AMMONIA NEUTRALIZATION IN HEART MUSCLE

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The pathways of ammonia neutralization in rat heart muscle were studied with the aid of ^{15}N. The effect of the ammonia concentration in the perfusion fluid on its utilization by the myocardium was studied within the range from 1.6 to 3.4 mM. Neutralized ammonia was found in large quantities in glutamine and glutamic acid and in smaller quantities in alanine and aspartic acid. Isoproterenol-induced necrosis of heart muscle led to a decrease in the binding of ammonia by glutamine (20-40%) and alanine (30-45%). The effect of necrosis on ammonia binding by glutamic acid was not significant, and incorporation of ^{15}N into aspartic acid was reduced in the presence of necrosis, but was increased in the presence of high concentrations.

KEY WORDS: ammonia; amino acids; isoproterenol necrosis; nitrogen-15.

The pathways of ammonia neutralization in heart muscle have still received comparatively little study, although evidence of disturbance of the mechanisms regulating ammonia formation and its level in heart diseases has been obtained. Foremost among this evidence is the rise in the concentration of ammonia in blood from the coronary sinus [1, 2], and an increase in its formation in the heart in hypoxia [7] and myocardial infarction [6]. An increase in ammonia liberation by the myocardium during loading of the heart has been observed [8].

Investigation of the coronary arteriovenous difference in ninhydrin-positive compounds in the blood of patients with various heart diseases has shown that only a negligible fraction of the ammonia is excreted into the blood stream in a free form [1, 8]. The ammonia formed during contraction of the heart muscle may be bound by 2-oxoglutarate by the glutamate-dehydrogenase reaction [3]. Another method of removal of ammonia from the heart is by glutamine formation [3, 5]. There is evidence that ammonia can be removed in the form of transamination products of glutamic acid [8]. It appeared interesting to study the degree of participation of each of the above-mentioned mechanisms in the process of ammonia neutralization in the heart, using ^{15}N.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250 g. Necrosis of the myocardium was induced by three intraperitoneal injections of isoproterenol (25 mg/kg) at intervals of 24 h. The presence of necrosis was confirmed morphologically. The heart was removed from the animals, anesthetized with ether, and perfused by Langendorff's method for 30 min, with recirculation, by oxygenated (95% O_2:5% CO_2) Krebs-Henseleit solution under a pressure of 60 mm Hg. Ammonium-^{15}N acetate (95 g-atom % ^{15}N) was added to the solution to concentrations of 1.6, 2.2, 2.8, and 3.4 mM. The heart was then quickly weighed, frozen in liquid nitrogen, and ground into powder. The protein-free tissue extract was applied to a preparative column (2.5×60 cm) of an M-121 amino-acid analyzer (Beckman) with M-72 resin. Elution was carried out with lithium-citrate buffer, pH 2.8, at the rate of 260 ml/h at 40°C. Amino-acid fractions were purified from buffer components by means of ion-exchange resins. A CHN-analyzer was used for the preparative isolation of nitrogen enriched with the isotope ^{15}N. The isotope composition of the nitrogen of the amino acids was determined on the MI-1305 mass spectrometer.

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TABLE 1. Incorporation of ^{15}N (in g-atom % excess ^{15}N*) into Amino Acids of Rat Heart (M±m)

Amino acid	Concentration of ammonium-^{15}N acetate in perfusion fluid, μmoles/ml			
	1,6	2,2	2,8	3,4
Aspartic acid	0,43±0,01	0,40±0,01	0,38±0,01	0,338±0,01
	0,233±0,07	0,211±0,007	0,36±0,001	0,41±0,01
Alanine	0,272±0,006	0,316±0,007	0,347±0,007	0,41±0,007
	0,171±0,005	0,181±0,007	0,250±0,008	0,32±0,01
Glutamic acid	0,53±0,01	0,56±0,01	0,638±0,008	0,68±0,01
	0,236±0,007	0,46±0,001	0,53±0,01	0,41±0,01
Glutamine	0,42±0,009	0,48±0,01	0,540±0,01	0,64±0,01
	0,34±0,01	0,37±0,01	0,50±0,01	0,59±0,01

*g-atom % excess ^{15}N denotes difference between number of g-atoms % ^{15}N in sample and number of g-atoms % ^{15}N in standard (0.365).

