Journal of Chromatography, 276 (1983) 19—27
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1703

GAS—LIQUID CHROMATOGRAPHY OF FREE AMINO ACIDS IN THE CYTOSOL OF MAMMALIAN ATRIUM AND VENTRICLE OF THE HEART

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(First received November 30th, 1982; revised manuscript received March 11th, 1983)

SUMMARY

N-Trifluoroacetyl *n*-butyl ester derivatives of amino acids were studied. The investigations, carried out on random slaughter-house material, were based on the results of histological and biochemical differences between the atrium and ventricle of the mammalian heart muscle. The data indicate a high level of glutamic acid, and a predominance of tyrosine in the atrium and phenylalanine in the ventricle. The results provide evidence for metabolic differences between the atrium and the ventricle of the heart. This finding may have some significance in heart muscle defects.

INTRODUCTION

Gehrke and Lamkin [1] developed the basis of amino acid derivatization which led to the development of methods for the investigation of volatile derivatives. Amino acids require derivatization of the functional groups before their analysis so that they can be separated by gas—liquid chromatography (GLC). A detailed review of this problem was published by Hušek and Macek [2].

In our investigations we used the technique of Gehrke and co-workers [3, 4], who found that separation on two columns was adequate for quantitative amino acid determinations. The work of Casagrande [5], Raulin et al. [6], Amico et al. [7], Adams [8] and Pellizzari et al. [9] was also very helpful with regard to the analysis of non-protein amino acids. The function and role of amino acids in metabolic processes were considered in detail by Meister [10].

In our previous work [11—13] we separated amino acids in mammalian tissues by GLC, and determined the amino acid composition of the tissues. The aim of this work was to indicate the quantitative difference in the amino

0378-4347/83/$03.00

acid composition in the atrium and ventricle of mammalian heart muscle. We based this investigation on results of histological pictures of muscular cells of the atrium and ventricle, from which we concluded that contractile cells of the atria differed from those of the ventricle in the following respects: (1) a smaller diameter (5—6 μm) and shorter length; (2) a less developed T system, or a lack of it; (3) the presence of grains of diameter 300—400 μm, which showed ATPase activity; (4) polar localization of mitochondria; (5) cytoplasm containing a greater number of thick, flattened vessicles; and (6) a well developed Golgi apparatus.

The results of biochemical investigations carried out by Rumyantsev [14, 15] with experimentally administered [^{3}H] thymidine indicate that the number of isotope impulses and mitotically divided cell nuclei in myocytes of the atrium of rats affected by acute left ventricular infarct is greater than that in the perinecrotic ventricular myocardium. In this work we intended to demonstrate that the quantitative amino acid composition of the atrial muscle differs from that of the ventricular muscle.

Literature data provide valuable information on the participation of the amino acid pool of the heart muscle in myogenesis and regeneration of muscular fibres [16, 17], hypertrophy of myocardium [18—20] and protein synthesis in sarcolemma [21, 22]. The important role of alanine [23, 24] in processes of glucogenesis, taurine [25] in the increased influx of amino acids, creatine in energetic processes [26, 27] and oxidation of leucine [28] in enzymatic changes in the heart muscle has been emphasized. The speed, sensitivity and high precision of GLC were advantageous in finding significant differences in the quantitative amino acid composition of the heart muscle of random animal material obtained from the slaughter-house at Bytom.

EXPERIMENTAL

Animals

The sample material was taken from pigs, sheep and cows at the time of slaughter. The purpose of such a choice was to investigate material from animals subjected to different feeding conditions. From each animal examined about 20 g of tissue were taken.

