Glutamine synthesis from aspartate in guinea-pig renal cortex

Gabriel BAVEREL,* Guy MARTIN and Christian MICHOUDET

Laboratoire de Physiologie Rénale et Métabolique, INSERM U80 et CNRS URA 1177, Faculté de Médecine Alexis Carrel, 69008 Lyon, France

1. Glutamine was found to be the main carbon and nitrogen product of the metabolism of aspartate in isolated guinea-pig kidney-cortex tubules. Glutamate, ammonia and alanine were only minor products. 2. Carbon-balance calculations and the release of $^{14}\text{CO}_2$ from $[\text{U-}^{14}\text{C}]$ aspartate indicate that oxidation of the aspartate carbon skeleton occurred. 3. A pathway involving aspartate aminotransferase, glutamate dehydrogenase, glutamine synthetase, phosphoenolpyruvate carboxykinase, pyruvate kinase, pyruvate dehydrogenase and enzymes of the tricarboxylic acid cycle is proposed for the conversion of aspartate into glutamine. 4. Evidence for this pathway was obtained by: (i) inhibiting aspartate removal by amino-oxyacetate, an inhibitor of transaminases, (ii) the use of methionine sulphoximine, an inhibitor of glutamine synthetase, which induced a large increase in ammonia release from aspartate, (iii) the use of quinolinate, an inhibitor of phosphoenolpyruvate carboxykinase, which inhibited glutamine synthesis from aspartate, (iv) the use of α -cyano-4-hydroxycinnamate, an inhibitor of the mitochondrial transport of pyruvate, which caused an accumulation of pyruvate from aspartate, and (v) the use of fluoroacetate, an inhibitor of aconitase, which inhibited glutamine synthesis with concomitant accumulation of citrate from aspartate.

INTRODUCTION

It is well established that aspartate, which is filtered through the renal glomerular barrier, is almost completely re-absorbed by the renal tubule [1]. Although its endogenous blood concentration is low, this amino acid has been shown by arteriovenous-difference measurements to be extracted from circulating blood by the kidney of various species including man, sheep and rabbit [2–4]. A significant renal extraction of aspartate has also been demonstrated in the dog when the concentration of plasma amino acids was increased to post-prandial levels [5]. It is therefore of physiological and nutritional interest to determine the renal metabolic fate and pathways of both aspartate nitrogen and carbon fragments.

In a previous paper [6], we have shown that, under appropriate experimental conditions, aspartate was synthesized from glutamate by isolated guinea-pig kidney-cortex tubules. Since aspartate aminotransferase (EC 2.6.1.1) is a near-equilibrium enzyme, we considered that aspartate could also be a substrate for the guinea-pig renal cortex. In the present study, we have tested this hypothesis and determined the metabolic fate of aspartate in this tissue. The data obtained do not support the conclusion of Krebs [7] that aspartate cannot be converted into glutamine in guinea-pig renal cortex; on the contrary, glutamine was found to be the main end-product of aspartate metabolism in this tissue. A pathway for such a conversion is proposed, and experimental evidence for this proposal presented, but we failed to find the reason why Krebs [7] did not observe glutamine synthesis from aspartate in this tissue.

MATERIALS AND METHODS

Guinea pigs

Guinea pigs (400–500 g), of the Dunkin–Hartley albino strain, were obtained from Iffa-Credo, St. Germain-sur-l'Arbresle, France, and were fed on a standard diet (U.A.R., Villemoisson-sur-Orge, France). Unless otherwise stated, all experiments

reported below were performed with kidney tubules from fed guinea pigs.

Reagents

L-Aspartate, D-aspartate, glutaminase (grade V), α -cyano-4-hydroxycinnamic acid, amino-oxyacetic acid, quinolinic acid, monofluoroacetic acid and methionine sulphoximine were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Other enzymes and coenzymes were supplied by Boehringer (Meylan, France). L- $[\text{U-}^{14}\text{C}]$ Aspartic acid (200 mCi/mmol) was obtained from the C.E.A. (Gif-sur-Yvette, France).

