Effects of Anoxia on Kinetics of $^{13}$N Glutamate and $^{13}$NH$_3$ Metabolism in Rabbit Myocardium

Janine Krivokapich, Randy E. Keen, Michael E. Phelps, Kenneth I. Shine, and Jorge R. Barrio

Positron emission tomography is a unique noninvasive imaging technique that provides cross-sectional images of radiotracer concentrations in myocardium and permits measurement of blood flow as well as metabolism. Ammonia and glutamate have been labeled with the positron-emitter $^{13}$N (half-life 10 minutes) for use with positron emission tomography as tracers of flow and metabolism, respectively. In order to characterize the fate of these $^{13}$N-labelled compounds in myocardium, isolated rabbit interventricular septa were used to study the kinetics of $^{13}$N glutamate ($^{13}$Nglu) and $^{13}$NH$_3$ under aerobic and anoxic conditions. Tissue analyses 6 minutes after injection of a $^{13}$Nglu bolus into myocardium revealed that 70% of the $^{13}$N-label was present in $^{13}$Nglu with 12%, 11%, and 4% in $^{13}$Nalanine ($^{13}$Nala), $^{13}$Naspartate ($^{13}$Nasp), and $^{13}$Nglutamine ($^{13}$Ngln), respectively. The corresponding relative specific activities were 1.0:0.4:0.5:0.01. Anoxia resulted in a significant increase in $^{13}$Nala with a reduction in $^{13}$Nglu. This was consistent with increased pyruvate production due to increased anaerobic glycolysis and transamination of pyruvate with $^{13}$Ngln to yield $^{13}$Nala. In support of this, addition of 2 mM pyruvate to the perfusate under control conditions produced a tissue distribution of $^{13}$N similar to that with anoxia. Six minutes after a bolus of $^{13}$NH$_3$ during both control and anoxic conditions, 60% of the tissue $^{13}$N-label was in $^{13}$Nglu with no detectable amounts in other amino acids. The rest of the $^{13}$N-label was in $^{13}$NH$_3$. Time-activity curve analyses demonstrated that anoxia significantly reduced the tissue retention of $^{13}$N-label from $^{13}$NH$_3$ but not from $^{13}$Nglu. Thus, $^{13}$N from $^{13}$NH$_3$ and $^{13}$Nglu was retained in tissue by different mechanisms involving glutamate, which were affected differentially by anoxia. These results suggest that positron emission tomography imaging with $^{13}$NH$_3$ and $^{13}$Nglu in combination may be useful in identifying ischemic myocardium. (Circulation Research 1987;60:505–516)

Positron emission tomography (PET) permits the noninvasive assessment of myocardial flow and metabolism in vivo using selected positron-emitting compounds.1,2 The positron-emitter nitrogen-13 (13N) can be used to label ammonia as well as various amino acids. The initial uptake and retention of 13N-labelled ammonia (13NH$_3$) has been shown to be dependent on flow and metabolism, resulting in the use of NH$_3$ as a myocardial flow tracer.3 Glutamate has been labelled with 13N (13Nglu) and has also been used with PET to image the heart.4-10 Canine studies with PET from our laboratory indicated that 13Nglu uptake was increased relative to 13NH$_3$ uptake during ischemia.9 These findings suggested that 13Nglu uptake may be a useful marker of ischemia when flow is decreased.

Glutamate metabolism is important in the heart for multiple reasons. It plays a key role in linking nitrogen metabolism with the citric acid cycle.11,12 The malate-aspartate shuttle, which is responsible for moving reducing equivalents across the mitochondria, is dependent on glutamate.13,14 In addition, the detoxification of ammonia by the glutamine synthetase reaction requires glutamate.15,16 This reaction is particularly important because elevated NH$_3$ levels can inhibit the tricarboxylic acid cycle and interfere with intermediary metabolism and energy production.17-19 Both anoxia and ischemia result in increased production of NH$_3$ in the heart.18,19

Glutamate is the only naturally occurring amino acid demonstrated to have a positive myocardial arteriovenous difference (A-V) in normal subjects as well as in patients with coronary artery disease.20 The A-V glutamate difference was significantly greater in patients with coronary artery disease.20,21 These studies suggested that chronic and/or repetitive myocardial ischemia might result in specific adaptations in myocardial amino acid metabolism.20,21 Furthermore, perfusion with glutamate has been shown to improve the recovery of mechanical function after anoxia and/or ischemia in the isolated rabbit interventricular septum,22 rabbit Langendorff heart,23 working rat heart,24 and canine hearts.25,26

The above information9,20-23,25,26 emphasizes the important role of glutamate metabolism in normal and oxygen-deprived myocardium. The ability to label glutamate with the positron-emitter 13N permits us to examine the fate of exogenous glutamate and trace the biochemical pathway of the nitrogen of glutamate.
using tracer quantities of $[^{13}\text{N}]\text{glu}$ that do not perturb the steady state kinetics of glutamate metabolism in the myocardium. This is an important distinction when compared to high mass levels required for isotopic studies with $^{15}\text{N}$ (measured by mass spectrometry or nuclear magnetic resonance).

