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# Myocardial amino acid metabolism in patients with chronic ischemic heart disease

## Myokardialer Aminosäuren-Stoffwechsel bei Patienten mit chronisch-ischämischen Herzleiden

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With 3 figures and 1 table

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*Summary*

In nine patients with ischemic heart disease the authors investigated the arterio-coronary venous difference of free amino acids in serum at rest and during pacing. At rest aspartate was the only amino acid with a marked positive arterio-coronary venous difference. At the peak of pacing, in addition to aspartate, there is a significant positive arterio-coronary venous difference in glutamate, leucine and isoleucine and a significantly negative difference in cystine-cysteine and glutamine with asparagine. When expressed in per cent of the arterial level, the negative difference in alanine is also significant. Among the mutual correlations of arterio-coronary venous differences the negative correlation between alanine and lactate is most significant, which suggests that under normal conditions pyruvate is transformed rather to alanine, while in ischemia lactate is formed from pyruvate, and released from the heart muscle. There is also a positive correlation between alanine and glutamine and between leucine, isoleucine and glutamate.

On the other hand, cystine-cysteine correlates very significantly but inversely with leucine, isoleucine and glutamate.

The arterio-coronary venous difference of aspartate, though significantly positive, does not correlate with any other amino acid. The arterio-coronary venous differences of ammonia and uric acid correlate inversely, whereby uric acid, contrary to ammonia, is practically not released from the heart muscle.

The importance of amino acids in the energy metabolism of the myocardium is not decisive from the quantitative aspect, various findings suggest, however, that amino acids play an important role in metabolic regulation and under certain conditions may become an essential substrate (8). Amino acids are linked at various sites with metabolic pathways, whereby a decisive role is played by transamination and oxidative deaminations. According to some findings it is probable that in the myocardium, similarly as in skeletal muscle, alanine is produced (9, 15, 24). The uptake of glutamate and release of alanine is probably associated with the necessity to

detoxicate in skeletal muscle and in the myocardium ammonia which is formed above all by the breakdown of adenine nucleotides (1, 31).

In ischemic heart muscle lactate is formed (3, 18, 21, 25) and at the same time according to some findings less alanine is released (4). The arterio-coronary venous (a-cv) difference of glutamate in subjects with myocardial ischemia remains positive during pacing (24). Little is known about the behaviour of other amino acids in ischemic heart muscle. We decided therefore to investigate changes in a a-cv differences of free amino acids during flow through the heart muscle at rest as well as at the peak of pacing in a group with ischemic heart disease. In our experiment we used the development of a negative a-cv difference of lactate and a higher lactate-pyruvate ratio in blood from the coronary sinus as compared with arterial blood as an objective metabolic criterium of the development of acute induced ischemia of the heart muscle (3, 11, 16, 25). Because the intensity of the response to pacing displays great individual differences, it is important to pay attention not only to mean values of a-cv differences for the whole group but also to individual investigations and mutual correlations of individual parameters and their relationship to possible lactate formation.

## Methods

Nine men aged 37–55 years were examined. All suffered from angina pectoris grade II to IV and all had a myocardial infarction in the case-history. None of the patients had signs of cardiac failure. It was a random selection from a group of 53 subjects about which we reported elsewhere (11).

A Goodale-Lubin catheter was inserted into the central portion of the coronary sinus. The stimulation catheter was introduced into the right auricle. The source of impulses was a cardiostimulator with a fixed smoothly adjustable frequency, designed in our department. Either a short or long specially shaped tube was inserted into the brachial artery which made intubation of the left ventricle possible. Heparin was administered in the usual amounts, i.e. 100 i.u. per kg were administered in a single dose and during the examination the catheters were rinsed with saline and heparin at a ratio of 500 i.u. per 250 ml. The values at rest were obtained 5–10 minutes after insertion of the catheter. Then pacing was started and the rate of impulses was increased up to the anginose threshold. However, a heart rate corresponding to 70 % of the maximum appropriate aerobic working capacity of the individual was never surpassed. Subsequent specimens were collected at the peak, i.e. during the 3rd to 6th minute of pacing.

In specimens of arterial blood and blood from the coronary sinus which were obtained by simultaneous suction, lactate and pyruvate were estimated by the enzymatic test of Boehringer Co., ammonia by the test of Hyland Co., uric acid according to *Hořejší* (19), free fatty acids according to *Dole* (10) and the amino acid spectrum in plasma on an amino acid analyzer of Beckmann Instruments (Beckmann 119 Automatic Amino Acid Analyser). Sodium citrate buffer was used. For each of these parameters the a-cv difference during flow through the heart muscle was calculated at rest and during pacing.

