOUS PLASMAS DURING ADMINISTRATION OF CARBOXYL-^{14}C-LABELED PYRUVATE INTO THE LEFT RENAL ARTERY AT A RATE OF 1.0 μC PER MINUTE.

Transamination in the renal production of alanine. The data presented above are consonant with the view that the production of alanine and its utilization as a source of ammonia nitrogen involve the reversible reaction of transamination. Figure 3 includes segments of the colorimetric Ninhydrin and ^{14}C flow scintillation ratemeter

[2] Minimal net alanine production is the sum of the alanine added to renal venous blood (— net extraction) and the amount of alanine utilized as a source of urinary ammonia. Since ammonia derived from alanine is no doubt also added to renal venous blood, a datum not measured in these experiments, the value for production is a minimal one. Furthermore, if plasma alanine were diverted to the formation of other amino acids or peptides, the true production of alanine would be still greater.

[3] Net extraction of an amino acid is calculated as the difference between the arterial plasma load into the kidney and the renal venous plasma load out of the kidney. The arterial plasma load is the product of renal arterial plasma inflow (milliliters per minute) and arterial plasma concentration (micromoles per milliliter) plus rate of intra-arterial infusion (micromoles per minute), the latter only of alanine. The renal venous plasma load is the product of renal venous plasma concentration (micromoles per milliliter) and renal venous plasma outflow (milliliters per minute). Renal venous plasma outflow equals arterial inflow, plus rate of intra-arterial infusion, minus urine flow, all in milliliters per minute. The Wolf equation (8) used in calculating renal plasma flow gives arterial inflow.

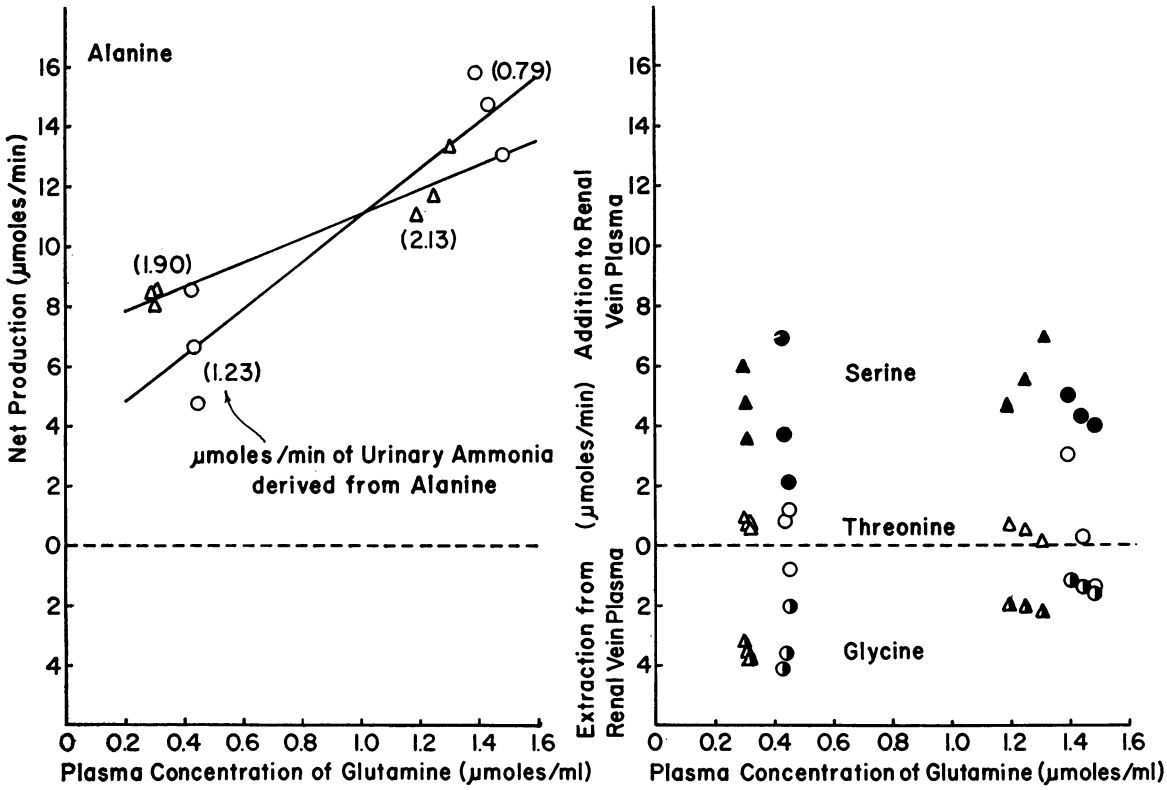

FIG. 4. LEFT: RELATIONSHIP BETWEEN ARTERIAL PLASMA CONCENTRATION OF GLUTAMINE AND MINIMAL NET RENAL PRODUCTION OF ALANINE. Figures in parentheses are rates of production of urinary ammonia from plasma alanine. Arterial plasma glutamine was increased above control values of 0.3 to 0.4 μmole per ml by the intravenous infusion of unlabeled glutamine.

