Glutamine synthesis from glucose and ammonium chloride by guinea-pig kidney tubules

Christian MICHOUDET, Marie-France CHAUVIN and Gabriel BAVEREL*

Physiopathologie Métabolique et Rénale et Spectroscopie RMN, CNRS E.P. 18, Faculté de Médecine Alexis Carrel, Lyon, France

1. At a physiological concentration (5 mM), glucose was found to be metabolized by isolated kidney cortex tubules prepared from fed guinea pigs. 2. The release of $^{14}\text{CO}_2$ from [U- ^{14}C]glucose indicated that oxidation of the glucose carbon skeleton represented about 50 % of the glucose removed; significant amounts of lactate and glutamine also accumulated. 3. Addition of 0.1–10 mM NH_4Cl led to a dose-dependent stimulation of glucose metabolism which was accompanied by a large increase in lactate and glutamine accumulation and, to a lesser extent, in glucose oxidation. 4. Comparison of the release of $^{14}\text{CO}_2$ from [1- ^{14}C] and [6- ^{14}C]glucose indicates that, in both the absence and the presence of NH_4Cl , the pentose phosphate shunt was only a minor pathway of glucose metabolism. 5. The central role of

pyruvate carboxylase in the conversion of glucose carbon into glutamine carbon was demonstrated by using a bicarbonate-free medium and measuring the fixation of $^{14}\text{CO}_2$ from [^{14}C]bicarbonate, which was recovered mostly at C-1 of glutamine plus glutamate. 6. The NH_4Cl -induced stimulation of glucose removal was secondary not only to increased glutamine synthesis, as shown by the effect of methionine sulfoximine, an inhibitor of glutamine synthetase, but also to the stimulation of phosphofructokinase activity by NH_4Cl . 7. Renal arterio-venous difference measurements revealed that, *in vivo*, the guinea-pig kidney removed glucose from the circulating blood, which suggests that glucose carbon may contribute to the carbon skeleton of the glutamine released by this organ.

INTRODUCTION

In previous studies [1,2], we have shown that, although they contain high activities of gluconeogenic enzymes [3] and although they form large amounts of oxaloacetate from alanine and aspartate, which are metabolized at high rates, guinea-pig kidney-cortex tubules do not synthesize glucose from these substrates even after 48 h of starvation. Since glucose has been shown to be synthesized from other substrates such as lactate and pyruvate in guinea-pig renal cortex [4], this led us to consider the possibility that the synthesis of glucose from alanine or aspartate in kidney tubules could be balanced by a simultaneous high glycolytic activity.

In the present study we have therefore tested the hypothesis that glucose could be a substrate for the guinea-pig renal cortex. The data obtained demonstrate that this is indeed the case *in vitro* and that the guinea-pig kidney also consumes glucose *in vivo*. They also show that addition of ammonium chloride, which greatly stimulates glucose utilization by guinea-pig kidney tubules, leads to a large production of glutamine. Furthermore, it is demonstrated that pyruvate carboxylase plays a critical role in the conversion of glucose carbon into glutamine carbon.

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). All experiments were performed with kidney tubules from fed guinea pigs. Access to water was not limited.

Reagents

Glutaminase (grade V), glutamate decarboxylase (grade V) and methionine sulfoximine were from Sigma Chemical Co. (St.

Louis, MO, U.S.A.). Other enzymes and coenzymes were supplied by Boehringer (Meylan, France). D-[U- ^{14}C]glucose (1–5 mCi/mmol), D-[1- ^{14}C]glucose (5–10 mCi/mmol), D-[6- ^{14}C]glucose (2–10 mCi/mmol) and $\text{NaH}^{14}\text{CO}_3$ (40–60 mCi/mmol) were obtained from C.E.A. (Gif-sur-Yvette, France).

Preparation of kidney tubules and incubations

Kidney-cortex tubules were prepared by collagenase treatment of renal-cortex slices as described by Baverel et al. [5]. Incubations other than those involving radioactive substrates were performed for 30 or 60 min 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 [6], supplemented or not with substrates or inhibitors. In all experiments, incubations with each experimental condition were performed in duplicate. Incubations were stopped by adding HClO_4 (final concn. 2 %, v/v) to each flask. Metabolite assays were conducted on the neutralized supernatant. In all experiments, zero-time flasks, with and without substrates, were prepared by adding HClO_4 before the tubules. When radioactive glucose or radioactive bicarbonate was present in the medium, incubation, deproteinization, and collection and measurement of the $^{14}\text{CO}_2$ formed were performed as described by Baverel and Lund [7]. After removal of the denatured protein by centrifugation, the supernatant was neutralized with 20 % (w/v) KOH for metabolite determination and measurement of $^{14}\text{CO}_2$ fixation.

