Glutamine catabolism by heart muscle

Properties of phosphate-activated glutaminase

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1. Rat heart homogenates catabolized glutamine in the presence of rotenone, an inhibitor of the respiratory chain. 2. The reaction was markedly stimulated by phosphate and inhibited by glutamate, but not greatly affected by ammonia. 3. Glutamine breakdown was enhanced by lactate, malate, citrate and ATP, and almost completely blocked by 1 mM-*N*-ethylmaleimide. 4. The K_m for glutamine measured in homogenates supplemented with either 50 mM- or 100 mM-phosphate and at pH 7.3 or 8.0 was about 4 mM, whereas the V_{max} was larger at higher anion concentrations and at the more alkaline pH. 5. Activity of glutaminase was 3-fold greater in isolated mitochondria than in muscle homogenates. Distribution of the activity was the same as that of a mitochondrial marker, cytochrome *c* oxidase. 6. Properties of glutaminase with respect to its dependence on the concentrations of phosphate, glutamine and H^+ were the same in intact and broken mitochondria and very similar to those in homogenates. However, the specific activity of the enzyme was considerably smaller in frozen-thawed than in intact organelles. 7. It is concluded that heart mitochondria possess a kidney-type phosphate-activated glutaminase that can serve as a source of myocardial glutamate.

INTRODUCTION

Glutamine is one of the most abundant amino acids in many organs, including heart (Krebs *et al.*, 1949). An important enzyme involved in its catabolism is phosphate-activated glutaminase, a mitochondrial protein that is intricately regulated by a number of low-molecular-mass effectors (Kovačević & McGivan, 1983). The enzyme exists in two forms, the kidney and the liver form, that differ not only in their regulation (Kovačević & McGivan, 1983) but also in the molecular structure (Banner *et al.*, 1988; Smith & Watford, 1990). In many tissues glutaminase is the crucial reaction generating glutamate, the key intracellular amino acid that links the metabolism of carbon and nitrogen. Although in heart glutamate can be produced by reductive amination of 2-oxoglutarate and through transamination reactions, catabolism of glutamine provides an attractive alternative. However, except for a short note by Ottaway (1969) on the ability of cardiac extracts to catabolize glutamine with 200 mM-phosphate in the medium, almost nothing is known about glutaminase activity of the heart.

The present study was designed to eliminate this oversight. It shows that glutamine is broken down by the heart and that phosphate-activated glutaminase is an important enzyme responsible for catabolism of this amino acid.

MATERIALS AND METHODS

Animals

Rats of both sexes, weighing 120–140 g (about 4 weeks of age, referred to as ‘young’ animals) or 220–300 g (3–4 months of age, referred to as ‘adult’ animals), were used throughout the study.

Preparation of heart homogenates

Rats were decapitated with a small-animal guillotine. Hearts were excised through a mid-sternal thoracotomy and placed immediately in ice-cold 50 mM-Tris/HCl, pH 7.4. The tissue was cut in small pieces and rinsed thoroughly with Tris/HCl buffer to

remove blood. A 14% (w/v) homogenate was then prepared in a two-step procedure. In the first, the tissue was minced in a Brinkman Polytron (30 s, rheostat setting at 6); in the second, it was further broken down in a Potter–Elvehjem homogenizer (8 passes at about 300 rev./min). The homogenate was filtered through cheesecloth and used immediately for the incubations.

Isolation of mitochondria

Mitochondria were isolated as described previously (Fuller *et al.*, 1985). In brief, hearts were excised as described above and placed in ice-cold isolation medium (225 mM-mannitol/75 mM-sucrose/10 mM-Mops/1 mM-EGTA, pH 7.4). The hearts were trimmed free of atria and large vessels; the ventricles were minced, exposed for 1 min to the proteolytic enzyme Nagase (Enzyme Development Corp, New York, NY, U.S.A.) and homogenized with a Polytron as described above. Proteolytic digestion was terminated by diluting the mixture with 10 vol. of the cold isolation medium containing 0.1% BSA. The mitochondria were then isolated in the same buffer by differential centrifugation. The final pellet was suspended at approx. 20 mg of protein/ml. In some experiments, mitochondrial integrity was destroyed by three cycles of freezing and thawing in an acetone/solid- CO_2 bath. The preparation was diluted with equal amounts of distilled water before treatment.