Apparatus

The following equipment was used: glass homogenizer (25 ml capacity) with a PTFE piston, K-24 and VAC 601 centrifuges produced by Janetzki Heinz (Ilmenau, G.D.R.), ion-exchange columns (150 × 12 mm I.D. and 300 × 15 mm I.D.), lyophilizing apparatus produced by VEB MLW Labortechnik (Engelsdorf, G.D.R.), reaction vessels of our own design equipped with PTFE-lined screw-caps, an ultrasonic cleaner produced by Bronson, Smith Kline Co. (Shelton, CN, U.S.A.), oil-baths with a thermoregulator, an evaporator produced by Pierce (Rockford, IL, U.S.A.), a gas chromatograph (Varian 3700) with a CDS 111 C data analyzer and A 25 recorder (Varian, Palo Alto, CA, U.S.A.), 10-μl syringes from Hamilton (Reno, NV, U.S.A.), and 1000- and 100-μl automatic pipettes produced by Eppendorf (Hamburg, G.F.R.).

Reagents

Ammonia solution (7 *N*), hydrogen chloride gas and Dowex 50W-X8 (H^+) (100—200 mesh) were obtained from Fluka (Buchs, Switzerland), pure picric acid from P.O.Ch. (Gliwice, Poland), *n*-butanol Seq. grade and trifluoroacetic anhydride (TFAA) from Pierce and standard amino acids from BDH (Poole, Great Britain), Mann Research Labs. (New York, NY, U.S.A.), Merck (Darmstadt, G.F.R.) and Pierce. A calibration mixture was prepared using Pierce Amino Acid Standard Physiological A/N.

Columns

Chromatographic packings for the analysis of amino acids as their N-trifluoroacetyl (TFA) *n*-butyl ester derivatives were as follows. Column A packing was 0.65% EGA-PS on 80—100 mesh Chromosorb W AW (Supelco, Bellefonte, PA, U.S.A.). Column B packing was 2% OV-17 + 1% OV-210 on 100—120 mesh Supelcoport (Supelco) for the separation only of His, Arg, Trp and Cys.

Preparation of amino acids, homogenization, centrifugation and ultracentrifugation

Tissues were homogenized in the ratio of 1:5 in 1% picric acid at 4°C. The homogenate was centrifuged at 600 *g* for 15 min and the supernatant was then decanted and ultracentrifuged at 105,000 *g* for 60 min. Cell hyaloplasm was obtained from the supernatant. The deproteinized supernatant containing an excess of picric acid was introduced into the ion-exchange column.

Ion exchange

In the following ion-exchange procedure a 60-fold excess of resin capacity to exchangeable ions placed in the column was maintained, i.e., 6 g of Dowex 50W-X8 (100—200 mesh) for 25 ml of the examined supernatant. The procedure was carried out according to Zumwalt et al. [29].

Lyophilization

A 55-ml volume of the eluate and washings was collected and quickly frozen in liquid nitrogen. The sample was placed in a 1000-ml condenser, and after lyophilization, the dry sediment was retained and transferred to an esterfication vessel.

Derivatization

The reaction was carried out according to the method of Kaiser et al. [30].

RESULTS AND DISCUSSION

The results of the investigations shown in the chromatograms in Figs. 1 and 2 and the results of the qualitative and quantitative evaluations are shown in Tables I and II. We selected 25 amino acids from the calibration mixture used in our laboratory. The results obtained provided basic data for evaluation of the amino acid pool in the cytosol of mammalian atrium and ventricle of heart muscle.