Measurement of enzyme activities

Pieces of guinea-pig renal cortex were rapidly excised, weighed and homogenized in 10 vol. of ice-cold 0.25 M sucrose medium (pH 7.2) buffered with 5 mM Tris/HCl and 0.5 mM EDTA in a Potter–Elvehjem glass homogenizer with a motor-driven Teflon

* To whom correspondence should be addressed: Laboratoire de Physiopathologie Métabolique et Rénale, Faculté de Médecine Alexis Carrel, 12 Rue Guillaume Paradin, 69372 Lyon Cedex 08, France.

Table 1 Effect of various concentrations of NH_4Cl on the metabolism of 5 mM D-[U- ^{14}C]glucose in guinea-pig kidney tubules

Kidney tubules (21.6 ± 2.5 mg dry wt. per flask) were incubated with 5 mM [U- ^{14}C]glucose plus the indicated concentration of NH_4Cl for 60 min as described in the Materials and methods section. Values ($\mu\text{mol/g}$ dry wt.) for metabolite removal (—) or production are reported as means $\pm$ S.E.M. for four experiments performed in duplicate. $^{14}\text{CO}_2$ means conversion of [U- ^{14}C]glucose into $^{14}\text{CO}_2$ (in μmol of glucose equivalents). Statistical differences were measured by the paired Student's *t* test against the control without NH_4Cl : * $P < 0.05$; ** $P < 0.01$. The carbon balance was calculated as described in the Materials and methods section.

[NH_4Cl] (mM)	Metabolite removal or production ($\mu\text{mol/g}$)						Carbon balance
	Glucose	Pyruvate	Lactate	Glutamate	Glutamine	$^{14}\text{CO}_2$	
0	-177.4 ± 11.3	4.7 ± 0.5	83.6 ± 15.0	13.2 ± 3.3	40.7 ± 3.5	82.7 ± 4.3	-3.4 ± 8.6
0.1	$-210.4 \pm 9.1^*$	5.8 ± 0.9	$149.3 \pm 29.8^*$	10.7 ± 3.2	$53.2 \pm 3.1^{**}$	88.5 ± 3.2	-19.6 ± 4.9
0.5	$-313.5 \pm 11.0^{**}$	4.1 ± 0.4	$300.2 \pm 25.5^{**}$	$-0.4 \pm 0.7^{**}$	$89.8 \pm 3.4^{**}$	$92.8 \pm 4.6^*$	-20.9 ± 8.8
1.0	$-368.7 \pm 14.4^{**}$	3.8 ± 0.5	$368.5 \pm 22.6^{**}$	$-0.2 \pm 1.1^{**}$	$106.9 \pm 3.8^{**}$	$99.7 \pm 3.9^{**}$	-23.9 ± 15.2
2.5	$-436.6 \pm 13.5^{**}$	3.7 ± 0.3	$438.2 \pm 25.7^{**}$	$1.1 \pm 1.3^{**}$	$121.0 \pm 8.7^{**}$	$101.1 \pm 14.1^{**}$	-7.6 ± 12.4
5.0	$-477.0 \pm 15.9^{**}$	3.9 ± 0.4	$471.1 \pm 20.4^{**}$	$4.4 \pm 2.0^{**}$	$119.9 \pm 10.0^{**}$	$105.0 \pm 5.0^{**}$	10.2 ± 10.3
10	$-506.9 \pm 24.4^{**}$	4.5 ± 0.5	$542.8 \pm 4.4^{**}$	$8.7 \pm 2.1^*$	$107.8 \pm 26.9^{**}$	$105.9 \pm 3.3^{**}$	10.8 ± 16.3

pestle. The homogenates were centrifuged at 38000 *g* for 15 min at 2 °C. The supernatant was immediately used for measurement of the activities of hexokinase (EC 2.7.1.1) as described by Bergmeyer et al. [8], phosphofructokinase (EC 2.7.1.11) as described by Bergmeyer et al. [9] and pyruvate kinase (EC 2.7.1.40) by the method of Gutmann and Bernt [10]. Measurements of enzyme activities were performed at 37 °C in the presence of 100 mM KCl and in ammonium-ion-free media; for this, the commercial enzyme preparations used were dialysed for 48 h against 4×1 litre of distilled water. One unit of enzyme activity is defined as the amount of enzyme that transforms 1 μmol of substrate/min under the conditions used.