The results were processed by routine statistical tests on a digital computer *MINSK* 22.

## Results

The first figure shows the mean a-cv differences of individual free amino acids in plasma of the investigated subjects, always ± 1 standard

Table 1. Correlation coefficients between the arterio-venous difference of amino acids across the heart muscle (which were on average significantly positive or negative) and the a-cv difference of lactate at rest and during pacing (n = 18). Coefficients underlined once are significant at the 5% and those underlined twice significant at the 1% level of significance.

| GLU | LEU | ILEU | CYS | ALA | GLU–$NH_2$ + ASP–$NH_2$ | Lactate | ALA + GLU-$NH_2$ + ASP–$NH_2$ | |
|---|---|---|---|---|---|---|---|---|
| 0.2034 | 0.2469 | 0.2036 | −0.1773 | 0.1946 | 0.0415 | 0.0826 | 0.1094 | ASP |
| | 0.7159 | 0.8212 | −0.7750 | 0.6272 | 0.6505 | −0.4516 | 0.5987 | GLU |
| | | 0.7368 | −0.6459 | 0.6025 | 0.4814 | −0.2619 | 0.5163 | LEU |
| | | | −0.7668 | 0.7445 | 0.7645 | −0.2116 | 0.7111 | ILEU |
| | | | | −0.5847 | −0.5445 | 0.4143 | −0.5368 | CYS |
| | | | | | 0.8804 | −0.6309 | 0.9675 | ALA |
| | | | | | | −0.5400 | 0.9568 | GLU–$NH_2$ + ASP–$NH_2$ |
| | | | | | | | −0.6416 | Lactate |

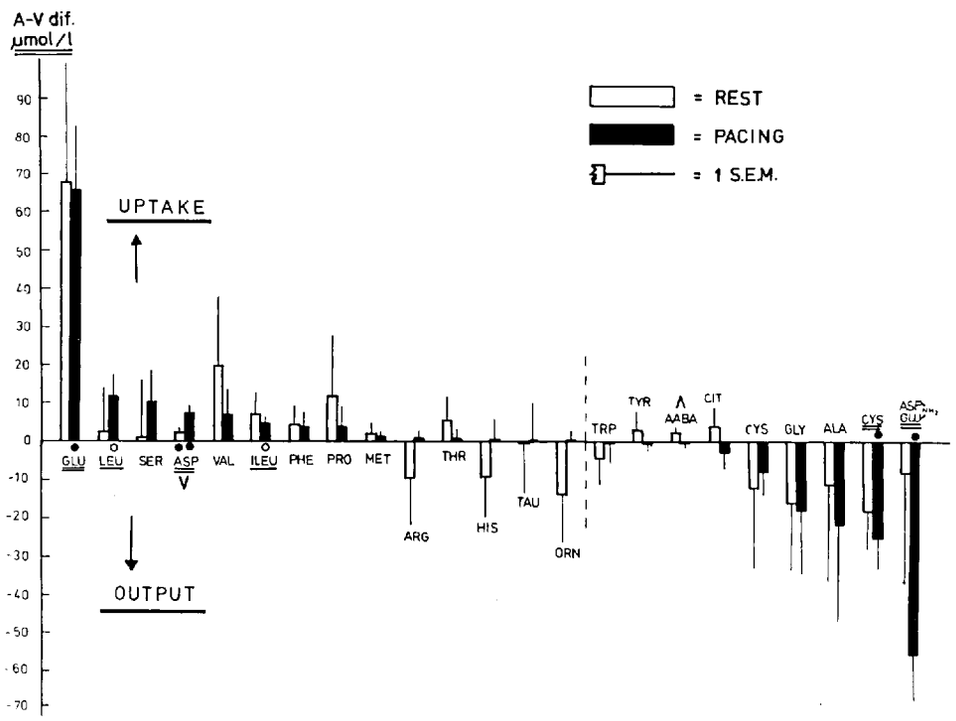


Fig. 1. Mean a-cv difference of amino acids across the heart muscle in micromoles/litre. The first column indicates values at rest, the second one the position at the peak of pacing. The vertical lines indicate the standard error of the mean. The amino acids are listed from the highest average positive to the greatest negative a-cv difference during pacing. o = significant at the 5 % level of significance. • = significant at the 1 % level of significance. V = significant difference between value at rest and after stimulation ($p < 0.05$). CYS = cystin + cystin. Dotted line indicates borderline between uptake and output during pacing.