RIGHT: SIMULTANEOUSLY OBSERVED RATES OF ADDITION OF SERINE TO RENAL VENOUS PLASMA, EXTRACTION OF GLYCINE FROM RENAL VENOUS PLASMA, AND EQUIVALENCE OF RENAL INFLOW AND OUTFLOW OF THREONINE AS FUNCTIONS OF ARTERIAL PLASMA GLUTAMINE CONCENTRATION.

chromatograms of arterial and renal venous plasmas from an experiment in which carboxyl-^{14}C–labeled pyruvate was infused into one renal artery of an acidotic dog at a rate of 1.0 μc per minute.[4] It is apparent that the ^{14}C of pyruvate was incorporated into the alanine that the kidney added to renal venous plasma. Thus, the rate-meter trace that recorded ^{14}C activity was off scale for venous alanine in comparison with arterial alanine. None of the pyruvate carboxyl-^{14}C appeared in threonine, serine, glutamine, or glycine. This experiment demonstrates that alanine is formed in the kidney from pyruvate and added to renal venous blood, and again is consonant with, although it does not prove, transamination as the biochemical pathway of production.

Figure 4 summarizes data obtained in two experiments similar to those presented in Table II and Figure 2, except that unlabeled glutamine rather than alanine was administered intravenously at a rate of 300 μmoles per minute. The rationale of these experiments was that if alanine is formed by the intrarenal transamination of pyruvate by glutamate, provision of excess glutamate might increase production of alanine. Glutamine was given rather than glutamate because of its more ready entry into renal tubular cells. It is evident, in accord with our expectations, that alanine production increased with increased load of glutamine, whereas the production of serine, the utilization of glycine, and the equivalence of renal inflow and outflow of threonine were unchanged.

[4] We are indebted to Dr. George Frimpter for the chromatograms and ^{14}C flow scintillation records of this experiment.

Elevation of plasma glutamine at constant plasma alanine did not appreciably alter the rate of production of ammonia from plasma alanine (figures in parentheses).

Discussion

After the work of Braunstein and Kritzmann (11), which demonstrated the ubiquity of tissue transaminases, the concept has developed that those amino acids which contribute their amino nitrogen as urinary ammonia do so indirectly by transaminating α-ketoglutarate to form glutamate (2). Glutamate is subsequently deaminated oxidatively to yield ammonia and regenerate α-ketoglutarate. This view is supported by the presence in renal tissue of glutamic dehydrogenase (12) in high activity and by the virtual absence of those L-amino acid oxidases (13) which might directly liberate the nitrogen of amino acids as ammonia. Our experiments on the utilization and production of alanine by the intact functioning kidney of the acidotic dog support and amplify this concept of the role of transamination in the metabolism of amino acids.

Since the transfer of the nitrogen of alanine to α-ketoglutarate to form glutamate is a reversible reaction involving only minor changes in free energy, the direction of the reaction, i.e., net production or net utilization of alanine, should be influenced to a major degree by the concentrations of the reactants. At normal plasma alanine concentrations, the kidney of the acidotic dog produces some 5 to 10 times as much alanine as it utilizes as a source of urinary ammonia. This excess alanine is added to renal venous blood. When plasma alanine is increased, utilization of plasma alanine as a precursor of ammonia increases and production diminishes. Minimal net production also decreases.

In previous work (6, 7) we have demonstrated that 35 to 50% of the urinary ammonia is derived from the amide nitrogen of glutamine extracted from the plasma. Only half of the amino nitrogen of the glutamine so extracted appears as urinary ammonia. The remaining half no doubt supplies the nitrogen that is added to renal venous blood as alanine and serine (4, 14, 15). There are two possible pathways by which this transfer might occur. Pyruvate and hydroxypyruvate might transaminate directly with the amino nitrogen of glutamine (glutaminase II reaction) to form alanine and serine and α-ketoglutaramate (16). The α-ketoglutaramate subsequently would liberate its amide nitrogen as ammonia (ω–amidase reaction). On the other hand, glutamine might liberate its amide nitrogen as ammonia by way of the glutaminase I reaction to form glutamate. Glutamate could then transaminate with pyruvate and hydroxypyruvate to form alanine and serine. Our finding that the infusion of glutamine increases the renal production of alanine could have either of the above explanations. Additionally, the increased production of α-ketoglutarate by either of the above pathways could increase the availability of pyruvate (17) and hence account for the increased production of alanine. It is evident, in accord with our previous findings, that provision of excess alanine like provision of excess glycine and glutamine (7) increases the utilization of the more abundant amino acid as a precursor of ammonia.

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