Our ultimate goal is to characterize ischemia in vivo using metabolic tracers with PET. To accomplish this, it is necessary to investigate the kinetics of each metabolic tracer in a controlled environment, such as an isolated heart preparation as we have previously done. $^5$$^7$$^2$ The purpose of the current study was specifically to characterize $^{13}\text{NH}_3$ and $[^{13}\text{N}]\text{glu}$ metabolism in isolated myocardium under anoxic conditions. Because anoxia results in increased glycolytic flux and increased pyruvate production, $^{28}$$^{29}$ we hypothesized that one of the effects of anoxia on $[^{13}\text{N}]\text{glu}$ metabolism would potentially be due to increased pyruvate production. Therefore, we studied the effects of the addition of 2 mM pyruvate to the perfusate on $[^{13}\text{N}]\text{glu}$ metabolism to determine if exogenous pyruvate produced results similar to those observed with anoxia.

Materials and Methods

Experimental Model

The arterially perfused interventricular septum of nonfasted male New Zealand white rabbits (1.5–2.0 kg) was the experimental model used in this work. $^5$$^7$$^2$ This model was chosen for the following reasons: it is ideally suited for on-line coincidence counting of positron emissions from tissue without interference from radioactivity in cardiac chambers (as in Langendorff preparations); flow can be delivered in the physiologic range; and the preparation is stable in terms of mechanical function, ATP and creatine-phosphate levels, and potassium losses for 4–6 hours. In addition, glutamate had been shown to improve mechanical recovery after anoxia in this preparation. $^{22}$ These advantages outweighed the disadvantage that the isolated rabbit septum is a low workload preparation.

The heart was rapidly removed from heparinized rabbits, which were anesthetized with intravenous pentobarbital sodium (180 mg). The septal artery was immediately cannulated, and constant perfusion without recirculation was begun at a flow of 1.5 ml/min to give a flow rate of 1–1.5 ml/g/min. The standard perfusate used in our experiments contained (in mM) NaCl 130, CaCl$_2$ 1.5, KCl 5, MgCl$_2$ 1, NaH$_2$PO$_4$ 0.435, NaHCO$_3$ 9, dextrose 5.56, NH$_4$HCO$_3$ 0.05, and insulin 5 U/l. Nonradioactive glutamate was not included in the perfusate because it markedly reduced the uptake of $[^{13}\text{N}]\text{glu}$, and $^{13}\text{N}$-radioactivity was difficult to measure in tissue and effluent. In previous unpublished experiments in rabbit, we have determined that in vivo there is a large gradient for glutamate between the tissue and vascular space with tissue concentrations 50–100 times higher than plasma concentrations. In the septal preparation, we have also demonstrated that between 1 and 3 hours of perfusion (the time during which the reported experiments took place), tissue concentrations of aspartate, alanine, glutamate, and glutamine were relatively stable.

The septum was isolated and suspended between 2 coincidence NaI (TI) detectors aligned 180° apart. The vertical vector of developed and rest tension was measured with a strain gauge transducer. Temperature was maintained at 37 ± 0.5°C by heating the perfusate, which was delivered without recirculation. The septa were paced at 72 beats/min.

Preparation of $^{15}\text{NH}_3$ and $[^{13}\text{N}]\text{glu}$

$^{15}\text{N}$-labelled ammonia ($^{15}\text{NH}_3$) was prepared by the UCLA biomedical cyclotron by bombarding water with protons at 21.0 MeV. $^{30}$ The gaseous $^{15}\text{NH}_3$ (175–200 mCi) was then bubbled into isotonic saline to be used for $^{15}\text{NH}_3$ injections or dissolved in 3 ml of a solution (pH 7.4) containing 30 mM sodium phosphate, 0.5 mM α-ketoglutarate, and 0.1 mM NADH. The second solution was passed by gravity through an enzyme column on which bovine liver glutamate dehydrogenase had been immobilized. $^{31}$$^{32}$ The column was then washed with 6 ml of 30 mM sodium phosphate buffer (pH 7.4), and the combined elutions were passed through a cation exchange resin that retained $^{15}\text{NH}_3$. The eluate was made isotonic and contained approximately 5–10 mCi/ml of $[^{13}\text{N}]\text{glu}$ at the time of bolus injection into the septa.

Experimental Protocols

Time-activity curves. Bolus injections (0.05 ml) of $[^{13}\text{N}]\text{glu}$ were rapidly delivered intra-arterially to septa first under control conditions and then approximately 1.5 hours later during perfusion with either 2 mM pyruvate added to the perfusate (n = 5) or an anoxic (n = 6) perfusate. The 2 mM pyruvate was present in the perfusate for at least 30 minutes prior to the second injection of $[^{13}\text{N}]\text{glu}$. Anoxia (oxygen content <0.1 vol%) was introduced by perfusing with the standard solution equilibrated with 98% N$_2$/2% CO$_2$ beginning 10 minutes prior to the second bolus injection, which was also nonoxygenated. In 4 additional septa, $^{13}\text{NH}_3$ was injected first under control conditions and then during anoxia using the same protocol.