Preparation of kidney-cortex tubules and incubations

Kidney-cortex tubules were prepared by collagenase treatment of renal-cortex slices as described by Baverel *et al.* [8]. Incubations other than those involving radioactive substrates were performed at 37 °C in a shaking water bath, in 25 ml stoppered Erlenmeyer flasks in an atmosphere of O_2/CO_2 (19:1). The flasks contained 1 ml of the tubule suspension plus 3 ml of Krebs–Henseleit medium [9] supplemented or not with substrates or inhibitors. In all experiments, each experimental condition was performed in duplicate. Incubation was stopped by adding HClO_4 (final concn. 2%, v/v) to each flask. Metabolite assays were conducted on the neutralized supernatant. When radioactive aspartate (0.2 μCi /flask) was the substrate, incubation, deproteinization, collection and measurement of the $^{14}\text{CO}_2$ formed were performed as described by Baverel & Lund [10].

Metabolite assays

Aspartate, glutamate, glutamine, alanine, ammonia, lactate, pyruvate, glucose, glycogen, citrate, 2-oxoglutarate, fumarate, malate and the dry weight of tubules added to the flasks were determined as described previously [6,8]. Asparagine was determined by the method of Bergmeyer *et al.* [11].

Calculations

Net substrate utilization and product formation were calculated as the difference between the total contents of

* To whom correspondence should be addressed.

the flask (tissue + medium) at the start (zero-time flasks) and after the period of incubation. The metabolic rates, reported as means $\pm$ S.E.M., are expressed in μ mol of substance removed or produced per g dry wt. of tubule fragments per unit time (30 or 60 min).

The metabolic fate of aspartate was established by calculating carbon and nitrogen balance. An estimate of oxidation of aspartate was obtained by carbon-balance calculation by using the following formula:

$$\text{Aspartate (in } C_4 \text{ units) oxidized} = \frac{4\text{ASP} - [5(\text{GLU} + \text{GLN}) + 3\text{ALA}]}{4}$$

where ASP is the rate of aspartate removal and GLU, GLN and ALA are the rates of formation of glutamate, glutamine and alanine respectively.

The rates of conversion of L-[U- 14 C]aspartate into $^{14}\text{CO}_2$ were calculated by dividing the radioactivity in $^{14}\text{CO}_2$ by the specific radioactivity of the labelled aspartate determined in the zero-time samples for each experiment.

RESULTS AND DISCUSSION

Metabolic fate of aspartate carbon and nitrogen

With 5 mM-L-aspartate as substrate, aspartate utilization was linear with time (Table 1). The high rate of aspartate removal was accompanied by a high rate of glutamine formation, which was also linear with time over a 60 min incubation period. Only small amounts of glutamate, ammonia and alanine accumulated. No accumulation of pyruvate, lactate, glucose, glycogen, or intermediates of the tricarboxylic acid cycle was observed (results not shown). Surprisingly, despite the presence of a high activity of asparagine synthetase in guinea-pig renal cortex [12], no asparagine was synthesized in the presence of aspartate and glutamine (formed from aspartate), the two substrates of this enzyme.

The nitrogen-balance calculations, given in Table 1, show that the amount of nitrogen found as glutamine, glutamate, ammonia and alanine is greater than the amount of nitrogen removed as aspartate; this indicates that the glutamine formation from endogenous sources, which is observed in the absence of added substrate (see Table 1), still occurs, at least partly, in the presence of aspartate as substrate. However, in the presence of aspartate as substrate, the very large amount of glutamine formed, which exceeds several-fold the amount of glutamine formed from endogenous sources in the absence of aspartate, clearly indicates that aspartate must be the precursor of both the carbon skeleton and the nitrogen groups of the glutamine formed. Neglecting the formation of glutamine from endogenous sources when aspartate is the added substrate, carbon- and nitrogen-balance calculations from the data of Table 1 show that the carbon skeleton of

glutamine and its nitrogenous groups accounted for 48.9% and 78.2% respectively of the carbon skeleton and amino group removed as aspartate after 30 min of incubation; the corresponding values were 56.5% and 90.4% after 60 min of incubation.