Individual amino acids are arranged according to the results obtained during pacing, from the highest positive to the highest negative difference. At rest only one a-cv difference is significant at the 1 % level, i.e. the positive difference of aspartate. The other significant a-cv differences develop only after pacing: positive for glutamate and aspartate at the 1 % level and for leucine and isoleucine at the 5 % level of significance. On the other hand a significant output of amino acids by the heart muscle, i.e. a negative a-cv difference develops during stimulation in cystine-cysteine and asparagine with glutamine. The decline of alanine in the blood from the coronary sinus during stimulation is not significant when expressed in absolute values but significant at the 5 % level when expressed in per cent of the arterial level. Alanine declines by about 10 % (9.86 ± 11.03 %).

The mean a-cv difference of lactate at rest is + 0.087 ± 0.092 µmol/l and during pacing 0.127 ± 0.191 micromoles/litre. The lactate-pyruvate ra-

tio at rest is 9.6 ± 2.4 in arterial blood and 10.3 ± 3.1 in the coronary sinus. At the peak of pacing this ratio in arterial blood is 10.9 ± 3.1 and in the coronary sinus 10.5 ± 4.5. The a-cv difference of lactate and the changes of the L : P ratio across the myocardium are thus not significant in the group as a whole.

Table 1 contains correlation coefficients between arteriovenous differences of all amino acids for which a significant uptake or outflow from the myocardium was found on pacing. The correlation is calculated for differences at rest and during stimulation (n = 18). Correlations with a-cv differences of lactate and with the sum of a-cv differences of alanine and glutamine were added to these parameters. In the table those correlation coefficients are marked which are significant at the 1 % and 5 % level of significance.

The arterial levels of free fatty acids in the group were on average 1492 ± 428 micromoles per litre at rest and 1511 ± 503 micromoles per litre during pacing and are thus markedly elevated, as compared with normal values. Figure 2 illustrates the negative correlation between a-cv differences of ammonia and urates. From this correlation it can also be concluded that ammonia is formed by the myocardium in some instances at rest and in particular during pacing, while this is practically not the case with uric acid. The a-cv difference of ammonia does not correlate with any other investigated parameter.

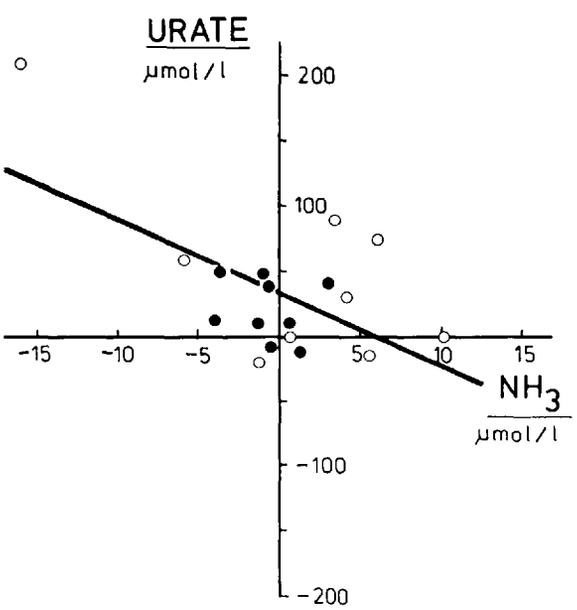


Fig. 2, Relationship between a-cv differences of ammonia (x) and urates (y)
$r_{xy} = -0.5674$, $p < 0.05$ $y = 34.60 - 5.57x$ $(x = 6.21 - 0.18y)$
○ = rert ● = pacing

## Discussion

For a number of years parameters of the carbohydrate and lipid metabolism stood in the foreground in investigations of the energy metabolism. In recent years it was revealed that although from the quantitative aspect in particular the role of amino acids is not primary, amino acids do play an important part in the intermediary metabolism (8) and as a substrate under some conditions (7).

Various findings were obtained in particular in the area of amino acid metabolism in striated muscle. In the foreground of these findings is in particular *Felig's* discovery of the glucose-alanine cycle, i.e. alanine production in muscle and its uptake in the liver where it becomes the substrate for gluconeogenesis (12, 13, 14). Along with alanine also glutamine (23) and asparagine are released. On the other hand, glutamate is taken up by the skeletal muscle (27). The differences of the other amino acids across skeletal muscle are variable with the exception of the branched amino acids, i.e. leucine, isoleucine and valine which can serve as a substrate in skeletal muscle (8).

There is less knowledge about the amino acid metabolism in the myocardium. The reason is methodical difficulties.