Legend. Here and in Table 2, numerator denotes normal myocardium, denominator myocardium with necrosis.

TABLE 2. Amino-Acid Concentrations in Rat Heart Tissues (in μmoles/100 ml intracellular fluid) after Perfusion for 30 min (M±m)

Amino acid	Concentration of ammonium-^{15}N acetate in perfusion fluid, μmoles/ml				
	0	1,6	2,2	2,8	3,4
Aspartic acid	204±9	180±20	151±7	100±7	123±7
	160±20	160±20	190±20	190±20	200±10
Alanine	402±5	370±10	390±20	410±20	440±10
	350±20	370±15	390±10	400±6	400±20
Glutamic acid	770±20	760±15	750±30	790±5	830±20
	780±10	800±20	810±20	800±25	750±20
Glutamine	800±30	920±10	950±50	960±20	1090±20
	690±10	720±20	760±20	820±10	960±20

Data on the intracellular content of amino acids in the myocardial tissue and incorporation of ^{15}N into these compounds were processed by two-factor dispersion analysis, at a 95% level of significance. Differences between the results for the normal heart and the heart with necrosis also were evaluated by the mean difference method for each compound. The calculations were done on the PDP-8i computer.

EXPERIMENTAL RESULTS

During perfusion of the hearts with ammonium-^{15}N acetate, incorporation of the label took place most actively into glutamic acid and glutamine, and there was less-marked incorporation into alanine and aspartic acid (Table 1).

For glutamic acid, glutamine, and alanine in the intact heart muscle and in isoproterenol-induced necrosis an increase was observed in the degree of incorporation of the isotope ^{15}N with an increase in the ammonium-^{15}N acetate concentration in the perfusion fluid. Enrichment of aspartic acid in the intact heart was reduced with an increase in the ammonium-^{15}N acetate concentration in the perfusion fluid, but the opposite relationship was found in necrosis.

In necrosis of the heart muscle the glutamine concentration fell significantly but the changes for glutamic acid and alanine were not significant. The aspartic acid concentration in the intact heart was reduced, but in the presence of necrosis it was increased (Table 2). An increase in the ammonium-^{15}N acetate concentration in the perfusion fluid led to a significant increase in the glutamine concentration in the normal and necrotic myocardium. Changes in the glutamic acid concentration during perfusion of the hearts with ammonium-^{15}N acetate were not significant (Table 2).

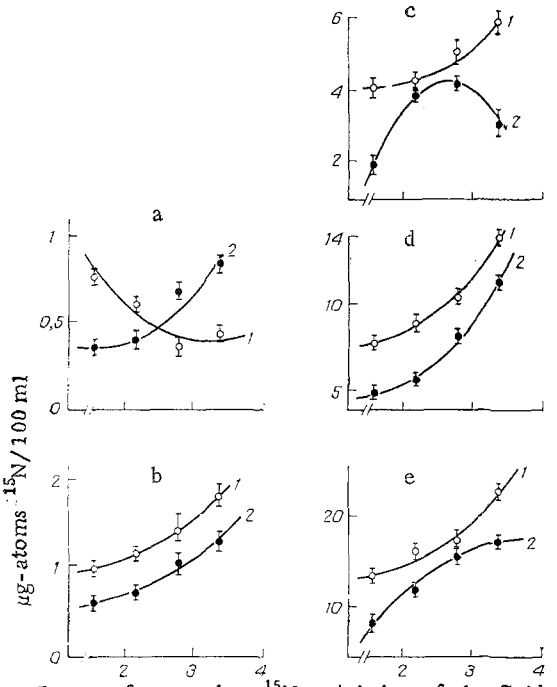

Fig. 1. Intracellular ^{15}N concentration in aspartic acid (a), alanine (b), glutamic acid (c), and glutamine (d) and total ^{15}N concentration in the above amino acids (e). Abscissa, concentration of ammonium-^{15}N acetate in perfusion fluid (in mM); ordinate, concentration of ^{15}N (in μg-atoms/100 ml). 1) Normal; 2) isoproterenol-induced necrosis.