The carbon-balance calculations from the data given in Table 1 also reveal that about 30% of the aspartate carbon removed was not accounted for by the non-volatile carbon products found, but was presumably oxidized. However, because of the formation of glutamine from endogenous sources as mentioned above, which occurs even in the presence of aspartate, the latter value (30%) is probably underestimated. This view is supported by the results obtained in an experimental series in which the conversion of 5 mM-L-[U- 14 C]aspartate into $^{14}\text{CO}_2$ represented 42.8% of the aspartate removed after a 60 min incubation period ($n = 5$).

The present results, which demonstrate that, in guinea-pig kidney tubules, glutamine is the main carbon and nitrogenous end product of aspartate metabolism, contradict those obtained by Krebs [7] and by Weil-Malherbe & Krebs [13], who concluded from their classical studies on renal glutamine synthesis that only glutamate, proline and hydroxyproline, but not aspartate, could give rise to glutamine formation in renal-cortex slices of the guinea pig.

Routes for the formation of glutamine from aspartate

The finding in the present study that most of the removal of aspartate (an amino acid whose molecule contains four carbon

Scheme 1. Pathway of glutamine synthesis from aspartate in guinea-pig kidney tubules

The numbers in parentheses indicate sites of inhibition: (1) of aspartate aminotransferase by amino-oxacetate, (2) of glutamine synthetase by methionine sulfoximine, (3) of phosphoenolpyruvate carboxykinase by quinolinate, (4) of mitochondrial pyruvate transport by α -cyano-4-hydroxycinnamate, (5) of aconitase by mono-fluoroacetate.

Table 1. Metabolism of 5 mM-L-aspartate in guinea-pig kidney tubules

Kidney tubules were incubated as described in the Materials and methods section. Values (μ mol/g dry wt.) for metabolite removal (–) or production (+) are reported as means $\pm$ S.E.M. for 4 experiments. Each flask contained 22.2 ± 1.7 mg dry wt. of tissue. Carbon and nitrogen balances were calculated as the sum of the carbon and nitrogenous substances removed minus the sum of those produced (in these calculations a – sign indicates a deficit and a + sign an excess of carbon skeletons or nitrogen found as compared with those removed).

Substrate added	Incubation (min)	Metabolite removal or production					Carbon balance (C_4 units)	Nitrogen balance
		Aspartate	Glutamate	Glutamine	Ammonia	Alanine		
L-Aspartate (5 mM)	30	-419.5 ± 25.4	$+63.6 \pm 6.0$	$+164.0 \pm 16.8$	$+44.0 \pm 7.5$	$+18.2 \pm 1.6$	-121.4	$+34.3$
None	30	-2.0 ± 0.3	-2.2 ± 1.0	$+47.1 \pm 9.8$	$+6.0 \pm 0.9$	$+2.6 \pm 0.1$	$+56.1$	$+98.6$
L-Aspartate (5 mM)	60	-757.4 ± 39.6	$+71.1 \pm 9.8$	$+342.4 \pm 22.8$	$+73.3 \pm 12.4$	$+18.1 \pm 1.4$	-227.0	$+89.9$
None	60	-1.2 ± 0.3	$+0.9 \pm 1.5$	$+83.4 \pm 12.9$	$+14.8 \pm 3.5$	$+5.4 \pm 2.9$	$+108.2$	$+186.7$

Table 2. Effect of amino-oxyacetate, methionine sulphoximine and NH_4Cl on the metabolism of 5 mM-L-aspartate in guinea-pig kidney tubules

Kidney tubules (18.9 ± 0.7 , 20.3 ± 1.1 and 20.2 ± 0.3 mg dry wt. per flask in the three successive experimental series, respectively) were incubated as described in the Materials and methods section. Results ($\mu\text{mol/g}$ dry wt.) for metabolite removal (–) or production (+) are reported as means $\pm$ S.E.M. for 4 experiments. Statistical difference was measured by the paired Student's *t* test against the control with aspartate alone: **P* < 0.05; ***P* < 0.01; ****P* < 0.001; N.S., statistically non-significant.