According to *Carlsten* there is no detectable uptake nor release of any amino acid except the release of alanine (9). *Mudge* et al. found in healthy subjects and in patients with coronary heart disease (CHD) a negative a-cv

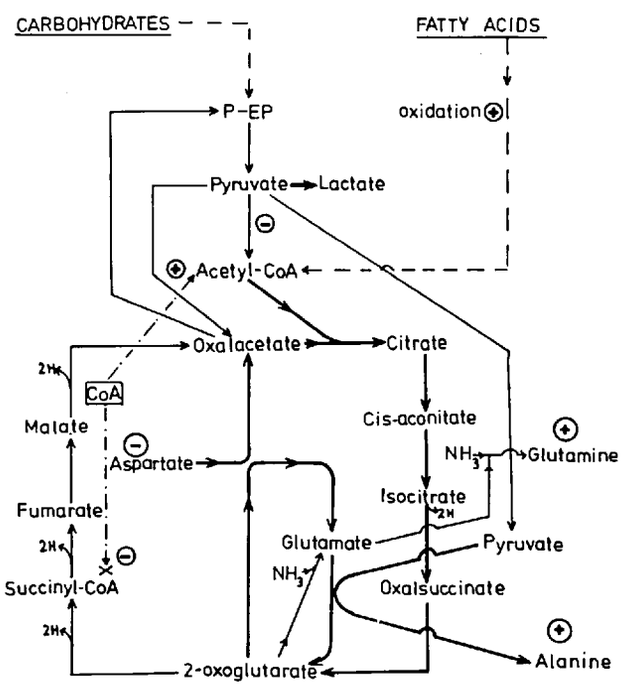


Fig. 3. Diagram of participation of some amino acids in the metabolic pathways of the myocardium; + rise, – decline, P-EP phosphoenol pyruvate.

difference for alanine only, which at rest was greater in patients with CHD. At the same time these authors found a significant glutamate uptake by the heart muscle, again in subjects with CHD as well as in controls (24).

During systole and diastole in the myocardium ATP is utilized and ADP is formed. Part of the ADP is transformed in the phosphohydrolase reaction to AMP. The decline in ATP content and rapid reutilization of inorganic phosphate activate AMP-deaminase which catalyzes the formation of IMP from AMP (28). In this reaction ammonia is formed (1, 22), the production of which in the myocardium was repeatedly demonstrated. Another portion of AMP is broken down to adenosine which has a vasodilating action (1).

Most of the adenosine is rephosphorylated back to AMP and also most of the IMP is transformed via adenyl succinate back to AMP (22). Only a minor portion of IMP and of adenosine is broken down to inosine. Uric acid is formed from inosine via hypoxanthine and xanthine. Our results provided evidence that while in skeletal muscle the ammonia and urate formation run parallel (5), practically no uric acid is produced in the myocardium. This is suggested by a negative correlation between urate and ammonia, urate in some instances – contrary to uric acid – being released from the myocardium, during pacing in particular.

While the ammonia formed is released from the myocardium to a minor extent, most of it is detoxicated on the spot. A small portion of the ammonia in the heart muscle can be transformed into urea, the formation of which in heart muscle was previously proved (30). Most of the ammonia is, however, detoxicated by combinations with 2-oxoglutarate whereby glutamate is formed, and by combination with glutamate whereby glutamine is formed (20). Glutamate participates at the same time in the transamination reaction where it is transformed back to 2-oxoglutarate and pyruvate is transformed at the same time to alanine (fig. 3). Alanine and glutamine + asparagine production runs parallel; the a-cv differences of these metabolites correlate closely in a positive way (30) and increase moreover during pacing. In this case also, alanine formation from pyruvate may be considered part of *Felig's* cycle where alanine is transported from the myocardium to the liver (12, 13, 14).

Pyruvate is formed in the myocardium by glycolysis and also from lactate which under aerobic conditions is one of the main substrates for the myocardium (25). During ischemia the sequence of the reaction catalyzed by lactate dehydrogenase is reversed and lactate is formed from pyruvate and is released from the heart muscle (3, 16, 25). Therefore it may be assumed that the greater the tendency to the development of ischemia or hypoxia in the myocardium, the more lactate and less alanine will be formed and vice versa. One of the reasons may also be the observation that a drop in pH leads to a reduction in transaminase activity (26). The existence of this relationship between lactate and alanine is confirmed by the highly significant negative correlation between the a-cv difference of alanine and lactate. The decline of alanine formation with a parallel rise of lactate formation may suggest the presence of myocardial ischemia. Similarly, a decline of the a-cv difference of glutamine may serve as an indicator of

ischemia; it is also accompanied by a rise of lactate formation in the heart muscle. Alanine and glutamine formation are closely associated and thus even a negative correlation of the a-cv difference of the sum of these two amino acids with a-cv differences of lactate is of importance.