Analytical methods

Glutaminase activity was measured as the amount of either glutamate formed or glutamine consumed. When the former method was used, the amount of glutamate present at zero time was subtracted from that found at the end of incubation. The latter method was employed when glutamine was added to the medium at 1 mM (Table 2). At higher levels of the amino acid (10 mM in most experiments), its utilization amounted to only 5–10% of the initial content and therefore could not be measured with sufficient accuracy. The basic medium consisted of 50 mM-Tris/HCl, pH 7.3, variable concentrations of potassium phosphate (pH 7.3), 10 μ M-rotenone, 2 mM-amino-oxyacetate

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Fig. 1. Effect of anions on glutaminase activity in homogenates (a) and mitochondria (b) from hearts of adult rats

Rates of glutaminase activity were measured as the amount of glutamate formed from 10 mM-glutamine in heart homogenates (a) with either phosphate (○) or sulphate (□) and in intact (▲) or frozen-and-thawed (△) mitochondria (b) in the presence of phosphate. Data represent means $\pm$ S.E.M. for five and three experiments for homogenates and mitochondria respectively.

and glutamine (1–20 mM). When the effect of pH was investigated, Tris/HCl was omitted from the buffer, potassium phosphate was increased to 100 mM and the pH was adjusted to the appropriate value. All other test compounds (lactate, malate, citrate, glutamate, ATP) were neutralized to pH 7.3 with NaOH. Incubation time was usually 30 or 60 min at 37 °C. In experiments in which kinetic constants were measured, 15 min incubations were used.

Cytochrome *c* oxidase activity was measured at 25 °C with a Clark-type O_2 electrode in a solution containing 0.05 M-potassium phosphate (pH 7.4), 20 μ M-cytochrome *c*, 10 mM-ascorbate and 0.23 mM-*NNN'*-tetramethyl-*p*-phenylenediamine. All fractions (i.e. cell debris, mitochondria and post-mitochondrial supernatant) were frozen and thawed; determinations were carried out in duplicates at two different protein concentrations.

Glutamate, glutamine and other amino acids were measured by h.p.l.c. (Jarrett *et al.*, 1986). P_i was assayed by the method of Lowry & Lopez (1946) and protein by the biuret reaction using bovine serum albumin as a standard.

RESULTS

Externally added glutamine was catabolized to glutamate in the presence of rotenone, an inhibitor of the mitochondrial respiratory chain. In all experiments described in this study, the reaction rates were linear with respect to protein concentration (1–5 mg/ml) and incubation time (up to 60 min for homogenates and 30 min for mitochondria).

Catabolism of glutamine in the presence of phosphate and sulphate

The rate of glutamate formation from 10 mM-glutamine with chloride as the sole anion was 0.26 ± 0.02 nmol/min per mg of protein and it declined to 0.11 ± 0.01 nmol/min per mg in the presence of 2 mM-amino-oxyacetate. With amino-oxyacetate, the level of aspartate did not change; however, without the inhibitor, it decreased at a rate of 0.16 ± 0.01 nmol/min per mg of protein. Since amino-oxyacetate does not affect glutaminase activity (which is the subject of this investigation) but eliminates the possible contribution from transaminases, it was used routinely in all subsequent experiments.

Addition of increasing concentrations of phosphate or sulphate caused marked enhancement of glutamate production from 10 mM-glutamine (Fig. 1a). The plots of [anion] versus glutaminase activity were markedly sigmoid, with half-maximal stimulation at 60–70 mM of each anion. However, the extent of

stimulation was greater for phosphate than for sulphate at each concentration tested. When the data for phosphate were transformed by the Hill equation, the resulting plot had a slope of about 1.5 (result not shown).

Activation of glutamine breakdown by phosphate in homogenates from hearts of adult male and female rats was identical. However, in younger animals (120–140 g) of both sexes, the activity was 30–40 % higher at each [phosphate].

Inclusion of 0.1 % Triton X-100 into the incubation mixture containing 50 mM-phosphate markedly decreased (over 50 %) the amount of glutamate produced from 10 mM-glutamine.

Kinetic parameters of glutamine breakdown

Kinetic constants for glutaminase activity were measured under a variety of conditions. In all experiments 15 min incubations were used to minimize possible inhibition from reaction products. The results were analysed by double-reciprocal plots ($1/v$ versus $1/[S]$). In homogenates from hearts of adult rats, with 50 mM-phosphate present, the K_m was 4.4 ± 0.3 mM and the V_{max} was 4.6 ± 0.5 nmol/min per mg of protein (Fig. 2).