Experimental condition	Metabolite removal or production				
	Aspartate	Alanine	Ammonia	Glutamate	Glutamine
L-Aspartate (5 mM)	–726.5 $\pm$ 14.2	+11.7 $\pm$ 0.6	+113.0 $\pm$ 1.0	+63.6 $\pm$ 3.3	+290.9 $\pm$ 4.3
L-Aspartate (5 mM) + amino-oxyacetate (0.5 mM)	–74.9 $\pm$ 27.9***	+8.8 $\pm$ 0.5*	+96.6 $\pm$ 3.7*	+13.5 $\pm$ 1.0**	+21.6 $\pm$ 2.6***
L-Aspartate (5 mM)	–761.6 $\pm$ 42.0	+15.9 $\pm$ 1.6	+46.1 $\pm$ 21.0	+65.5 $\pm$ 5.1	+321.3 $\pm$ 16.6
L-Aspartate (5 mM) + methionine sulphoximine (5 mM)	–615.5 $\pm$ 33.7***	+46.0 $\pm$ 3.6***	+371.6 $\pm$ 44.7**	+222.1 $\pm$ 2.8***	–4.7 $\pm$ 3.5***
L-Aspartate (5 mM)	–763.9 $\pm$ 24.5	+16.9 $\pm$ 3.0	+81.3 $\pm$ 18.5	+85.3 $\pm$ 10.6	+331.1 $\pm$ 29.9
L-Aspartate (5 mM) + NH_4Cl (5 mM)	–617.8 $\pm$ 30.6**	+32.1 $\pm$ 4.6**	+4.6 $\pm$ 11.1 (N.S.)	+10.1 $\pm$ 8.1**	+230.0 $\pm$ 20.5**

atoms and one of nitrogen) is accounted for by the formation of glutamine (an amino acid whose molecule contains five carbon atoms and two nitrogen atoms) raises the question of which pathways are involved in the metabolism of both the amino group and the carbon skeleton of aspartate. The sequence of reactions proposed to explain the conversion of aspartate into glutamine is shown in Scheme 1. In this sequence of reactions, two aspartate molecules give one glutamine molecule plus three CO_2 molecules. In agreement with this proposal is the observation that the amount of glutamine synthesized by the tubules is about half the aspartate removed (see Table 1).

Role of aspartate aminotransferase. Table 2 shows that amino-oxyacetate, a well-established inhibitor of transaminases [14], decreased by about 90 % the utilization of aspartate in guinea-pig kidney tubules. This observation indicates that, in these tubules, aspartate metabolism is probably exclusively initiated by aspartate aminotransferase and not by the purine nucleotide cycle, whose involvement in ammoniogenesis has been proposed by others in the rat kidney [15,16].

Role of glutamate dehydrogenase. Of the two glutamate molecules formed by the transamination of two aspartate molecules with two 2-oxoglutarate molecules, one can be converted into glutamine by glutamine synthetase, which is very active in guinea-pig renal cortex [6,7]. Evidence is presented in Table 2 that glutamate dehydrogenase, which plays a major role in glutamate metabolism in guinea-pig tubules [6], provides from the second glutamate molecule available the ammonia needed for the formation of the glutamine amide nitrogen. The suppression of glutamine synthesis by methionine sulphoximine, a potent inhibitor of glutamine synthetase [17], was accompanied by a large accumulation of the ammonia released by glutamate dehydrogenase, whose role was masked by the very efficient operation of glutamine synthetase.