Analytical methods

Glucose, glycogen, lactate, pyruvate, glutamate, glutamine, alanine, aspartate, citrate, 2-oxoglutarate, fumarate, malate and the dry weight of tubules added to the flasks were determined as described previously [11]. Fixation of $^{14}\text{CO}_2$ from [^{14}C]bicarbonate was calculated by dividing the acid-stable radioactivity present in the incubation medium after incubation by the specific radioactivity of the total 'bicarbonate + CO_2 ' pool measured in the zero-time flasks. The measurement of the radiolabelled bicarbonate carbon incorporation into C-1 of glutamate and glutamine was determined by the method described by Squires and Brosnan [12]. The tubular concentration of fructose 2,6-bisphosphate was measured at the end of the incubation by the method of Van Schaftingen et al. [13] in tubule extracts prepared by homogenizing tubule pellets (obtained by centrifugation at 4000 *g* for 2 min) in 3 ml of 0.1 M NaOH.

Calculations

Net substrate utilization and product formation were calculated as the difference between the total flask contents (tissue plus 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 as μmol of substance removed or produced per unit time (30 or 60 min) per *g* dry wt. of tubule fragments.

The rates of release of $^{14}\text{CO}_2$ from D-[1- ^{14}C]glucose and D-[6- ^{14}C]glucose and conversion of D-[U- ^{14}C]glucose into $^{14}\text{CO}_2$ (in μmol of glucose equivalents) were calculated by dividing the radioactivity (c.p.m.) in $^{14}\text{CO}_2$ by the specific radioactivity of the labelled glucose determined in the zero-time samples for each experiment.

When [U- ^{14}C]glucose was added as substrate, the carbon balance was calculated by using the following formula:

$$GLC - [(PYR + LAC + ALA)/2 + GLU + GLN + ^{14}\text{CO}_2]$$

where *GLC* is the rate of glucose removal; *PYR*, *LAC*, *ALA*, *GLU* and *GLN* are the rates of pyruvate, lactate, alanine, glutamate and glutamine accumulation respectively; and $^{14}\text{CO}_2$ is the rate of conversion of [U- ^{14}C]glucose into $^{14}\text{CO}_2$ (in μmol of glucose equivalents).

When unlabelled glucose was used as the substrate, the carbon balance was calculated by using the above formula, but without $^{14}\text{CO}_2$. A negative carbon balance means that the sum of the products measured (in μmol of glucose equivalents) exceeds the amount of glucose removed. In these calculations, it was assumed that one glucose molecule yields two pyruvate, lactate or alanine molecules, and one glutamate or glutamine molecule.

The nitrogen balance was calculated as the difference between the nitrogen removed and the nitrogen found as alanine, glutamate and glutamine (two nitrogen atoms per glutamine molecule) and, under certain conditions, as NH_4^+ . A negative nitrogen balance indicates that the nitrogen found as nitrogenous intermediates or end products exceeds the nitrogen removed.

RESULTS AND DISCUSSION

Metabolism of glucose by guinea-pig kidney tubules: effect of various concentrations of NH_4Cl

Table 1 shows that, at a physiological concentration, glucose was readily used as a substrate by guinea-pig kidney-cortex tubules, and Table 2 shows that glucose utilization was approximately linear with time. Thus, like rabbit kidney-cortex tubules [14], guinea-pig kidney tubules have the capacity of both synthesizing [4,11,15] and degrading glucose. [U- ^{14}C]Glucose utilization was accompanied by lactate and glutamine formation and by $^{14}\text{CO}_2$ release, which represented about half the glucose used (Tables 1 and 2). The fact that the accumulation of lactate was less after 60 min than after 30 min of incubation (Table 2) indicates that lactate re-utilization occurred during the second half of the incubation period.