Coincidence counting (time-window = 10 nanoseconds) of $^{15}\text{N}$ radioactivity from the septa was recorded for 30 minutes after each bolus and stored on line in 0.1-second increments on a digital computer (Scintigraphic Data Analyzer 5407, Hewlett Packard). The coincidence counting system used for these experiments included 2 NaI (TI) scintillation detectors (1.5 inches by 3.0 inches) aligned 180° apart with relatively fast photomultipliers (RCA 4855, which have a 2-nanosecond rise time). The anode signal from the photomultipliers was directed into a fast (×10) preamplifier (Ortec 9301) and from there to a fast constant fraction discriminator (> 100 MHz) (Ortec 934) using the dual high/low threshold technique. The timing output pulse for the above discriminator was set at 6 nanoseconds to give an overlap coincidence timing of 10 nanoseconds with a LeCroy 365AL Regenerative
Majority Coincidence Module. The mass of the rabbit septum was small (approximately 1 g), and the detectors were well shielded; therefore, there was little scatter. Typically the coincidence counts were 10–15% of the singles' counts, which is a relatively large fraction compared to the 1% commonly found with PET. The primary source of dead-time in the counting system was due to the decay time of the NaI(Tl) detectors' scintillation light, and the dead-time for the counting system as a whole was measured to be 10 μsec/coincident count. Peak coincidence counts for 13NH₃ experiments were between 4,000–10,000 counts/sec and for [13N]glu experiments were in the 2,000–4,000 counts/sec range. At peak coincidence count rates of 2,000–10,000 counts/sec, the percentage dead-time losses were only 2–10%. Paralyzing dead-time losses were not observed. The random counts varied from 1% to a maximum of 2% of the total count rate in the count range we obtained.

The time-activity curves were corrected for radioactive decay. The Marquardt-Levenberg algorithm was used to fit each curve with a weighted sum of exponential components by a nonlinear regression program. The [13N]glu curves were variably fit best by 2 or 3 exponential components as illustrated in Figure 1. All 13NH₃ curves fit best with 3 exponential components. The extraction fraction (EF) of each component was calculated by dividing the weighted coefficient of each component by the sum of the weighted coefficients as has been previously described. The clearance half-time (t₁/₂) was calculated by dividing the natural logarithm of 2 by the rate constant from each component of the fitted curve. The nomenclature 1 and 3 denote the components with the shortest and longest t₁/₂’s respectively.

Chemical analyses of tissue and effluent. An initial bolus (1.0 ml) of [13N]glu, present in tracer quantities, was injected intra-arterially under control conditions, and a second bolus was injected intra-arterially 1.5 hours later during anoxia or perfusion with 2 mM pyruvate. A similar protocol was followed for injecting 13NH₃ under control conditions and during anoxia. These larger volume injections were needed to have sufficient radioactivity for well-counting of tissue and effluent samples after chemical analysis because of the 10-minute half-life of 13N. The boluses were oxygenated, or bubbled with 98% N₂/2% CO₂ for the neuous chromatographic analysis of tissue and effluent samples. The boluses were obtained 6 minutes after each bolus. Simultaneous chromatographic analysis of tissue and effluent samples was not possible after a single bolus of [13N]glu because of the short t₁/₂ of 13N. Therefore, one set of septa were used for tissue analyses and another set for the effluent analyses after [13N]glu boluses.

The tissue samples were immediately placed into liquid nitrogen after removal and prepared for homogenization as previously described. The tissue was added to 0.46 M perchloric acid (1.0 ml) prior to homogenization. The acid insoluble precipitate was separated from the supernatant, and the 13N-radioactivity was counted using a NaI(Tl) well-counter. Five percent or less of the total 13N-radioactivity was present in the protein precipitate. An aliquot of the neutralized supernatant was also counted. The remainder of the supernatant was placed on a cation-exchange resin, which retained 13NH₃. Column eluate was analyzed by reversed-phase, high-pressure liquid chromatography (HPLC) using o-phthaldialdehyde (OPT) pre-column fluorescence derivatization to determine the amino acid concentrations. The concentrations of the nonradioactive amino acids were reported in μmoles/g tissue (× 10⁵). Effluent samples were collected in 0.46 M perchloric acid and analyzed as above with the omission of the homogenization step.

Statistical analysis was performed using paired t

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**Figure 1.** Time-activity curves after bolus injections of [13N]glu during control conditions plotted semilogarithmically. Straight dark lines represent the mathematically derived components that best fit the data. The time-activity curve on top was best fit by 3 components; the bottom curve was best fit by 2 components. EF(1), EF(2), and EF(3) represent extraction fractions for components 1, 2, and 3, respectively. t₁/₂(1), t₁/₂(2), and t₁/₂(3) represent half-times of clearance for components 1, 2, and 3, respectively.
Table 1. Extraction Fractions and Tissue Clearance Half-times for [13N]Glutamate