The addition of aspartate (5 mM) plus NH_4Cl (5 mM) did not increase glutamine synthesis, as has been observed when glutamate was the substrate [6], and no ammonia was removed (Table 2); on the contrary, both glutamine synthesis and aspartate removal were inhibited. This probably resulted from the decrease in the availability of 2-oxoglutarate for the aspartate aminotransferase reaction secondary to the end-product inhibition of glutamate dehydrogenase by high concentrations of ammonia, as previously demonstrated with glutamate as substrate [6]; calcu-

lations from the data of Table 2 show that flux through glutamate dehydrogenase, taken as the glutamine plus the ammonia found, decreased from a mean value of $412.4 \mu\text{mol/g}$ dry wt. with aspartate alone to a mean value of $234.6 \mu\text{mol/g}$ dry wt. of tubule fragments with aspartate plus NH_4Cl .

Conversion of oxaloacetate into 2-oxoglutarate. In the sequence of reactions proposed, one molecule of 2-oxoglutarate is required for each molecule of aspartate transaminated. Since two aspartate molecules are metabolized to form one glutamine molecule, two molecules of 2-oxoglutarate are also needed. Thanks to the glutamate dehydrogenase reaction, one is re-formed in a cyclic way. Therefore, another molecule of 2-oxoglutarate must be synthesized from aspartate, which is the only exogenous carbon precursor. Since two molecules of oxaloacetate are formed during the transamination of two molecules of aspartate, the absence of accumulation of pyruvate, lactate, glucose or glycogen led us to consider oxaloacetate as the possible source of the second molecule of 2-oxoglutarate needed for glutamine synthesis. The only pathway for the formation of 2-oxoglutarate from oxaloacetate appeared to involve the operation of phosphoenolpyruvate carboxykinase (EC 4.1.1.32), pyruvate kinase (EC 2.7.1.40) and the pyruvate dehydrogenase complex, allowing citrate to be formed via citrate synthase (EC 4.1.3.7) as well as the operation of the enzymes of the tricarboxylic cycle responsible for the conversion of citrate into 2-oxoglutarate.

Such a pathway, in which citrate formation (besides alanine accumulation, which is negligible) is the only way to remove the oxaloacetate formed from aspartate, implies that the rates of utilization of oxaloacetate through the combined action of phosphoenolpyruvate carboxykinase, pyruvate kinase and pyruvate dehydrogenase on the one hand and through citrate synthase on the other hand are identical.

Effect of quinolinate. Addition of quinolinate, an inhibitor of phosphoenolpyruvate carboxykinase [18] whose inhibitory effect is established in guinea-pig renal cortex [6], caused a large inhibition of both aspartate removal and glutamine synthesis (Table 3), indicating that phosphoenolpyruvate carboxykinase plays a role in the provision of the 2-oxoglutarate needed for the aspartate aminotransferase reaction.

Effect of α -cyano-4-hydroxycinnamate. Table 3 shows that α -cyano-4-hydroxycinnamate, a potent inhibitor of pyruvate trans-

Table 3. Effect of quinolinate, α -cyano-4-hydroxycinnamate and monofluoroacetate on the metabolism of 5 mM-L-aspartate in guinea-pig kidney tubules

Kidney tubules (21.6 ± 2.0 , 17.6 ± 2.4 and 21.6 ± 2.0 mg dry wt. per flask in the three successive experimental series, respectively) were incubated for 60 min as described in the Materials and methods section. Results ($\mu\text{mol/g dry wt.}$) for metabolite removal (–) or production (+) are reported as means $\pm$ S.E.M. for 4 experiments. Statistical difference was measured by the paired Student's *t* test against the control with aspartate alone: **P* < 0.05; ***P* < 0.01; ****P* < 0.001; N.D., not determined; N.S., statistically non-significant.