The data presented in Tables 1 and 2 also show that addition of both low (physiological) and high concentrations of NH_4Cl caused a dose-dependent stimulation of glucose utilization which was more than doubled in the presence of the highest concen-

Table 2 Time course of the effect of 5 mM NH₄Cl on the metabolism of 5 mM D-glucose in guinea-pig kidney tubules

Kidney tubules (24.7 ± 0.9 mg dry wt. per flask) were incubated as described in the Materials and methods section. Values ($\mu\text{mol/g dry wt.}$) for metabolite removal (—) or production are reported as means $\pm$ S.E.M. for four experiments performed in duplicate. Statistical differences were measured by the paired Student's *t* test against the control without NH₄Cl: *P < 0.05; **P < 0.01. The carbon and nitrogen balances were calculated as described in the Materials and methods section.

Substrate(s) added	Incubation period (min)	Metabolite removal or production ($\mu\text{mol/g}$)					Glutamine	NH ₄ ⁺	Carbon balance	Nitrogen balance
		Glucose	Pyruvate	Lactate	Glutamate	Glutamine				
Glucose (5 mM)	30	—98.5 $\pm$ 5.7	5.4 $\pm$ 0.9	62.0 $\pm$ 7.1	8.2 $\pm$ 1.8	33.1 $\pm$ 0.9	—6.9 $\pm$ 1.2	23.5 $\pm$ 2.4	—67.5 $\pm$ 0.5	
Glucose (5 mM)	60	—160.7 $\pm$ 8.8	4.3 $\pm$ 0.8	48.9 $\pm$ 3.0	12.4 $\pm$ 4.2	56.7 $\pm$ 2.0	—7.0 $\pm$ 1.0	65.0 $\pm$ 9.6	—118.8 $\pm$ 1.0	
Glucose (5 mM) + NH ₄ Cl (5 mM)	30	—289.3 $\pm$ 12.6**	4.8 $\pm$ 0.6	300.4 $\pm$ 7.3**	0.5 $\pm$ 0.7*	99.7 $\pm$ 1.7**	—146.1 $\pm$ 15.1**	36.5 $\pm$ 10.9	—53.8 $\pm$ 16.2	
Glucose (5 mM) + NH ₄ Cl (5 mM)	60	—554.2 $\pm$ 15.4**	4.2 $\pm$ 0.6	522.2 $\pm$ 24.7**	4.8 $\pm$ 0.9	167.0 $\pm$ 1.7**	—240.9 $\pm$ 9.2**	119.2 $\pm$ 3.8*	—97.9 $\pm$ 9.6	
None	30	—	0.4 $\pm$ 0.5	—11.3 $\pm$ 0.7	—7.7 $\pm$ 0.4	39.1 $\pm$ 0.6	4.8 $\pm$ 2.3	—	—75.3 $\pm$ 1.4	
None	60	—	0.3 $\pm$ 0.2	—12.0 $\pm$ 0.8	—8.1 $\pm$ 0.7	55.1 $\pm$ 3.0	27.0 $\pm$ 4.0	—	—129.1 $\pm$ 3.6	
NH ₄ Cl (5 mM)	30	—	0.4 $\pm$ 0.1	—12.0 $\pm$ 1.1	—6.7 $\pm$ 0.4	35.3 $\pm$ 1.9	—2.6 $\pm$ 39.0	—	—61.3 $\pm$ 40.6	
NH ₄ Cl (5 mM)	60	—	0.2 $\pm$ 0.1	—12.2 $\pm$ 0.8	—5.7 $\pm$ 0.9	46.4 $\pm$ 2.2	2.5 $\pm$ 4.5*	—	—89.6 $\pm$ 7.3*	

trations of NH₄Cl used. The NH₄Cl-induced stimulation of glucose removal, which was also approximately linear with time (Table 2), was accompanied by a very large increase in lactate accumulation and, to a lesser extent, by a stimulation of glutamine synthesis and ¹⁴CO₂ release. The proportion of glucose carbon converted into CO₂ progressively decreased to about 20% with the highest concentration (10 mM) of NH₄Cl added.

Pyruvate and glutamate were only minor products of glucose and/or NH₄Cl metabolism, and under no conditions did we observe any net formation of glycogen, alanine, aspartate or tricarboxylic acid cycle intermediates.