Fig. 2. Kinetic constants for glutaminase activity in homogenates and mitochondria from hearts of adult rats

Glutamate production was measured in a medium containing 50 mM-phosphate. Reciprocal velocities were calculated after subtracting the control background, and kinetic constants were determined from each experiment, with the error bars showing the range of those values. Data for homogenates are means $\pm$ S.E.M. from four experiments and those for mitochondria are from a single experiment. K_m is in mM and V_{max} in nmol/min per mg of protein.

Fig. 3. Effect of pH on glutaminase activity in homogenates (a) and mitochondria (b) from hearts of adult rats

Glutaminase activity was measured as the amount of glutamate formed from 10 mM-glutamine in 100 mM-phosphate buffer of an appropriate pH value. The results are means $\pm$ S.E.M. of four experiments for homogenates (a) and two experiments for both intact (■) and frozen-and-thawed (□) mitochondria (b) that gave results within 5% of each other.

In homogenates from organs of young animals, the K_m was the same but the V_{max} was substantially higher (10.6 ± 1.0 nmol/min per mg of protein). With 100 mM-phosphate and at pH 7.3, the K_m was slightly lower (3.8 ± 0.4 mM) and V_{max} was larger (8.0 ± 0.8 nmol/min per mg of protein), whereas at pH 8.0 (100 mM-phosphate) the values for the kinetic constants were 4.2 ± 0.3 mM and 15.8 ± 1.6 nmol/min per mg of protein.

Effectors of glutaminase activity

The effect of pH on glutamine breakdown is shown in Fig. 3(a). In media containing 100 mM-phosphate and 10 mM-glutamine, glutaminase activity exhibited a maximum at pH > 8.0 . At pH 6.7 and lower, glutamine catabolism was very slow.

The ability of several other cations to influence glutamine catabolism was tested in a medium containing 50 mM-phosphate and 10 mM-glutamine. Na^+ , K^+ and Tris were without effect; Ca^{2+} stimulated glutaminase activity at concentrations between 0.2 and 2.0 mM and inhibited it at higher levels (Fig. 4). Mg^{2+} at 1 mM did not alter the rate of glutamate formation ($95 \pm 6\%$ of control; mean $\pm$ S.E.M., $n = 3$), but decreased it to $64 \pm 5\%$ of control at 10 mM.

The effect of several organic anions (20 mM), such as lactate, malate and citrate, was tested in the presence of 10 mM-phosphate (Table 1). All these compounds increased glutamine breakdown, and the extent of stimulation was proportional to the number of carboxylic groups; the largest enhancement was observed with the tricarboxylic acid, citrate.

An interesting finding was a strong stimulation of glutaminase activity by 20 mM-ATP. To exclude the possibility that activation was caused by phosphate generated from trinucleotide hydrolysis, the concentration of P_i was measured after a 30 min incubation with and without ATP. Without ATP (control samples), phosphate concentration was 10.8 ± 0.3 mM, whereas in the presence of the nucleotide it was 20.2 ± 1.5 mM. However, when the same amount of phosphate (20 mM) was added to the incubation mixture at the beginning of the experiment, the increase in glutamate formation was less than 2-fold. This change was considerably smaller than that produced by ATP (over 5-fold stimulation). Moreover, ATP enhanced glutaminase activity when oligomycin (5 μ g/ml) was present in the incubation mixture (results not shown).

Glutaminase activity was markedly inhibited by 1 mM-*N*-

Fig. 4. Effect of Ca^{2+} on glutaminase activity in rat heart homogenates

Control levels of glutamate production from glutamine were determined in 50 mM-phosphate buffer at pH 7.3 with no Ca^{2+} added. Results were expressed as percentages of control and represent means $\pm$ S.E.M. for three experiments.

ethylmaleimide, irrespective of the amount of phosphate in the medium. Moreover, the inhibition was unaffected by the presence of 1 mM- Ca^{2+} .