Experimental condition	Metabolite removal or production						
	Aspartate	Alanine	Ammonia	Glutamate	Glutamine	Pyruvate	Lactate
L-Aspartate (5 mM)	–733.0 $\pm$ 35.0	+15.1 $\pm$ 1.1	+92.6 $\pm$ 15.9	+59.4 $\pm$ 5.2	+310.6 $\pm$ 4.9	N.D.	N.D.
L-Aspartate (5 mM) + quinolinate (5 mM)	–436.7 $\pm$ 25.3***	+7.3 $\pm$ 0.6**	+95.9 $\pm$ 14.7 (N.S.)	+57.4 $\pm$ 5.4 (N.S.)	+195.3 $\pm$ 5.3***	N.D.	N.D.
L-Aspartate (5 mM)	–785.8 $\pm$ 18.7	+13.2 $\pm$ 13.2	+126.4 $\pm$ 30.1	+76.4 $\pm$ 7.2	+310.7 $\pm$ 13.8	+2.0 $\pm$ 1.1	–3.1 $\pm$ 1.1
L-Aspartate (5 mM) + α -cyano-4-hydroxycinnamate (2.5 mM)	–577.1 $\pm$ 15.3**	+15.7 $\pm$ 1.4 (N.S.)	+276.5 $\pm$ 26.6***	+65.0 $\pm$ 5.4 (N.S.)	+155.6 $\pm$ 15.1***	+229.5 $\pm$ 23.9**	+32.8 $\pm$ 6.4**
L-Aspartate (5 mM)	–733.0 $\pm$ 35.0	+15.1 $\pm$ 1.1	+92.6 $\pm$ 15.9	+59.4 $\pm$ 5.2	+310.6 $\pm$ 4.9	N.D.	N.D.
L-Aspartate (5 mM) + monofluoroacetate (1 mM)	–229.1 $\pm$ 2.5**	+14.7 $\pm$ 2.2 (N.S.)	+186.4 $\pm$ 7.3**	+30.8 $\pm$ 4.9 (N.S.)	+28.5 $\pm$ 4.4***	N.D.	+1.6 $\pm$ 1.1
							+107.1 $\pm$ 18.2***

port across the mitochondrial membrane [19], caused a large accumulation of pyruvate in tubules metabolizing aspartate; this effect was accompanied by an inhibition of both aspartate removal and glutamine synthesis. This observation clearly demonstrates that pyruvate and the mitochondrial pyruvate transporter are involved in the conversion of aspartate into glutamine in guinea-pig kidney tubules. The small accumulation of lactate caused by α -cyano-4-hydroxycinnamate suggests that very little reducing equivalents were available in the cytosol at the level of lactate dehydrogenase. The small accumulation of alanine caused by α -cyano-4-hydroxycinnamate, despite a large availability of extramitochondrial pyruvate, is in agreement with the preferential localization of alanine aminotransferase in the mitochondria [20]. The large accumulation of ammonia from aspartate observed in the presence of α -cyano-4-hydroxycinnamate can be explained by a preferential utilization by glutamate dehydrogenase of the glutamate formed by aspartate transaminase to compensate for the decreased provision of 2-oxoglutarate secondary to pyruvate accumulation.

Effect of fluoroacetate. The results of Table 3 show that fluoroacetate, an inhibitor of aconitase [21], caused an almost complete inhibition of glutamine synthesis from aspartate; as expected, this effect was accompanied by citrate accumulation as well as by a decrease in aspartate removal. These data provide evidence that citrate and aconitase are on the pathway of aspartate conversion into glutamine. In the presence of monofluoroacetate, almost all the glutamate formed by the aspartate aminotransferase reaction was metabolized by glutamate dehydrogenase without concomitant glutamine synthesis, which explains why monofluoroacetate caused an accumulation of ammonia from aspartate (see Table 3).

Effect of D-aspartate

In an attempt to identify the reasons why Krebs [7] failed to demonstrate the synthesis of glutamine from aspartate in guinea-pig renal-cortex slices, we first thought that he used DL-aspartate as substrate and that, in some way, D-aspartate might have inhibited the conversion of L-aspartate into glutamine. But D-aspartate, which was probably metabolized by guinea-pig kidney tubules, because it caused an accumulation of ammonia and glutamate and a small increase in glutamine synthesis when it was the sole added substrate (Table 4), only slightly inhibited the synthesis of glutamine from L-aspartate.