The nitrogen balance calculations reveal that more nitrogen was found as glutamine plus glutamate than was removed as NH₄Cl (Table 2). This means that part of the glutamine nitrogen found in the presence of glucose plus NH₄Cl came from endogenous substrates; this observation is in agreement with the relatively high rate of glutamine synthesis observed in the absence of exogenous substrates (Table 2).

It should be noted that the amount of glutamine synthesized in the presence of glucose as the sole exogenous substrate was in the same range as that formed from endogenous sources (Table 2). This suggests that the glutamine carbon synthesized in the presence of glucose alone did not necessarily come from the glucose carbon removed but rather, at least in part, from the carbon skeleton of endogenous substrates. In contrast, the fact that the rate of glutamine synthesis from glucose plus NH₄Cl was about three times higher than that found under other conditions (in the presence of glucose alone or in the absence of exogenous substrates) strongly suggests that, under these conditions, glucose carbon contributed to a large extent to glutamine carbon. In agreement with this view is (i) the observation that, at least in the presence of the highest concentrations of NH₄Cl used (2.5–10 mM), the glucose removed was virtually completely accounted for by the sum of the accumulation of lactate plus pyruvate (in C₆ units), glutamine, glutamate and the conversion of glucose into ¹⁴CO₂ (Table 1), (ii) the absence of stimulation of glutamine synthesis by the addition of NH₄Cl alone (Table 2).

Importance of the pentose-phosphate pathway in glucose metabolism by guinea-pig kidney tubules

Table 3 shows the results obtained in experiments in which [U-¹⁴C]-, [1-¹⁴C]- or [6-¹⁴C]glucose were used as substrates in parallel in the absence and the presence of NH₄Cl. It can be seen that, in both the absence and the presence of NH₄Cl, the release of ¹⁴CO₂ from the C-1 of glucose was significantly higher than that from the C-6 of glucose (*P* < 0.01), which indicates that the pentose-phosphate pathway functioned in our guinea-pig kidney tubules. However, the release of CO₂ from the C-1 minus that from the C-6 of glucose was extremely small, and identical in the absence and the presence of NH₄Cl when compared with the rate of glucose removal, which means that the pentose-phosphate pathway contributed only to a limited extent to glucose metabolism and that it was not stimulated by the addition of NH₄Cl.

Importance of the presence of bicarbonate for glutamine synthesis from glucose and NH₄Cl: role of pyruvate carboxylase

The synthesis of glutamine from glucose plus NH₄Cl observed in our study implies that an adequate amount of 2-oxoglutarate was diverted from the tricarboxylic acid cycle to undergo a reductive amination, as a result of the glutamate dehydrogenase reaction. Thus citrate and therefore oxaloacetate must have been synthesized from glucose.

Tables 4 and 5 present results of experiments designed to test

Table 3 Release of $^{14}\text{CO}_2$ from 5 mM D-[U- ^{14}C]glucose and D-[6- ^{14}C]glucose in guinea-pig kidney tubules

Kidney tubules (24.2 ± 1.1 mg dry wt. per flask) were incubated for 60 min as described in the Materials and methods section. Values ($\mu\text{mol/g}$ dry wt.) for metabolite removal (—) or production are reported as means $\pm$ S.E.M. for four experiments performed in duplicate. Since the removal of glucose and the accumulation of products (except $^{14}\text{CO}_2$) obtained with the three different species of labelled glucose were not statistically different, results were pooled and are presented together. The release of $^{14}\text{CO}_2$ from [U- ^{14}C]glucose was obtained by multiplying the conversion of [U- ^{14}C]glucose into $^{14}\text{CO}_2$ by 6. In the absence of exogenous substrate and in the presence of 5 mM NH_4Cl , glutamine synthesis was 47.6 ± 4.7 and 39.6 ± 4.1 $\mu\text{mol/g}$ dry wt. respectively. Statistical differences were measured by the paired Student's *t* test against the control without NH_4Cl : *P < 0.05; **P < 0.01. The carbon and nitrogen balances were calculated as described in the Materials and methods section.