<table>
<thead>
<tr>
<th></th>
<th>Control*</th>
<th>Anoxia†</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. n = 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF (1)</td>
<td>.41 ± .14</td>
<td>.51 ± .18</td>
<td>NS</td>
</tr>
<tr>
<td>EF (2)</td>
<td>.24 ± .15</td>
<td>.32 ± .12‡</td>
<td>NS</td>
</tr>
<tr>
<td>EF (3)</td>
<td>.35 ± .06</td>
<td>.32 ± .09</td>
<td>NS</td>
</tr>
<tr>
<td>t½ (1)</td>
<td>.20 ± .11</td>
<td>.27 ± .21</td>
<td>NS</td>
</tr>
<tr>
<td>t½ (2)</td>
<td>.91 ± .75</td>
<td>.37 ± .19‡</td>
<td>NS</td>
</tr>
<tr>
<td>t½ (3)</td>
<td>416 ± 201</td>
<td>259 ± 90</td>
<td>.05</td>
</tr>
<tr>
<td>B. n = 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF (1)</td>
<td>.53 ± .05</td>
<td>.51 ± .03</td>
<td>NS</td>
</tr>
<tr>
<td>EF (3)</td>
<td>.47 ± .05</td>
<td>.49 ± .03</td>
<td>NS</td>
</tr>
<tr>
<td>t½ (1)</td>
<td>.27 ± .08</td>
<td>.27 ± .06</td>
<td>NS</td>
</tr>
<tr>
<td>t½ (3)</td>
<td>293 ± 70</td>
<td>120 ± 41</td>
<td>.05</td>
</tr>
</tbody>
</table>

*All 6 controls best fit to 3 components; 12 component fits used for 3 anoxia runs that could not be fit to 3 components; f = 3; all runs best fit to 2 components.

Values are means ± SD; EF, extraction fraction; t½, clearance half-time in minutes; NS, no significant difference; p, level of significance.

tests. Results are reported as means ± standard deviation.

Results

Control [13N]glu (Preanoxia)

Time-activity curves obtained under control conditions after a bolus of [13N]glu in 6 septa prior to anoxia were all best fit to 3 exponential components as illustrated in Figure 1A. The 2 fastest components, denoted as 1 and 2, each had average clearance half-times (t½'s) of 1.2 minutes or less (Table 1A). Thus, by 5 minutes after a bolus of [13N]glu (or 4–5 clearance t½'s), these 2 components were reduced to insignificant levels. The average half-time of the third component was 416 ± 201 minutes. The extraction fractions of these 3 components are listed in Table 1A. The third component, which represents the longest retention of 13N-label in myocardium, had an extraction fraction of 0.35 ± 0.06.

Chemical analyses to determine the distribution of 13N-radioactivity was performed on tissue sampled 6 minutes after a bolus of [13N]glu was given. Six minutes was chosen because it permitted clearance of the first 2 components and, therefore, permitted examination of the 13N-radioactivity in the retained component; it was also short enough to have adequate counts for well-counting after chemical analysis. A representative example of the chromatographic results from a control experiment is illustrated in the top panel of Figure 2. The tissue analyses from 3 septa revealed that the majority (70%) of the 13N-label was present in [13N]glu (Table 2A). Twelve percent was present in [13N]ala and 11% in [13N]asp. Thus, exogenously delivered glutamate that is extracted by myocardium remains predominantly as [13N]glu at 6 minutes after bolus injection. It is also converted to aspartate and alanine via transaminase reactions, presumably using aspartate aminotransferase (E.C.2.6.1.1) and alanine aminotransferase (E.C.2.6.1.2). 13N-labelled glutamine ([13N]gln) accounted for 5% of the 13N-radioactivity and provided evidence for the presence of glutamine synthetase activity (E.C.6.3.1.2). The distribution of the 13N-label in the effluent collected during the 1-minute interval between 5 and 6 minutes was very similar to the tissue distribution under control conditions (Table 2A). Thus, under control conditions, 13N-labelled amino acids are cleared from the myocardium in proportion to their tissue concentrations. The results for tissue and effluent distributions of 13N-label listed in Table 2B for the control experiments that preceded the pyruvate studies were very

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Distribution of 13N in tissue 6 minutes after bolus of [13N]glu as determined by HPLC. The peaks representing aspartate, glutamate, glutamine, and alanine are indicated by the following respective abbreviations: Asp, Glu, Gin, and Ala. The control distribution appears on top panel, and the distribution during anoxia in the same septum appears on bottom panel. The percent distribution of 13N in [13N]ala increased with anoxia.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Distribution of 13N in tissue 6 minutes after bolus of [13N]glu as determined by HPLC. The peaks representing aspartate, glutamate, glutamine, and alanine are indicated by the following respective abbreviations: Asp, Glu, Gin, and Ala. The control distribution appears on top panel, and the distribution during anoxia in the same septum appears on bottom panel. The percent distribution of 13N in [13N]ala increased with anoxia.
similar to those detailed in Table 2A but were derived from a separate set of 3 septa.

The relative specific activities for these control experiments (A and B) were calculated by dividing the percent distributions of $^{13}$N-label in each amino acid by its measured concentration listed in Table 3. The calculated specific activities are reported in Table 4. In addition, the calculated specific activities were divided by the specific activity for glutamate to obtain normalized specific activities (Table 4). The normalized tissue specific activities for aspartate and alanine from the 2 sets of control experiments ranged from 0.46-0.51 and 0.33-0.39, respectively, compared with 1.0 for glutamate. The glutamine tissue specific activities were 0.02.

Effluent specific activities are also listed in Table 4 (A and B) for the control experiments. Even though the distribution of $^{13}$N-radioactivity in the amino acids was remarkably similar for tissue and effluent, the specific activity for effluent alanine was 4-9% of the specific activity for effluent glutamate, whereas the specific activity for tissue alanine was 33-39% of the specific activity of tissue glutamate. This occurred because the concentration ratio of alanine to glutamate in the effluent was approximately 3-4:1, whereas the same ratio in tissue was approximately 0.5:1. (Concentrations are presented in Table 3.) Thus, a large proportion of the alanine that effluxed from myocardium was apparently not from the compartment that contained $^{13}$Nala.