Incubation of tubules and slices in phosphate medium

Careful examination of the experimental conditions used by Krebs [22] revealed that incubation occurred in phosphate-buffered saline medium with an atmosphere of pure O_2 . As shown in Table 5, this does not explain why Krebs [7] did not observe glutamine synthesis from aspartate, because guinea-pig kidney tubules incubated under identical conditions formed glutamine from aspartate at rates about 11 times those observed from endogenous substrates in the absence of added aspartate. Taking a fresh-weight/dry-weight ratio of 4.95 for the guinea-pig renal cortex [6], slices (300 mg fresh wt. per flask) incubated for 30 min under the same experimental conditions were found to synthesize glutamine from 5 mM-L-aspartate at a rate of $74.4 \mu\text{mol/g dry wt. per flask}$ ($n = 2$; results not shown); this is only 2.2 times less than glutamine synthesis from L-aspartate by tubules after 30 min of incubation (see Table 1); in the absence of exogenous substrate, the corresponding value was $10.7 \mu\text{mol/g dry wt per flask}$. Thus the reason why Krebs [7] concluded that aspartate is not a precursor of glutamine in guinea-pig renal cortex remains to be identified.

Table 4. Effect of D-aspartate on the metabolism of 5 mM-L-aspartate in guinea-pig kidney tubules

Kidney tubules (21.9 ± 1.7 mg dry wt. per flask) were incubated for 60 min as described in the Materials and methods section. Results ($\mu\text{mol/g}$ dry wt.) for metabolite removal (–) or production (+) are reported as means $\pm$ S.E.M. for 4 experiments. Statistical difference was measured by the paired Student's *t* test against the control without D-aspartate: **P* < 0.05; ***P* < 0.01; ****P* < 0.001; N.S., statistically non-significant.

Substrate added	Metabolite removed or production				
	L-Aspartate	Alanine	Ammonia	Glutamate	Glutamine
L-Aspartate (5 mM)	-756.4 ± 10.4	$+19.2 \pm 0.2$	$+75.6 \pm 0.1$	$+82.0 \pm 0.9$	$+335.4 \pm 13.3$
L-Aspartate (5 mM) + D-aspartate (5 mM)	$-605.1 \pm 16.6^{***}$	$+23.9 \pm 1.1^*$	$+120.4 \pm 7.3^{**}$	$+116.7 \pm 3.9^{**}$	$+301.4 \pm 9.4^{**}$
None	-1.4 ± 0.2	$+2.5 \pm 0.1$	$+12.7 \pm 0.4$	-0.4 ± 0.2	$+103.0 \pm 3.5$
D-Aspartate (5 mM)	-0.8 ± 3.0 (N.S.)	$+8.3 \pm 1.5^*$	$+118.4 \pm 5.7^{***}$	$+33.2 \pm 2.3^{***}$	$+111.0 \pm 2.9^{**}$

Table 5. Metabolism of 5 mM-L-aspartate in guinea-pig kidney tubules incubated in phosphate/saline buffer

Kidney tubules (24.4 ± 3.1 mg dry wt. per flask), previously washed three times and resuspended in phosphate/saline buffer, pH 7.4 [22], were incubated in 4 ml of the same buffer in an atmosphere of pure O_2 . Results ($\mu\text{mol/g}$ dry wt.) for metabolite removal (–) or production (+) are reported as means $\pm$ S.E.M. for 4 experiments; N.D., not determined.

Substrate added	Metabolite removal or production				
	Aspartate	Alanine	Ammonia	Glutamate	Glutamine
L-Aspartate (5 mM)	-573.6 ± 23.2	$+16.0 \pm 2.4$	$+128.0 \pm 8.7$	$+38.8 \pm 1.5$	$+231.4 \pm 2.2$
None	N.D.	$+0.5 \pm 0.5$	$+63.0 \pm 0.6$	-5.3 ± 1.2	$+22.0 \pm 3.6$

Synthesis of glutamine from other amino acids

The results given in Table 6 show that, besides glutamate and proline, whose conversion into glutamine in guinea-pig renal cortex is known since the work of Krebs [7] and Weil-Malherbe & Krebs [13], aspartate and alanine are the two other amino acids converted into glutamine at high rates. Small but statistically significant amounts of glutamine were also formed from arginine, asparagine and ornithine, but not from the other amino acids tested.