Detailed evaluation of the results, common for atrium and ventricle, pre-

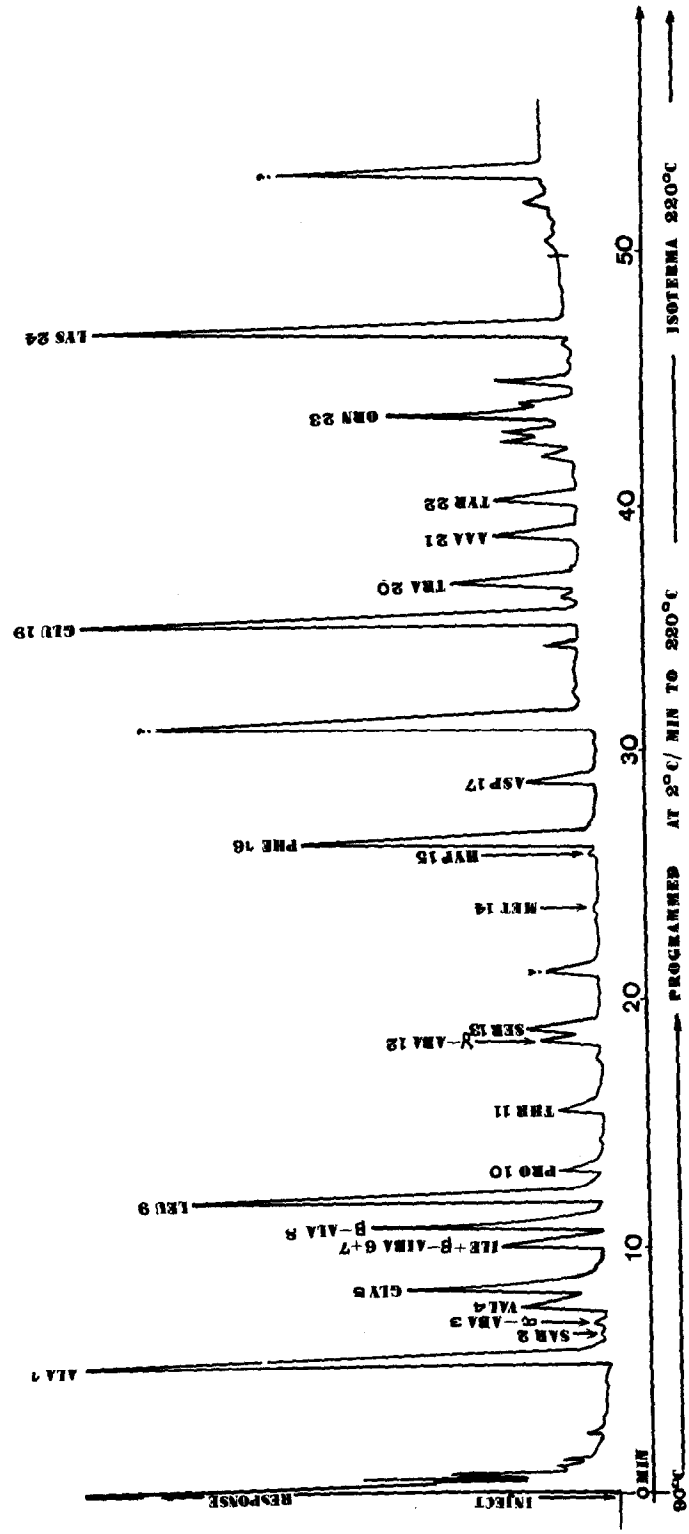

Fig. 1. Simultaneous GLC separation of N-TFA *n*-butyl esters of amino acids in the cytosol of the cow heart atrium. Sample injected: ca. 1 μl. Column: Pyrex (200 cm × 6.35 mm O.D. × 2 mm I.D.), filled with 0.65% EGA-PS on 80—100 mesh Chromosorb W AW (Supelco). Attenuation, 2×10^{-10} a.u.f.s.; initial temperature, 90°C; programming rate, 2°C/min; final temperature, 220°C, flow-rates, carrier gas (helium) 10 ml/min, hydrogen 30 ml/min and air 300 ml/min; internal standard tranexamic acid (peak 20, TRA). High disproportion in amino acid tissue levels makes the correct separation of high and low peaks difficult.