### Table 2. Tissue and Effluent Percent Distribution of N-13 Label 6 Minutes After Bolus of $[^{13}]$N Glutamate

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Tissue</th>
<th>Effluent</th>
<th>Tissue</th>
<th>Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Anoxia</td>
<td>p</td>
<td>Control</td>
</tr>
<tr>
<td>ASP</td>
<td>11.0 ± 3.3</td>
<td>5.4 ± 2.1</td>
<td>NS</td>
<td>12.8 ± 5.2</td>
</tr>
<tr>
<td>GLU</td>
<td>70.4 ± 7.3</td>
<td>40.5 ± 5.1</td>
<td>.05</td>
<td>61.1 ± 9.3</td>
</tr>
<tr>
<td>GLN</td>
<td>4.6 ± 0.6</td>
<td>6.3 ± 1.3</td>
<td>.05</td>
<td>3.3 ± 2.6</td>
</tr>
<tr>
<td>ALA</td>
<td>12.2 ± 5.2</td>
<td>38.8 ± 2.4</td>
<td>.05</td>
<td>15.5 ± 8.6</td>
</tr>
<tr>
<td>UC</td>
<td>1.9 ± 0.6</td>
<td>9.4 ± 3.2</td>
<td>.05</td>
<td>7.3 ± 2.7</td>
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### Table 3. Tissue and Effluent Amino Acid Concentrations

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Tissue (μmoles/kg wet tissue)</th>
<th>Effluent (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Anoxia</td>
</tr>
<tr>
<td>ASP</td>
<td>590 ± 126</td>
<td>325 ± 72</td>
</tr>
<tr>
<td>GLU</td>
<td>1699 ± 281</td>
<td>682 ± 219</td>
</tr>
<tr>
<td>GLN</td>
<td>5909 ± 543</td>
<td>4250 ± 1627</td>
</tr>
<tr>
<td>ALA</td>
<td>723 ± 159</td>
<td>1238 ± 235</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Pyruvate</td>
</tr>
<tr>
<td>ASP</td>
<td>522 ± 159</td>
<td>445 ± 20</td>
</tr>
<tr>
<td>GLU</td>
<td>1524 ± 237</td>
<td>640 ± 66</td>
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<tr>
<td>GLN</td>
<td>5439 ± 2012</td>
<td>4129 ± 1156</td>
</tr>
<tr>
<td>ALA</td>
<td>804 ± 276</td>
<td>1628 ± 494</td>
</tr>
</tbody>
</table>

Values are means ± SD.
ASP, aspartate; GLU, glutamate; GLN, glutamine; ALA, alanine; UC, unidentified compounds; p, significance level using paired t test.
Amino acid | Tissue (× 10⁻³) | Effluent | p
--- | --- | --- | ---
A. n = 3 | n = 3 | p
ASP | 19.3±8.0 .(46±.19) | 17.7±8.6 .(28±.14) NS | 5.5±3.2 .(50±.29) | 16.0±8.3 .(42±.22) NS
GLU | 42.0±3.0 .(100±.07) | 62.6±1.5 .(100±.02) NS | 11.1±0.3 .(100±.03) | 38.3±23.6 .(100±.62) NS
GLN | 0.8±0.2 .(0.02±0.005) | 1.6±0.8 .(0.03±.01) NS | 0.1±0.04 .(0.009±0.004) | 0.2±0.06 .(0.005±.002) NS
ALA | 16.4±3.8 .(39±.09) | 32.5±8.9 .(52±.14) NS | 1.0±0.5 .(09±.04) | 2.0±0.6 .(05±.02) .05

B. n = 3 | n = 3 | p
ASP | 23.0±1.0 .(51±.02) | 19.0±17.0 .(35±.31) NS | 8.1±3.9 .(29±.13) | 3.9±0.5 .(06±.007) NS
GLU | 45.0±0.5 .(100±.01) | 55.0±21.0 .(100±.38) NS | 32.8±14.6 .(100±.54) | 68.7±37.8 .(100±.51) NS
GLN | 0.9±0.6 .(02±.01) | 1.4±0.7 .(03±.01) .05 | 0.13±0.14 .(004±.005) | 0.48±0.18 .(007±.002) .05
ALA | 14.8±9.0 .(33±0.20) | 26.2±6.0 .(48±.11) NS | 1.0±0.9 .(04±.03) | 1.9±0.9 .(03±.01) NS

Values are means ± SD. Relative specific activities were calculated by dividing the percent distribution of \(^{13}\text{N}\)-label (Table 2) in each amino acid by its measured concentration (Table 3).

ASP, aspartate; GLU, glutamate; GLN, glutamine; ALA, alanine; p values are for comparisons between relative specific activities; NS, not significant. Relative specific activities were divided by the relative specific activity of glutamate for each set of septa and each set of conditions to yield the normalized specific activities, which are listed in parentheses.