The ^{15}N concentration in the amino acids (in μg-atoms/100 ml) was calculated by the equation:

$$q = \frac{\text{m} \cdot \text{g-atoms } \%^{15}N}{100} \cdot n$$

Total incorporation of ^{15}N by the amino acids of the intact heart studied was about 0.1% of ^{15}N contained in the perfusion fluid. In the case of necrosis this fraction was significantly reduced to 0.07-0.08%. The total quantity of heavy nitrogen incorporated into amino acids increased with an increase in the concentration of ammonium-^{15}N acetate in the perfusion fluid.

Most of the ^{15}N (over 50% of the quantity incorporated) was found in glutamine, and less of the added ammonium was utilized by glutamic acid (about 25%). The rest of the ^{15}N was distributed between alanine and aspartic acid.

Isoproterenol necrosis led to a significant decrease in ^{15}N incorporation into glutamine by 20-40% and into alanine by 30-45% compared with normal. The effect of necrosis for glutamic acid was not significant. Incorporation of ^{15}N into aspartic acid was reduced in necrosis, but in high concentrations it was significantly increased (Fig. 1).

Disparity between its synthesis as a result of perfusion with labeled ammonia and the increase in its absolute concentration in the heart tissues, whether under normal or pathological conditions, was more marked for glutamine than for the other amino acids. For example, during perfusion of the necrotic heart with a 1.6 mM solution of ammonium-^{15}N acetate, 4.9 μmoles glutamine-^{15}N was formed (Fig. 1), whereas the increase in its concentration in the myocardium with the same concentration of ammonium-^{15}N acetate corresponded to approximately 30 μM (Table 2). This fact made it necessary to analyze the possible behavior of glutamine under these experimental conditions and to choose mutually compatible views in order to explain it.

The increase in the concentration of glutamine – the principal ammonia carrier in the myocardium – during perfusion with ammonium acetate may be due to activation of glutamine synthetase under the influence of ammonia (but to a negligible degree), by an increase in proteolysis, inhibition of protein synthesis [8], inhibition of glutaminase, and a decrease in the liberation of glutamine into the perfusion fluid, although this effect of ammonia was not confirmed by the results obtained in [4]. Further experiments are required to assess the contribution of the above-mentioned factors more definitely.

The results with respect to synthesis of amino acids from ammonia-^{15}N are evidence that ammonia is neutralized in the myocardium in the same ways as the corresponding processes in the liver, brain, and muscles, although changes in the concentration of these compounds in the heart during perfusion with ammonium acetate are determined to a greater degree by other causes. Ammonia metabolism in necrosis of heart muscle is substantially modified, and this calls for further study.

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CALCIUM TRANSPORT AND ATPase ACTIVITY IN MITOCHONDRIA AND FRAGMENTS OF SARCOPLASMIC RETICULUM OF THE HEART AND MUSCLES OF RABBITS WITH HYPERCHOLESTEREMIA

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Keeping rabbits on a high-cholesterol diet (1 g/kg) for 3-7 months led to an increase in cholesterol concentration in the mitochondrial membranes and fragments of the sarcoplasmic reticulum (SPR) of the myocardium and skeletal muscles. Saturation of the membranes with cholesterol led to a decrease in efficiency of the Ca-pump of the SPR, as reflected in lowering of the Ca/ATP ratio and an increase in the outflow of Ca^{++} from the SPR. Under these conditions the rate of accumulation of Ca^{++} was higher in SPR than in the mitochondria. Activity of mitochondrial Mg^{++}-activated 2,4-DNP-ATPase was reduced in hypercholesteremia.

KEY WORDS: calcium transport; Ca-ATPase; mitochondria; fragments of sarcoplasmic reticulum; heart; skeletal muscle; hypercholesteremia.

Investigations have shown the role of calcium ions in reactions of carbohydrate, lipid, and protein metabolism and in the processes of muscular contraction and oxidative phosphorylation. Accordingly the study of Ca^{++} transport through subcellular membranes is of special interest. One function of Ca^{++} is to regulate muscle contraction and relaxation. The sarcoplasmic reticulum (SPR) of muscles plays a leading role in this process [7, 8]. However,

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