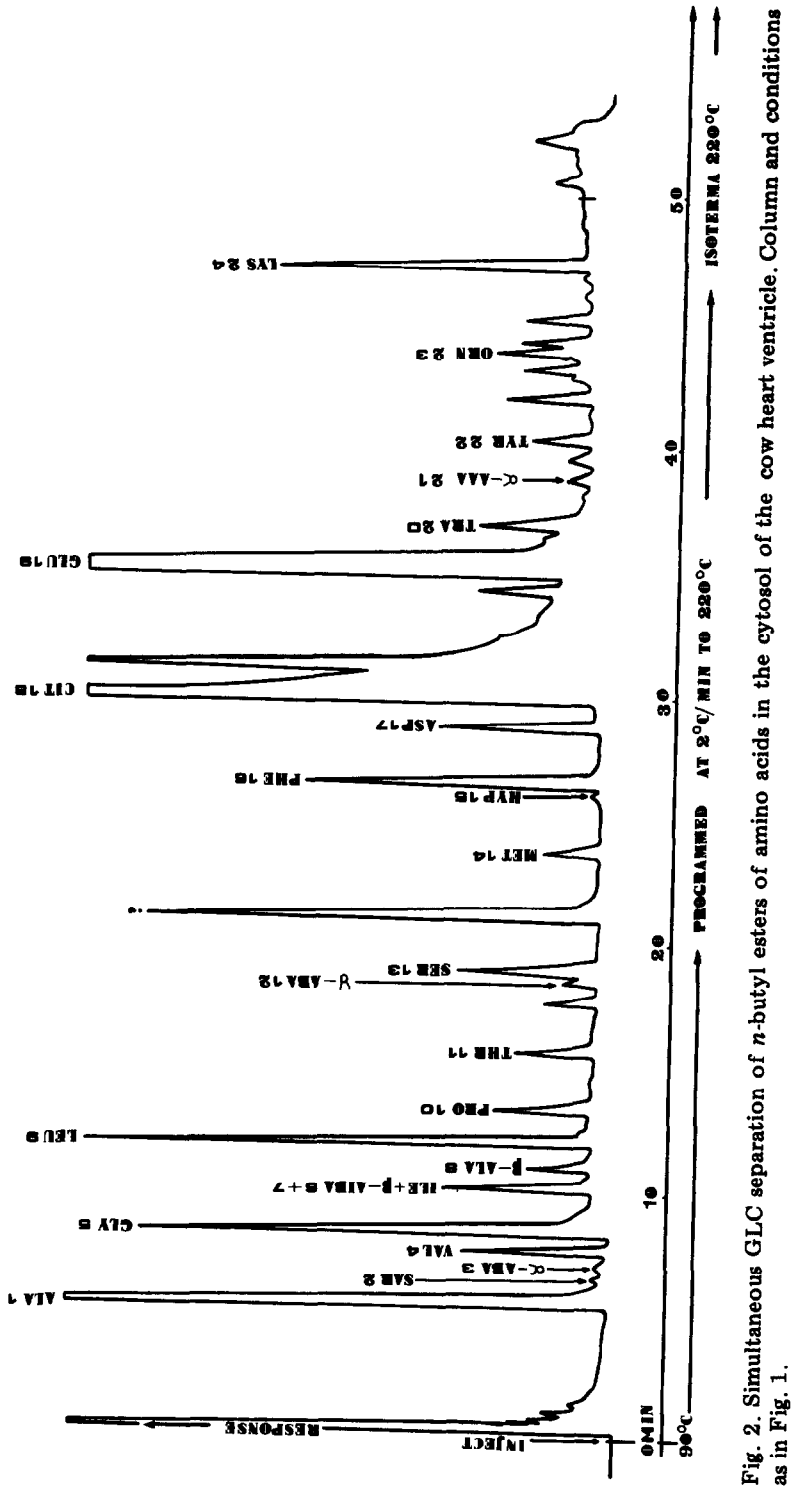

Fig. 2. Simultaneous GLC separation of *n*-butyl esters of amino acids in the cytosol of the cow heart ventricle. Column and conditions as in Fig. 1.