Physiological significance

The observation in the present study that, in guinea-pig renal cortex, the metabolism of both the carbon skeleton and the amino group of aspartate is almost completely directed to the synthesis of glutamine is of great interest. Such a synthesis may represent one mechanism by which, *in vivo*, the guinea-pig kidney, which produces an alkaline urine [23] and therefore does not need to excrete large amounts of ammonia, prevents or limits the synthesis and subsequent release into the renal vein of

Table 6. Glutamine synthesis from various L-amino acids in guinea-pig kidney tubules

Kidney tubules (14.8 ± 1.0 mg dry wt. per flask) were incubated as described in the Materials and methods section in the absence or the presence of various amino acids (5 mM). Results ($\mu\text{mol/g}$ dry wt.) for metabolite removal (–) or production (+) are reported as means $\pm$ S.E.M. for 4 experiments. Statistical difference was measured by the paired Student's *t* test against the control without any added substrate: **P* < 0.05; ***P* < 0.01; ****P* < 0.001; N.S., statistically non-significant.

Amino acid	Metabolite removal or production		
	Ammonia	Glutamate	Glutamine
None	$+24.3 \pm 1.9$	-3.1 ± 0.4	$+40.7 \pm 1.2$
Proline	$-7.7 \pm 0.5^{***}$	$+103.5 \pm 8.2^{**}$	$+252.8 \pm 6.3^{***}$
Hydroxyproline	$+6.7 \pm 0.8^{***}$	$+20.7 \pm 2.0^{**}$	$+50.4 \pm 2.0$ (N.S.)
Histidine	$+58.1 \pm 1.1^{***}$	-2.7 ± 0.5 (N.S.)	$+21.8 \pm 2.2^{**}$
Arginine	$+41.5 \pm 1.8^{***}$	-2.6 ± 0.6 (N.S.)	$+60.3 \pm 2.1^{**}$
Asparagine	$+126.1 \pm 12.3^{**}$	$+3.1 \pm 1.4^*$	$+69.9 \pm 6.7^*$
Cysteine	$+96.8 \pm 1.8^{***}$	-1.9 ± 0.8 (N.S.)	$-4.3 \pm 1.6^{***}$
Ornithine	$+56.4 \pm 2.5^{**}$	$+9.0 \pm 0.7^{**}$	$+79.4 \pm 1.8^{***}$
Methionine	$+61.7 \pm 1.3^{**}$	$+6.3 \pm 2.0^*$	$+37.4 \pm 0.7$ (N.S.)
Valine	$+19.4 \pm 1.0^*$	$+6.6 \pm 2.1^*$	$+42.5 \pm 2.0$ (N.S.)
Leucine	$+75.8 \pm 1.3^{***}$	$+12.1 \pm 2.1^{**}$	$+45.7 \pm 1.5$ (N.S.)
Isoleucine	$+8.0 \pm 0.6^{**}$	$+6.6 \pm 2.0^*$	$+46.0 \pm 2.6$ (N.S.)
Alanine	$+63.1 \pm 6.8^*$	$+9.3 \pm 0.6^{***}$	$+317.0 \pm 8.1^{***}$
Aspartate	$+96.3 \pm 10.1^{**}$	$+59.2 \pm 3.8^{***}$	$+266.7 \pm 7.3^{***}$
Glutamate	$+25.4 \pm 19.9$ (N.S.)	$-578.1 \pm 21.9^{***}$	$+313.6 \pm 13.9^{***}$

ammonia, a potentially harmful compound. The great efficiency of such a mechanism probably explains why no glucose was found to be formed from aspartate in the present study (even in tubules from 48 h-fasted guinea pigs; $n = 2$; results not shown), despite the presence of gluconeogenic enzymes in this tissue [24] and the demonstration of gluconeogenic capacity of guinea-pig kidney tubules [6].

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