Table 4. Tissue and Effluent Relative (and Normalized) Specific Activities

There was a significant increase in \(^{13}\text{N}\)-ala (218%) and \(^{13}\text{N}\)-glu (37%) with a significant decrease in \(^{13}\text{N}\)-glu (−42%). This was accomplished by an increase in nonradioactive tissue concentrations of alanine (71%, p < 0.05) compared to control conditions, as shown in Table 3A. These concomitant changes resulted in no significant increase in the tissue specific activity of alanine, as shown in Table 4A. Glutamate and aspartate tissue concentrations decreased 59% and 44%, respectively, but these changes were not significant at the p < 0.05 level (Table 3A).

Effluent studies in 3 additional septa did not reveal an increase in the \(^{13}\text{N}\) distribution into \(^{13}\text{N}\)-ala compared with \(^{13}\text{N}\)_glu as noted in the tissue analyses (Table 2A). In addition, the concentration ratio of alanine to glutamate in the effluent during anoxia did not increase from control conditions despite the striking increase in the tissue ratio (Table 3A). Moreover, the concentration ratio of effluent to tissue glutamate remained essentially unchanged from control to anoxia, whereas that of alanine fell substantially. Thus, the increased radioactive and nonradioactive alanine produced in tissue as a result of anoxia may be in a compartment(s) that is not cleared of alanine as rapidly as alanine is cleared from the tissue under control conditions.

Pyruvate \(^{13}\text{N}\)_ glu

The addition of 2 mM pyruvate to the perfusate in 5 septa did not change the extraction fractions derived from time-activity curves obtained after a bolus of \(^{13}\text{N}\)_glu (Table 1B and Figure 4). The \(t_{1/2}\) of the slow component was, however, significantly (p < 0.05) faster with pyruvate present (Table 1B).

Two millimolar pyruvate resulted in a 218% increase in tissue \(^{13}\text{N}\)-radioactivity in \(^{13}\text{N}\)-ala and a 49% decrease in tissue \(^{13}\text{N}\)_glu in 3 septa (Table 2B and Figure 5), which were almost identical to the results...
with anoxia. Correspondingly, nonradioactive tissue alanine increased by 103%, and glutamate decreased by 58% (Table 3B). The normalized specific activities for tissue revealed that there were no significant changes in the specific activity of alanine relative to that for glutamate (Table 4B). The relative amount of \(^{13}\text{N}\)-label in \([^{13}\text{N}]\text{gln}\) increased significantly as also occurred during anoxia.

In contrast to the effluent studies with anoxia, studies with 2 mM pyruvate present demonstrated an increase in the percent of \([^{13}\text{N}]\text{ala}\) present in effluent (Table 2B) as well as an increased concentration ratio of alanine to glutamate when compared with controls (Table 3B). However, the concentration ratio of effluent to tissue glutamate remained stable, whereas the same ratio for alanine fell, as noted with anoxia. The normalized specific activities of glutamate and alanine did not change (Table 4B).

Anoxia \(^{13}\text{NH}_3\)

Time-activity curves from a representative septum after bolus injections of \(^{13}\text{NH}_3\) during control conditions and during anoxia are presented in Figure 6. A summary of the analyses of \(^{13}\text{NH}_3\) time-activity curves from 4 septa during control and anoxia appears in Table 5. As we have previously reported, \(^{13}\text{NH}_3\) curves are best fit by 3 components, of which the fastest 2 have clearance \(t_{1/2}\)’s of approximately 1 minute or less. With anoxia, the extraction of \(^{13}\text{NH}_3\) into the retained component \(\text{EF}(3)\) was significantly reduced \((p < 0.05)\) by 42%.

Chemical analysis revealed that 62% of the tissue \(^{13}\text{N}\)-label was in \([^{13}\text{N}]\text{gln}\) 6 minutes after the \(^{13}\text{NH}_3\) bolus under control conditions, and 61% was in \([^{13}\text{N}]\text{gln}\) under anoxic conditions (Table 6). The rest of the \(^{13}\text{N}\)-label was in \(^{13}\text{NH}_3\) or in the protein fraction. Anoxia did increase the percent of \(^{13}\text{N}\)-label in \(^{13}\text{NH}_3\) in the effluent (Table 6).

Discussion

The clinical application of PET requires use of positron-labelled compounds such as \(^{13}\text{NH}_3\) and \([^{13}\text{N}]\text{glu}\) and knowledge of their metabolic pathways in the organ being studied. Although pathways of ammonia and glutamate metabolism are well known, the specific fate and kinetics of \(^{13}\text{N}\)-labelled ammonia and glutamate in terms of the \(^{13}\text{N}\)-label, in particular, must be characterized to accurately interpret PET images. Detailed studies with the \(^{13}\text{N}\)-label are hampered by the 10-minute half-time for \(^{13}\text{N}\) decay, but there is no other nitrogen isotope available that can be used in tracer quantities.

Control \([^{13}\text{N}]\text{glu}\)

\([^{13}\text{N}]\text{glu}\) is extracted by myocardium, and the \(^{13}\text{N}\)-label is retained predominantly as \([^{13}\text{N}]\text{glu}\), \([^{13}\text{N}]\text{ala}\), and \([^{13}\text{N}]\text{asp}\), with only small amounts of \([^{13}\text{N}]\text{gln}\). The

\begin{figure}

\textbf{FIGURE 4.} Normalized time-activity curves from a septum after injection of \([^{13}\text{N}]\text{glu}\) under control conditions (XXX) and during perfusion with 2 mM pyruvate (...). \(\text{EF}(3)\) s and \(t_{1/2}(3)\) s for each curve are denoted. Pyruvate did not significantly change \(\text{EF}(3)\) but accelerated \(t_{1/2}(3)\).