TABLE I

COMPARISON OF GAS–LIQUID CHROMATOGRAPHIC ANALYSIS OF ATRIAL AMINO ACIDS IN THE HEART MUSCLES OF MAMMALIANS

Abbreviations of non-protein amino acids: α-ABA = α-aminobutyric acid; SARC = sarcosine; α-AAA = α-aminoadipic acid. Values are expressed in mg of amino acid and represent the mean ± S.D. of five independent determinations.

No.	Amino acid	In atrium of cow (mg ± S.D.)	Amino acid	In atrium of pig (mg ± S.D.)	Amino acid	In atrium of sheep (mg ± S.D.)
1	βALA	0.585 ± 0.065	GLU	1.141 ± 0.074	GLU	4.325 ± 0.183
2	GLU	0.582 ± 0.029	ALA	0.500 ± 0.056	ALA	2.650 ± 0.092
3	LYS	0.525 ± 0.022	TYR	0.145 ± 0.038	SER	0.775 ± 0.090
4	ALA	0.515 ± 0.058	CYS	0.130 ± 0.000	GLY	0.610 ± 0.018
5	LEU	0.451 ± 0.059	PRO	0.128 ± 0.001	THR	0.184 ± 0.022
6	TYR	0.412 ± 0.108	GLY	0.121 ± 0.019	ASP	0.168 ± 0.007
7	PHE	0.390 ± 0.019	PHE	0.100 ± 0.019	βALA	0.155 ± 0.043
8	ARG	0.215 ± 0.005	ARG	0.090 ± 0.006	TYR	0.143 ± 0.018
9	GLY	0.209 ± 0.032	THR	0.088 ± 0.016	PRO	0.039 ± 0.014
10	VAL	0.148 ± 0.010	LEU	0.086 ± 0.008	LEU	0.136 ± 0.012
11	HIS	0.135 ± 0.025	LYS	0.082 ± 0.015	PHE	0.105 ± 0.020
12	ILE	0.105 ± 0.014	αAAA	0.058 ± 0.027	αAAA	0.096 ± 0.009
13	SER	0.105 ± 0.001	ORN	0.057 ± 0.009	VAL	0.080 ± 0.001
14	THR	0.077 ± 0.003	SER	0.056 ± 0.016	LYS	0.080 ± 0.002
15	γABA	0.070 ± 0.002	HPR	0.040 ± 0.005	HIS	0.070 ± 0.030
16	ASP	0.061 ± 0.009	βALA	0.040 ± 0.010	ORN	0.067 ± 0.008
17	CYS	0.050 ± 0.020	ASP	0.035 ± 0.019	HPR	0.060 ± 0.016
18	ORN	0.049 ± 0.001	ILE	0.029 ± 0.008	ILE	0.060 ± 0.034
19	PRO	0.047 ± 0.006	VAL	0.028 ± 0.003	CYS	0.055 ± 0.005
20	TRY	0.035 ± 0.025	MET	0.010 ± 0.003	γABA	0.050 ± 0.030
21	HPR	0.020 ± 0.005	TRY	0.010 ± 0.000	ARG	0.050 ± 0.020
22	MET	0.016 ± 0.002	γABA	0.009 ± 0.001	MET	0.040 ± 0.010
23	αABA	0.015 ± 0.003	αABA	0.003 ± 0.001	TRY	0.030 ± 0.000
24	αAAA	0.008 ± 0.001	SARC	0.001 ± 0.000	αABA	0.020 ± 0.010
25	SARC	0.003 ± 0.001	HIS	0.000	SARC	0.004 ± 0.000
	Total	4.934	Total	2.995	Total	10.154

sented in Tables I and II indicates a predominant role of glutamic acid and alanine, which constituted about 50% of the total amino acid pool. Some workers [31, 32] reported a particular function of the compounds during the fasting period connected with substantial energetic expenditure and insulin synthesis. The level of glutamic acid was actively regulated by alanine and glycine as a result of polyvalent allosteric anhibition of glutamine synthetase. Alanine is the product resulting from D-glucose degradation. Glycine synthesis was preceded by glycolysis and 3-phosphoglycerine acid production. Combination of alanine and glycine with glutamic acid suggests that the energy was possibly taken from processes of degradation of amino acids and sugars.