Effect of glutamate and NH_4Cl on glutamine catabolism

The effect of glutamate and NH_4Cl on glutaminase activity was measured in incubations containing 1 mM-glutamine (Table 2). At this glutamine concentration, 2 mM-glutamate produced about 50% inhibition. Addition of 20 mM-glutamate blocked activity by over 90%. Inhibition of glutamine breakdown by glutamate was slightly but not significantly smaller at pH 8.0 and when the concentration of phosphate was raised to 100 mM. In contrast, NH_4Cl had very little influence on glutaminase activity. In all experiments in this series, the amount of glutamine utilized during 60 min incubation (Δ glutamine) was used as a measure of enzyme activity. It should be noted that in experiments without added glutamate the amount of glutamate formed (Δ glutamate) was equal, within the limits of the experimental error, to that of glutamine disappeared (Δ glutamine).

Table 1. Modulators of cardiac glutaminase activity

All test compounds were added together with glutamine at zero time. Glutamate was measured in neutralized heart extracts by h.p.l.c. Δ Glutamate represents the difference in amino acid level at zero time and that after 30 min incubation, and is expressed as nmol/mg of protein. Values are means $\pm$ S.E.M. for three experiments. Abbreviation: NEM, *N*-ethylmaleimide.

Addition	Δ Glutamate	
	(nmol/30 min per mg of protein)	(%)
10 mM- P_i		
None (Control)	11.9 ± 1.2	100
Lactate (20 mM)	15.0 ± 1.2	126
Malate (20 mM)	16.1 ± 0.7	135
Citrate (20 mM)	25.5 ± 0.8	214
ATP (20 mM)	63.0 ± 1.7	529
20 mM- P_i	22.7 ± 2.0	191
50 mM- P_i		
None	65.7 ± 5.6	100
NEM (1 mM)	12.2 ± 0.5	19
150 mM- P_i		
None	128 ± 3	100
NEM (1 mM)	39.9 ± 3	18

Table 2. Effect of glutamate and NH₄Cl on glutamine catabolism in heart homogenates

Glutamine was measured in neutralized heart extracts by h.p.l.c. Δ Glutamine represents the difference between the amount found at zero time (added concn. ~ 1 mM) and that after a 60 min incubation. Protein levels were measured in each flask and were found to differ by 10% or less within a single experiment. The range of values for all experiments was 3.2–3.8 mg/ml. Values represent means $\pm$ S.E.M. for the numbers of experiments indicated in parentheses.

Addition	Δ Glutamate	
	(nmol/30 min per mg of protein)	(%)
50 mM-P _i , pH 7.3	48.5 $\pm$ 4.6 (4)	100
+ 2 mM-glutamate	25.7 $\pm$ 2.3 (4)	53
+ 20 mM-glutamate	5.5 $\pm$ 0.9 (4)	9
50 mM-P _i , pH 8.0	61.0 (2)	100
+ 2 mM-glutamate	36.0 (2)	49
100 mM-P _i , pH 7.3	78.3 $\pm$ 4.8 (3)	100
+ 2 mM-glutamate	44.0 $\pm$ 7.4 (3)	56
50 mM-P _i , pH 7.3	46.9 $\pm$ 4.8 (4)	100
+ 2 mM-NH ₄ Cl	40.2 $\pm$ 4.9 (4)	86
+ 20 mM-NH ₄ Cl	32.0 $\pm$ 5.3 (4)	68

Catabolism of glutamine by intact and frozen and thawed mitochondria

Mitochondria isolated from adult rats produced glutamate from glutamine rapidly; the phosphate-activation curve was sigmoid and very similar in shape to that obtained for the homogenates. The activity at each phosphate concentration was, however, 2.5–3-fold greater (Fig. 1*b*). The K_m value for glutamine with 50 mM-phosphate was 4.7 mM, and V_{max} was 12.1 nmol/min per mg of protein (Fig. 2). The difference in the maximal velocities between homogenates and mitochondria is consistent with heart tissue containing about 30% mitochondrial protein. Mitochondrial glutaminase activity, like that in homogenates, showed higher velocities at more alkaline pH values, with an optimum at pH 8.0–8.5 (Fig. 3*b*).

The capability to catabolize glutamine was compared in parallel experiments on the same mitochondrial preparations that were either untreated (and presumably 'intact' or minimally damaged) or broken by freeze-thawing. In broken mitochondria, the phosphate-activation curve (Fig. 1*b*) and the pH-dependence curve (Fig. 3*b*) were identical in shape with those for the intact preparation, but levelled off at lower velocities; however, they

were not shifted. The lack of a significant shift to the right under conditions when the permeability barrier is destroyed suggests that the gradient of phosphate ($[P_i]_{in} > [P_i]_{out}$) or pH ($pH_{in} > pH_{out}$) that may exist in untreated mitochondria is not large enough to affect the results reported here.