Substrate added	Metabolite removal or production ($\mu\text{mol/g}$)							Carbon balance	Nitrogen balance
	Glucose	Lactate	Glutamate	Glutamine	NH_4^+	$^{14}\text{CO}_2$ from [U- ^{14}C]Glc	$^{14}\text{CO}_2$ from [6- ^{14}C]Glc		
[^{14}C]Glucose (5 mM)	-173.8 ± 11.0	43.4 ± 10.0	8.6 ± 1.1	48.3 ± 2.9	-7.1 ± 0.2	554.4 ± 21.2	77.0 ± 1.4	2.8 ± 4.7	-98.1 ± 5.3
[^{14}C]Glucose (5 mM) + NH_4Cl (5 mM)	$-510.5 \pm 9.2^{**}$	$426.6 \pm 22.9^{**}$	$3.8 \pm 0.8^{**}$	$168.8 \pm 10.2^{**}$	$-267.2 \pm 11.5^{**}$	$776.4 \pm 15.0^{**}$	$85.2 \pm 1.2^*$	-4.8 ± 5.8	$-74.2 \pm 9.2^{**}$

Table 4 Effect of bicarbonate on the metabolism of 5 mM D-[U- ^{14}C]glucose and 5 mM NH_4Cl in guinea-pig kidney tubules incubated for 60 min in phosphate/saline buffer

Kidney tubules (23.2 ± 0.5 mg dry wt. per flask), previously washed three times and resuspended in phosphate/saline buffer, pH 7.4 (see [17]), were incubated in 4 ml of the same buffer in an atmosphere of pure O_2 . When NaHCO_3 was added to the phosphate/saline buffer, the NaCl concentration was decreased to keep the osmolality of the medium constant, and the buffer as well as the flasks were gassed with a mixture of O_2 and CO_2 in appropriate concentrations to maintain the pH at 7.4 under all conditions. $^{14}\text{CO}_2$ means conversion of [U- ^{14}C]glucose into $^{14}\text{CO}_2$ (in μmol of glucose equivalents). Results ($\mu\text{mol/dry wt.}$) for metabolite removal (—) or production are reported as means $\pm$ S.E.M. for four experiments performed in duplicate. Statistical differences were measured by the paired Student's *t* test against the control without bicarbonate: *P < 0.05; **P < 0.01. The carbon and nitrogen balances were calculated as described in the Materials and methods section.

Buffer system	Substrate added	Metabolite removal or production ($\mu\text{mol/g}$)								Carbon balance	Nitrogen balance
		Glucose	Pyruvate	Lactate	Alanine	Glutamate	Glutamine	NH_4^+	$^{14}\text{CO}_2$		
Phosphate/saline; pure O_2	[U- ^{14}C]Glc (5 mM) + NH_4Cl (5 mM)	-432.2 ± 13.2	2.8 ± 0.1	545.2 ± 17.7	12.4 ± 1.2	6.5 ± 1.5	30.2 ± 1.9	-20.5 ± 9.9	78.4 ± 2.9	36.9 ± 4.0	-58.8 ± 9.8
Phosphate/saline + 10.5 mM HCO_3^- ; 2.1% CO_2 in O_2	[U- ^{14}C]Glc (5 mM) + NH_4Cl (5 mM)	$-550.5 \pm 21.9^{**}$	3.2 ± 0.1	589.1 ± 19.5	$2.8 \pm 0.6^{**}$	3.4 ± 1.9	$104.3 \pm 4.9^{**}$	$-149.1 \pm 14.9^{**}$	$130.8 \pm 2.2^{**}$	14.4 ± 10.3	-65.7 ± 5.5
Phosphate/saline + 25 mM HCO_3^- ; 5% CO_2 in O_2	[U- ^{14}C]Glc (5 mM) + NH_4Cl (5 mM)	$-608.2 \pm 23.2^{**}$	3.8 ± 0.2	615.5 ± 35.2	$0.3 \pm 0.4^{**}$	6.7 ± 2.0	$138.0 \pm 4.7^{**}$	$-222.5 \pm 19.0^{**}$	$141.2 \pm 3.1^{**}$	$12.4 \pm 4.0^*$	-60.5 ± 9.9
Phosphate/saline; pure O_2	None	—	0.1 ± 0.1	-2.4 ± 0.3	-2.2 ± 0.5	-5.4 ± 1.0	13.4 ± 3.0	62.7 ± 2.7	—	—	-81.9 ± 6.6
Phosphate/saline + 10.5 mM HCO_3^- ; 2.1% CO_2 in O_2	None	—	0.2 ± 0.1	-2.9 ± 0.2	-3.7 ± 0.4	-5.3 ± 1.2	$25.6 \pm 4.1^{**}$	$46.8 \pm 2.6^{**}$	—	—	-89.0 ± 7.0
Phosphate/saline + 25 mM HCO_3^- ; 5% CO_2 in O_2	None	—	0.1 ± 0.2	-2.8 ± 0.2	-3.7 ± 0.7	-5.2 ± 1.4	$23.6 \pm 2.0^{**}$	$42.7 \pm 2.0^{**}$	—	—	-81.0 ± 5.1