The position of glycine in Table I is important because it is the precursor of some biochemically important compounds such as purine, glutathione, creatine, phosphocreatine and tetrapyrrole, all of which take part in the synthesis of proteins and amino acids.

Successive positions in Table I are occupied by lysine, leucine, phenylalanine

TABLE II

COMPARISON OF GAS–LIQUID CHROMATOGRAPHIC ANALYSIS OF VENTRICLE AMINO ACIDS OF THE HEART MUSCLES OF MAMMALIANS

For abbreviations, see Table I. Values are expressed in mg of amino acid and represent the mean ± S.D. of five independent determinations.

No.	Amino acid	In ventricle of cow (mg ± S.D.)	Amino acid	In ventricle of pig (mg ± S.D.)	Amino acid	In ventricle of sheep (mg ± S.D.)
1	GLU	4.417 ± 0.035	GLU	1.430 ± 0.067	GLU	2.260 ± 0.183
2	ALA	1.625 ± 0.091	ALA	0.373 ± 0.033	ALA	1.460 ± 0.173
3	LEU	0.553 ± 0.024	LEU	0.154 ± 0.010	GLY	0.515 ± 0.056
4	GLY	0.485 ± 0.088	GLY	0.149 ± 0.018	LEU	0.465 ± 0.023
5	PHE	0.305 ± 0.019	LYS	0.146 ± 0.009	PHE	0.375 ± 0.023
6	LYS	0.288 ± 0.016	PHE	0.138 ± 0.011	SER	0.260 ± 0.096
7	βALA	0.259 ± 0.053	TYR	0.072 ± 0.005	βALA	0.240 ± 0.023
8	TYR	0.220 ± 0.046	TRY	0.065 ± 0.015	TYR	0.195 ± 0.015
9	ILE	0.195 ± 0.107	ORN	0.056 ± 0.003	TRY	0.165 ± 0.036
10	SER	0.189 ± 0.006	PRO	0.050 ± 0.002	ORN	0.140 ± 0.006
11	THR	0.189 ± 0.010	ARG	0.045 ± 0.015	LYS	0.135 ± 0.013
12	VAL	0.155 ± 0.033	SER	0.041 ± 0.003	ILE	0.115 ± 0.013
13	PRO	0.132 ± 0.002	THR	0.039 ± 0.001	VAL	0.105 ± 0.020
14	ARG	0.100 ± 0.000	ASP	0.037 ± 0.001	PRO	0.090 ± 0.006
15	MET	0.099 ± 0.036	ILE	0.036 ± 0.003	ASP	0.080 ± 0.003
16	ASP	0.097 ± 0.002	CYS	0.030 ± 0.000	THR	0.065 ± 0.016
17	ORN	0.093 ± 0.014	VAL	0.024 ± 0.013	αAAA	0.045 ± 0.005
18	CYS	0.050 ± 0.000	βALA	0.016 ± 0.004	CYS	0.035 ± 0.005
19	γABA	0.040 ± 0.016	αAAA	0.015 ± 0.006	MET	0.025 ± 0.005
20	αAAA	0.025 ± 0.001	MET	0.006 ± 0.002	ARG	0.015 ± 0.005
21	HPR	0.016 ± 0.010	γABA	0.005 ± 0.003	γABA	0.010 ± 0.000
22	TRY	0.015 ± 0.005	αABA	0.004 ± 0.002	αABA	0.010 ± 0.006
23	SARC	0.004 ± 0.000	HPR	0.003 ± 0.002	SARC	0.000
24	αABA	0.004 ± 0.000	SARC	0.001 ± 0.000	HPR	0.000
	Total	9.559	Total	2.940	Total	6.795

and tyrosine, which participate in many metabolic pathways and are endogenic amino acids. Degradation of these amino acids leads to the formation of ketogenic products and acetyl-CoA. Alanine, glycine, serine and threonine make up another group of amino acids, which produce an indirect metabolite, i.e. pyruvate, which has glucogenic properties utilized in the synthesis of sugar compounds.