The increased fatty acid supply in our investigation (17) leads to enhanced fatty acid oxidation and inhibits at the same time the decarboxylation system of pyruvate (fig. 3). The enhanced acetyl coenzyme A production leads to a deficiency of coenzyme A for the formation of succinyl CoA which may lead to a block of the tricarboxylic acid cycle at the level of 2-oxoglutarate. Via the transamination reaction, however, 2-oxoglutarate is linked with oxalacetate whereby at the same time aspartate is used and glutamate is formed (29).

The aspartate consumption in our investigation is also typical – it is highly significant at rest as well as during pacing where it increases significantly. While the absolute aspartate consumption is relatively small, the rise of the a-cv difference of aspartate expressed in per cent of the arterial level is considerable (at rest about 20 and after stimulation almost 50 %!). Although the a-cv difference of aspartate is significantly positive, similar to the a-cv differences of leucine, isoleucine or glutamate, it does not correlate significantly with any of these amino acids (which otherwise correlate very closely), and it may thus be concluded that it is quite independent regarding the functional aspect. The product of the transamination reaction in which aspartate is consumed is glutamate which is also formed at the same time by direct amination from oxoglutarate. Despite this, glutamate is not produced, but consumed (it has a significantly positive a-cv difference after pacing). This is due to the binding of ammonia and the participation in another transamination where alanine is formed from pyruvate. The pyruvate consumption eliminates at the same time its cumulation which could occur during partial block of decarboxylation during preferential fatty acid oxidation (29).

The significantly positive a-cv difference of leucine and isoleucine which are associated with an insignificantly positive difference in valine suggest the retention of branched amino acids in the myocardium. We know that these amino acids may serve as a substrate in muscle (8), that their entry into muscle is regulated by insulin and glucagon and that the isolated intake of these amino acids can eliminate a negative nitrogen balance during fasting (8). Therefore an ever increasing theoretical and clinical importance is ascribed to them (7). In our opinoin the finding of the output by the heart muscle of the redox pair cystine-cysteine is very important. It was proved that the level of this amino acid declines after insulin and rises significantly after glucagon administration (6). In the response to these regulatory hormones cystine-cysteine is the only amino acid which displays such a defined reverse behaviour (6).. It cannot be ruled out that the output of cystine-cysteine by the heart muscle which rises and is significant during stimulation is associated with the rise of the serum glucagon level during stress (2).

Free plasma amino acids are not parameters of the myocardial metabolism which are investigated routinely. It was, however, revealed that the importance at least in some amino acids or groups of the latter will

not be purely theoretical and may be a diagnostic contribution. An example is the inverse relation between a-cv differences of alanine, glutamine and the sum of alanine and glutamine on the one hand and the a-cv differences of lactate on the other hand which may contribute to the diagnosis of myocardial ischemia.

*Zusammenfassung*

Die Autoren untersuchten bei 9 Patienten mit ischämischen Herzleiden die arterio-koronarvenöse Differenz von freien Fettsäuren im Serum unter Ruhebedingungen und während künstlichem Schrittmacherantrieb. Bei körperlicher Ruhe war Aspartat die einzige Aminosäure mit einer ausgesprochenen positiven arterio-koronarvenösen Differenz. Auf dem Höhepunkt des Schrittmacherantriebs war zusätzlich zum Aspartat eine positive arteriovenöse Differenz für Glutamat, Leucin und Isoleucin und eine signifikant negative Differenz für Cystin-Cystein und Glutamin zusammen mit Asparagin zu verzeichnen. Ausgedrückt als Prozentsatz des arteriellen Spiegels war die negative Differenz auch bei Alanin signifikant. Bei wechselseitiger Korrelation der arteriovenösen Differenzen war die negative Beziehung zwischen Alanin und Lactat am eindeutigsten, was dafür spricht, daß unter normalen Bedingungen Pyruvat eher zu Alanin umgeformt wird, während im Zustand der Ischämie Lactat aus Pyruvat gebildet und vom Herzmuskel freigesetzt wird. Auch ergibt sich eine positive Korrelation zwischen Alanin und Glutamat und zwischen Leucin, Isoleucin und Glutamat. Andererseits korreliert Cystin-Cystein hoch signifikant, aber invers mit Leucin, Isoleucin und Glutamat.

Die arterio-koronarvenöse Differenz von Aspartat, obwohl signifikant positiv, korreliert nicht mit irgendeiner anderen Aminosäure. Die arteriovenösen Differenzen von Ammoniak und Harnsäure korrelieren invers, wobei Harnsäure im Gegensatz zu Ammoniak praktisch nicht aus dem Herzmuskel freigesetzt wird.

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