Consistent with the above findings, measurements of the kinetic parameters for glutamine showed that the maximum velocity in freeze-thawed preparations was 2–3-fold slower. By contrast, the K_m was about the same (4.7 mM). The latter findings suggest that glutamine transport in untreated mitochondria does not limit glutaminase activity.

Glutaminase activity in frozen and thawed mitochondria could not be restored to that observed in intact organelles by addition of 20 mM-citrate or 20 mM-ATP (with oligomycin present), or both.

The ability of cardiac mitochondria to catalyse breakdown of glutamine was eliminated by addition of 0.05% Triton X-100. A similar response was noted in liver mitochondria by Joseph & McGivan (1978).

Localization of phosphate-activated glutaminase

To confirm the presumed mitochondrial localization of phosphate-activated glutaminase, its distribution among various fractions of the heart homogenate was compared with that of cytochrome *c* oxidase (Table 3). The fractions used were: mitochondria (pellet after 7 min spin at 8000 *g*), post-mitochondrial supernatant (after centrifugation for 7 min at 8000 *g*) and low-speed residue (i.e. the pellet remaining after a 5 min spin at 600 *g*). Specific activity of glutaminase was measured with 50 mM-phosphate and 10 mM-glutamine, and that of cytochrome *c* oxidase with reduced cytochrome *c* as the substrate (see the Materials and methods section for details). Total activity in each fraction was then calculated by multiplying the protein content of the fraction by its specific activity.

The results displayed in Table 3 show that the distribution of glutaminase was essentially the same as that of cytochrome *c* oxidase, a mitochondrial marker. The relatively low total activity (about 40%) of both enzymes found in the mitochondrial fraction can be explained by their loss to the low-speed pellet, since the procedure was optimized for purity and not the maximum number of organelles.

DISCUSSION

Cardiac phosphate-activated glutaminase as a source of myocardial glutamate

Three enzymes that catalyse glutamine breakdown were considered as potential sources of myocardial glutamate:

Table 3. Distribution of glutaminase and cytochrome *c* oxidase in various fractions of the heart homogenate

A heart of initial weight 850 mg was homogenized in a mannitol/sucrose/Mops buffer ('homogenate') and centrifuged for 5 min at 600 *g*. The supernatant was then spun for 7 min at 8000 *g* yielding mitochondria (pellet) and 'cytosol' (supernatant). Protein and specific activity were measured in each fraction as described in the Materials and methods section.

Fraction	Total protein (mg)	Specific activity (nmol/min per mg of protein)		Total activity			
		Glutaminase	Cytochrome <i>c</i> oxidase	Glutaminase		Cytochrome <i>c</i> oxidase	
				(nmol/min)	(%)	(nmol/min)	(%)
Pellet after 600 <i>g</i>	86	2.43	77.5	209	58.4	6665	54.8
Mitochondria	19	7.60	260	144	40.2	4940	40.6
Cytosol	33	0.14	17.0	4.6	1.4	561	4.6
Total	138			358	100	12166	100
Homogenate	144	2.53	86.7	364		12485	
Recovery (%)	96			98		97	

glutaminase I, or phosphate-activated glutaminase (Errera, 1949; Krebs, 1935), maleate-activated glutaminase (Katunuma *et al.*, 1966) and glutaminase II (Errera, 1949), which represents a combined action of glutamine-ketoacid transaminase (Greenstein & Carter, 1946) and ω -amidase (Meister & Tice, 1950). The maleate-activated enzyme, reported in extracts of kidney by Katunuma *et al.* (1966), was later found to be identical with γ -glutamyltranspeptidase (Curthoys & Kuhlenschmidt, 1975; Tate & Meister, 1975). Glutaminase II, or the transamination pathway, is active in many tissues (Cooper & Meister, 1981) and can be distinguished from the phosphate-activated glutaminase by its sensitivity to inhibition by amino-oxyacetate (Deaciuc & Petrescu, 1980). Glutaminase I, or phosphate-activated glutaminase, is the only true hydrolytic enzyme.