Table 5 $^{14}\text{CO}_2$ fixation during the metabolism of 5 mM D-glucose plus 5 mM NH_4Cl in guinea-pig kidney tubules

Kidney tubules (24.0 ± 2.9 mg dry wt. per flask) were incubated for 60 min in Krebs–Henseleit medium containing 25 mM $\text{NaH}^{14}\text{CO}_3$ 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 four experiments performed in duplicate. $^{14}\text{CO}_2$ fixation and incorporation into glutamine and glutamate were measured as described in the Materials and methods section. The carbon and nitrogen balance were calculated as described in the Materials and methods section.

Substrate added	Metabolite removal or production ($\mu\text{mol/g}$)					$^{14}\text{CO}_2$ fixed		Carbon balance	Nitrogen balance
	Glucose	Lactate	Glutamate	Glutamine	NH_4^+	Total	$[1-^{14}\text{C}]\text{Glu} + [1-^{14}\text{C}]\text{Gln}$		
Glucose (5 mM) + NH_4Cl (5 mM) + $\text{NaH}^{14}\text{CO}_3$ (25 mM)	-390.3 ± 33.7	360.3 ± 49.8	5.9 ± 0.8	114.4 ± 12.4	-154.8 ± 20.2	79.9 ± 4.3	68.3 ± 5.2	89.9 ± 17.9	-79.9 ± 11.8
$\text{NaH}^{14}\text{CO}_3$ (25 mM)	—	-7.2 ± 0.7	-4.1 ± 0.8	43.2 ± 1.3	26.1 ± 1.9	34.3 ± 2.4	27.4 ± 1.8	—	-108.4 ± 2.7

Table 6 Effect of methionine sulfoximine on the metabolism of 5 mM D-glucose in the absence and the presence of 5 mM NH_4Cl in guinea-pig kidney tubules

Kidney tubules (24.0 ± 2.9 mg dry wt. per flask) were incubated for 60 min as described in the Materials and methods section. Values ($\mu\text{mol/g}$ dry wt.) for metabolite removal (—) or production are reported as means $\pm$ S.E.M. for four experiments performed in duplicate. Statistical differences were measured by the paired Student's *t* test against the control without methionine sulfoximine (MSO): *P < 0.05; **P < 0.01.

Experimental condition	Metabolite removal or production ($\mu\text{mol/g}$)			
	Glucose	Glutamate	Glutamine	NH_4^+
Glucose (5 mM)	-166.3 ± 18.5	8.4 ± 1.1	47.5 ± 3.8	-4.9 ± 1.5
Glucose (5 mM) + MSO (5 mM)	-164.7 ± 16.1	$23.8 \pm 2.3^{**}$	$-9.6 \pm 0.9^{**}$	$134.3 \pm 5.3^{**}$
Glucose (5 mM) + NH_4Cl (5 mM)	-390.3 ± 33.7	5.9 ± 0.8	114.4 ± 12.4	-154.8 ± 20.2
Glucose (5 mM) + NH_4Cl (5 mM) + MSO (5 mM)	$-267.8 \pm 26.3^{**}$	$58.7 \pm 9.5^{**}$	$-7.1 \pm 1.4^{**}$	$19.0 \pm 12.4^{**}$
No added substrate	—	-4.1 ± 0.8	43.2 ± 1.3	26.1 ± 1.9
No added substrate + MSO (5 mM)	—	$5.3 \pm 1.5^{**}$	$-12.9 \pm 1.7^{**}$	$186.9 \pm 8.2^{**}$

the importance of CO_2 fixation in forming the oxaloacetate necessary for the synthesis of the glutamine carbon skeleton from the glucose carbon skeleton. When guinea-pig kidney tubules were incubated under conditions used by Krebs [16] (phosphate medium with an atmosphere of pure O_2 [17]), the rate of glutamine synthesis in the presence of glucose plus NH_4Cl , although higher than that observed in the absence of exogenous substrate, remained low (Table 4).