\end{figure}
presence of $^{13}$Nala and $^{13}$Nasp indicates that exogenous $^{13}$Nglu is a substrate for both alanine aminotransferase and aspartate aminotransferase. These 2 enzymes are located in both the cytoplasm and mitochondria, but the enzyme activity of alanine aminotransferase is significantly lower than that of aspartate aminotransferase in heart muscle. The alanine transferase is significantly lower than that of aspartate after injection of $^{13}$NH$_3$ under control conditions (XXX) and during anoxia (...). EF(3) and $t_{1/2}$s for each curve are denoted. Anoxia resulted in a significant reduction in EF(3) without a significant change in $t_{1/2}$.

The small amount of $^{13}$Nglu produced suggests that exogenous $^{13}$Nglu is not as readily available to glutamine synthetase (located in endoplasmic reticulum) as it is to the aminotransferases. However, glutamine becomes labelled rapidly after a bolus injection of $^{13}$NH$_3$. The glutamine synthetase catalyzed formation of $^{13}$Nglu requires endogenous glutamate. Thus, exogenously provided $^{13}$Nglu appears to enter a compartment in which the aminotransferases are more active than glutamine synthetase. This further confirms compartmentalization of glutamate in myocardium.

Table 5. Extraction Fractions and Tissue Clearance Half-times for $^{13}$NH$_3$

<table>
<thead>
<tr>
<th>Component</th>
<th>Control</th>
<th>Anoxia</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF (1)</td>
<td>0.38 ± 0.21</td>
<td>0.56 ± 0.10</td>
<td>NS</td>
</tr>
<tr>
<td>EF (2)</td>
<td>0.18 ± 0.13</td>
<td>0.19 ± 0.09</td>
<td>NS</td>
</tr>
<tr>
<td>EF (3)</td>
<td>0.44 ± 0.13</td>
<td>0.23 ± 0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>$t_{1/2}$ (1)</td>
<td>0.34 ± 0.09</td>
<td>0.36 ± 0.13</td>
<td>NS</td>
</tr>
<tr>
<td>$t_{1/2}$ (2)</td>
<td>1.05 ± 0.32</td>
<td>1.58 ± 0.14</td>
<td>NS</td>
</tr>
<tr>
<td>$t_{1/2}$ (3)</td>
<td>93 ± 24</td>
<td>90 ± 21</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD.
EF, extraction fraction; $t_{1/2}$, clearance half-time in minutes; NS, not significant; $p$, level of significance.

Table 6. Tissue and Effluent Percent Distribution of $^{13}$N-Label 6 Minutes After Bolus of $^{13}$NH$_3$

<table>
<thead>
<tr>
<th>Component</th>
<th>Tissue</th>
<th>Effluent</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_3$</td>
<td>33 ± 13</td>
<td>33 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td>Gln</td>
<td>62 ± 13</td>
<td>61 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td>PP</td>
<td>5 ± 0.5</td>
<td>6 ± 2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean percent ± SD.
Gln, glutamine; NS, not significant; PP, protein pellet; $p$, level of significance.
Anoxia \[^{13}\text{N}\]glu

In our studies with \[^{15}\text{N}\]glu, anoxia resulted in an increased production of \[^{15}\text{N}\]ala. Our data are consistent with the hypothesis that the lack of oxygen in the perfusate resulted in increased pyruvate production secondary to enhanced anaerobic glucose metabolism. The increased pyruvate production resulted in increased production and accumulation of radioactive and nonradioactive tissue alanine via alanine aminotransferase. This agrees with the studies by Galis and Benmouyal\(^{44}\) using perfused guinea pig hearts in which increased glycolytic flux and increased pyruvate concentrations resulted in increased alanine production. Similarly, Mudge et al\(^{50}\) observed an increase in alanine release from human myocardium during pacing, which could also increase pyruvate production. Increased alanine production has also been noted in autolyzing\(^{46}\) and anoxic rabbit myocardium\(^{47,48}\) as well as in hypoxic perfused rat hearts.\(^{49,50}\) In addition, patients with coronary artery disease release more alanine at rest than subjects without coronary artery disease.\(^{70,21}\) The amount of alanine released was related to the severity of the coronary artery stenoses.\(^{21}\) Thus, increased alanine production may result whenever pyruvate production is increased beyond the metabolic capabilities of the tricarboxylic acid cycle, such as during exercise, pacing, anoxia, and ischemia.