The results in the present paper show that the ability of cardiac homogenates to catabolize glutamine in the absence of added phosphate, both with and without amino-oxyacetate, is extremely limited. This means that neither γ -glutamyltranspeptidase nor glutaminase II is active in the heart. On the other hand, this organ can catabolize glutamine to glutamate in the presence of phosphate, which indicates that a phosphate-activated glutaminase is responsible for this activity. The heart glutaminase appears to be located in the mitochondria, as shown by the similarity of its distribution to that of cytochrome *c* oxidase, a mitochondrial marker. The enzyme is readily inhibited by glutamate, but is not stimulated by ammonia; thus the cardiac protein can be classified as the kidney-type glutaminase (Kovačević & McGivan, 1983).

Properties of cardiac phosphate-activated glutaminase

The K_m value for cardiac glutaminase measured in this work, 3.8–4.8 mM, is essentially the same as that reported previously by Ottaway (1969), which was determined in 200 mM-phosphate buffer at pH 7.6. It is also very similar to the Michaelis constants for the kidney (Haser *et al.*, 1985) and brain (Benjamin, 1981; McGeer & McGeer, 1979; Weil-Malherbe, 1969) enzymes, both of which lie in the range 1.6–6.0 mM. The V_{max} of 4.6 nmol/min per mg of protein (276 μ mol/h per g of protein) obtained in this work in the presence of 50 mM-phosphate is, however, considerably larger than the value of 42 μ mol/h per g of protein reported by Ottaway (1969). Nevertheless, even this much larger value is 10–20-fold smaller than the velocities of glutamine breakdown in kidney and brain homogenates (Erecińska & Silver, 1990).

The cardiac phosphate-activated glutaminase shares many properties with the kidney and brain enzymes. (i) It is stimulated by mono-, di- and tri-carboxylic acids, and the extent of stimulation increases with the number of carboxyl groups (Curthoys *et al.*, 1976; Weil-Malherbe, 1972); (ii) it shows a biphasic response to non-physiological $[Ca^{2+}]$, with enhancement in the 0.2–2 mM range and inhibition at the higher levels (Benjamin, 1981); (iii) it is inhibited by the thiol reagent *N*-ethylmaleimide (Haser *et al.*, 1985; Kvamme & Olsen, 1979; Sayre & Roberts, 1958; Weil-Malherbe, 1969). Its sensitivity to stimulation by phosphate and sulphate is, however, lower than that of the brain glutaminase (Bradford & Ward, 1976; Haser *et al.*, 1985), but similar to that of the kidney protein (Godfrey *et al.*, 1977; Haser *et al.*, 1985). By contrast, the enhancement of activity by ATP appears to be more pronounced than that seen in either kidney or brain (Curthoys *et al.*, 1976; Weil-Malherbe, 1972).

A comparison of the behaviour of glutaminase in intact and broken mitochondria with respect to its dependence on [phosphate], [glutamine] and pH indicates that permeability characteristics of the mitochondrial membrane do not greatly

affect the enzyme activity. However, the enzyme appears to be unusually sensitive to any condition that disrupts mitochondrial integrity, more so than glutaminases from other organs (Kovačević & McGivan, 1983). The reason(s) for this behaviour is (are) not clear at present.

The activity of glutaminase was higher in 4–6 week-old rats than in adult (3–4 months old) animals. This may be a consequence of a higher content of mitochondria in younger animals (Vitorica *et al.*, 1981).

Is the heart a net glutamine consumer?

The demonstration of glutaminase activity in the heart raises the issue of whether or not this organ is a net glutamine consumer *in vivo*. Although a previous study by Iqbal & Ottaway (1970) could not detect any synthesis of glutamine in the heart homogenate, a later work by Cao Danh *et al.* (1985) found such activity in the soluble fraction from cardiac muscle. Nevertheless, the rate of this reaction in the heart was considerably slower than in comparable preparations from liver and brain. The capability to produce glutamine was also demonstrated in the isolated arterially perfused rabbit interventricular septum after administration of a bolus of $^{13}NH_3$ (Krivokapich *et al.*, 1984). The rate of glutamine synthesis was, however, not calculated and the experiment was performed in the absence of added amino acids (i.e. without glutamine). Interestingly, when a bolus of $[^{13}N]$ glutamine was injected, 7–8 % of the ^{13}N label was recovered in free $^{13}NH_3$, which supports our contention that glutaminase is present in cardiac muscle. Thus it seems that the heart possesses enzymes that can both form and utilize glutamine. However, the issue of whether this organ is a net consumer or producer of glutamine remains an open question.

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