Table 4 also shows that a large and progressive increase in glutamine synthesis occurred when increasing concentrations of bicarbonate were added to the medium. This observation suggests a critical role for CO_2 fixation in glutamine synthesis from glucose, presumably via the operation of pyruvate carboxylase which has been shown to be present in guinea-pig renal cortex by Robinson [3]. This observation may also explain why Krebs [16] did not observe any increase in glutamine synthesis from glutamate plus NH_4Cl upon addition of glucose in renal cortex slices of the guinea pig, because pyruvate carboxylase could not operate under his experimental conditions.

The data presented in Table 5 show that fixation of $^{14}\text{CO}_2$ did occur from $\text{NaH}^{14}\text{CO}_3$ during glutamine synthesis from glucose plus NH_4Cl , and that most of the CO_2 fixed was recovered as the C-1 of glutamine and glutamate.

Mechanism of the stimulation of glucose utilization by NH_4Cl

As shown in Table 6, addition of methionine sulfoximine, a potent inhibitor of glutamine synthetase [18], completely abolished the glutamine synthesis found in the presence of glucose as

sole exogenous substrate, without altering the rate of glucose removal. By contrast, addition of methionine sulfoximine in the presence of glucose plus NH_4Cl , which also resulted in the suppression of glutamine synthesis, significantly reduced but did not completely suppress the stimulation of glucose removal induced by NH_4Cl (Table 6). This indicates that the NH_4Cl -induced stimulation of glucose utilization observed in the present study was secondary to the stimulation of glutamine synthesis only in part. Thus some other step, presumably located between glucose and lactate [because the proportion of glucose converted into lactate increased considerably in the presence of NH_4Cl (see Tables 1–3)] must have been stimulated by NH_4Cl .

Neither the activity of hexokinase (8.8 ± 0.7 versus 8.6 ± 0.4 units/g fresh wt. in the presence of 5 mM NH_4Cl ; *n* = 5) nor that of pyruvate kinase (66.3 ± 2.5 versus 67.4 ± 1.0 units/g fresh wt. in the presence of 5 mM NH_4Cl ; *n* = 5) was significantly affected by the addition of NH_4Cl . By contrast, a significant stimulation of phosphofructokinase activity was observed in the presence of 5 mM NH_4Cl (14.1 ± 0.6 versus 11.7 ± 0.5 units/g fresh wt; *P* < 0.01; *n* = 5). Thus phosphofructokinase appears to be the key glycolytic enzyme responsible (at least in part) for the stimulation of glucose utilization observed in our study in the presence of NH_4Cl . It should be mentioned that the concentration of fructose 2,6-bisphosphate, a stimulator of phosphofructokinase activity [13], did not change in guinea-pig kidney tubules incubated with 5 mM glucose upon addition of 5 mM NH_4Cl (74.5 ± 4.7 nmol/g dry wt. in the presence of 5 mM glucose versus 76.7 ± 6.2 nmol/g dry wt. in the presence of 5 mM glucose plus 5 mM NH_4Cl ; *n* = 4).

Physiological significance

The present study, which demonstrates that, at a physiological concentration, glucose can be metabolized and converted into glutamine in the presence of NH_4Cl by isolated guinea-pig kidney tubules, raises the question of whether glucose is taken up from circulating blood by the intact functioning guinea-pig kidney. Renal arterio-venous concentration differences of glucose measured in five fed guinea pigs by a technique previously described [11] revealed that this is actually the case: the arterial and renal venous concentrations of glucose were 11.55 ± 0.44 and 9.51 ± 0.70 mM ($P < 0.01$), respectively. Thus, in contrast with what is known in other species (see [19] for a review), glucose is taken up in net amounts by the guinea-pig kidney *in vivo*. In addition, glucose appears to be a potential source of the carbon skeleton of the glutamine known to be released into the vein of the guinea-pig kidney [11]. Although this remains to be demonstrated, glucose might also be a substrate contributing, via renal glutamine synthesis, to the detoxification of elevated circulating concentrations of ammonium ions in the guinea pig.

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