In addition, a body of evidence is accumulating consistent with anoxia-induced increased succinate production with concomitant substrate-level phosphorylation in rabbit heart.\(^{48,51}\) The production of increased succinate begins with augmented conversion of aspartate to oxaloacetate via aspartate aminotransferase and of glutamate to \(\alpha\)-ketoglutarate via alanine aminotransferase.\(^{51}\) Pyruvate is the second substrate of the second reaction and alanine is the by-product. Thus, pyruvate utilization to form alanine facilitates substrate-level phosphorylation via succinate production. This utilization of glutamate may explain why exogenously supplied glutamate results in protection of myocardial mechanical function after oxygen deprivation.\(^{22-26}\)

The decreased tissue glutamate concentration noted with anoxia did not induce a detectable increase in the extraction fraction of the retained third component on \([^{13}\text{N}]\text{glu}\) time-activity curves. However, more important, anoxic conditions did not reduce the EF(3). Thus, retention of the \(^{13}\text{N}\)-label of \([^{13}\text{N}]\text{glu}\) was maintained despite anoxia although the \(^{13}\text{N}\) distribution was significantly changed due to increased formation of \([^{13}\text{N}]\text{ala}\). These results are in agreement with the continued extraction of \([^{13}\text{N}]\text{glu}\) noted by canine myocardium despite significant ischemia indicated by severe reduction in blood flow measured using \(^{13}\text{NH}_3\) as a flow tracer.\(^{9}\)

Effluent analyses on samples taken between 5–6 minutes after the bolus during anoxia indicated that \([^{13}\text{N}]\text{glu}\) was being cleared preferentially compared to \([^{13}\text{N}]\text{ala}\) despite nearly equal tissue \(^{13}\text{N}\)-label distributions. However, the ratio of effluent to tissue alanine fell dramatically from 21.6 \((\times 10^{-3})\) during control conditions to 4.1 \((\times 10^{-3})\) during anoxia, despite no change in the corresponding glutamate ratio. This suggests that anoxia may have induced alanine production in a tissue compartment that is cleared of alanine slower than the tissue as a whole was cleared of alanine under control conditions. The \(^{13}\text{N}\)-label distribution data (Table 2A) confirmed this finding because the percentage of \([^{13}\text{N}]\text{ala}\) in the effluent did not increase, even though there was a significant increase in the percentage of tissue \([^{13}\text{N}]\text{ala}\).
we observed. Anoxia would increase tissue pyruvate first in pyruvate pool (I), but this pool communicates with pool (II). If the exogenously provided [13]glu continues to communicate chiefly with pool (II) and with mitochondrial pyruvate, [13]ala would be synthesized in even greater quantities than under control conditions and would represent a higher percentage of the [13]ala in tissue and therefore have a higher tissue specific activity, as we observed. However, the newly synthesized [13]ala will be found predominantly in a pool(s) that is cleared more slowly of alanine than the tissue as a whole, and the effluent specific activity of alanine would be even less than under control conditions, which agrees with our data. Our data reemphasize the presence of compartmentation of amino acids but, because of limitations in using the short-lived [15]N, do not provide the detailed in situ measurements of metabolite concentrations and absolute tracer specific activities that are needed to further clarify the compartmentation of pyruvate and alanine in myocardium.

Pyruvate [13]glu

Pyruvate was added to the perfusate to test the hypothesis that the changes in [13]glu uptake and clearance induced by anoxia were due to a secondary increase in pyruvate formation. The results were similar to those found with anoxia, except the percentage of [13]N in [13]ala in effluent was increased compared with control values during pyruvate perfusion in contrast to the decrease in percentage noted with anoxia. Despite this, during perfusion with pyruvate, the percentage of effluent [13]N-label in [13]ala was still less than expected given the tissue distribution of the [13]N-label as was noted in the anoxia experiments. This again suggests compartmentation of [13]ala with reduced release of [13]ala from tissue relative to release of [13]glu from tissue.

Thus, the changes that were observed in [13]glu metabolism during anoxia appear to be predominantly related to anoxia-induced increased pyruvate production from increased anaerobic glycolysis. The data are consistent with exogenous pyruvate entering the same pool for alanine synthesis that pyruvate production induced by anoxia enters. This agrees with studies in rat heart in which extracellular pyruvate was directly in communication with the functional cytosolic pool of pyruvate that communicated with the intracellular pool of alanine.52

Anoxia [13]NH3

Anoxia resulted in a significant decrease in the EF(3) of [13]NH3, without a change in clearance half-time or in the distribution of tissue [15]N. The decrease in EF(3) may be related to increased endogenous ammonia production and clearance from tissue to the vascular space.16 It is also possible that the decrease in tissue glutamate (Table 3) we observed with anoxia was associated with a significant decrease in glutamate concentration specifically in the compartment containing glutamine synthetase. This could result in a significant diminution in [13]N-label retention. Finally, the activity of glutamine synthetase may have been reduced during anoxia because it is energy dependent and requires ATP.

Summary

In conclusion, the [15]N-label from [13]glu and [13]NH3 was retained in myocardium by different mechanisms. Anoxia differentially affected the uptake and retention of [13]NH3 and [13]glu. With [13]glu, the distribution of the [13]N-label changed due to a mass action effect on the near-equilibrium alanine aminotransferase reaction without changing the net extraction of [13]N. In contrast, with [13]NH3, the mechanism of retention of the [13]N-label, which uses endogenous glutamate, was unchanged but the net extraction decreased. Although anoxia and ischemia do not have identical effects on myocardial lactate and pyruvate metabolism, the important differences in uptake and retention of the [13]N-label from [13]NH3 (a flow tracer) and [13]glu (a metabolic tracer) during anoxia we observed encourage us to explore further the usefulness of PET with these tracers to diagnose ischemia in vivo. The degree and length of ischemia may well determine the exact pattern of uptake and retention that we will observe.

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