The positions of endogenic amino acids (3—25 in Table I) indicate that there is a great differentiation in the nutritional demands of the animals investigated. The pH properties in Table I show that basic lysine occupies a particularly high position. The results in Table II for ventricular muscle show that the similarity between the investigated animals (position 1) provides support for the need for metabolic stability.

Evaluation of the tyrosine and phenylalanine levels indicates that there is 50% more phenylalanine in the ventricles than in the atrium. It may be concluded that the atrial pathway of furan, to which both amino acids belong, differs from the ventricular pathway. This may be caused by the activity of

phenylalanine hydroxylase, which produces tyrosine by means of hydroacylation. This enzyme is of special interest because of the precursor role of tyrosine in the synthesis of the following pharmacological and biochemical compounds: adrenaline, noradrenaline, melanine, tyrosine, mescaline, tyramine, morphine, codeine and papaverine.

Leucine was found to be more abundant in the ventricular muscle. This amino acid is formed during the condensation of ketoisovaleric acid (which is the precursor of valine) with the pyruvate acetyl-CoA derivative. Further transformations are similar to the synthesis of α-ketoglutaric acid in the tricarboxylic acid cycle. This process provides evidence for the need to supply the organism with sugar compounds necessary for basic metabolism in the ventricular muscle.

There are also high levels of isoleucine and valine in the ventricular muscle. These are produced from the pyruvate derivative, i.e. active acetaldehyde combined with thiamine pyrophosphate. Subsequently, α-acetyl-α-hydroxy acids are formed, and aminated by aminotransferase to isoleucine and valine. Degradation of glucogenic compounds and D-glucose is necessary for these changes to occur.

The comparison of the final results for the analysis of amino acids found in the atria and ventricals shows a high content of histidine, the precursor of histamine, a vasodilating compound.

The amino acid positions in the atrial muscle in Table I indicate different qualitative compositions of the amino acids in the atria investigated (from position 3). The sequence of amino acids may indicate that energetic processes in the atrial muscle are less intense than those found in the ventricular muscle. It is difficult to make a comparison between the amino acid pools in the atria and ventricles. The amino acid composition in the atria allows the conclusion that the dynamic metabolic changes result from different metabolic properties, enzymatic composition and permeability of cell membranes of atrial and ventricle heart muscle.

Analysis of the chromatograms indicates that special attention should be paid to the peaks found between serine and methionine and between aspartic acid and glutamic acid. Also the peaks occupying positions lower than serine are of great interest, as the area of unidentified peaks undergoes continuous changes depending on the kind of tissue. The solvents applied and the TFAA do not affect the area of the unidentified peaks. The remaining unidentified peaks marked with question marks require the application of gas chromatography—mass spectrometry for elucidation.

CONCLUSIONS

The atria and ventricles of mammalian heart have a high level of glutamic acid and alanine, amounting to about 50% of the amino acid pool of the cell cytosol, with a predominance of tyrosine in the cytosol of the atrium and phenylalanine in cytosol of the ventricle. A high level of these compounds is connected with continuous regulation of energetic processes in the actively working heart muscle.

Differences in the metabolism are apparent from the sequence of amino

acids indicated in Tables I and II. The positions of the amino acids indicate that the enzymatic composition in the atria differs from that in ventricles. These differences are connected with the consequences of an acute heart muscle infarct which divides the nuclei in atrial cells as well as causing necrosis of ventricular muscle wall. Existing morphological, biochemical and regenerative differences may indicate significant participation of embryogenetic processes in the development of the mammalian heart muscle.

ACKNOWLEDGEMENT

The authors thank Mrs. Krystyna Bartnicka for technical assistance.

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