PART 9

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The Effect of Peritoneal Dialysis on Intracellular Free Amino Acids in Muscle from Uraemic Patients

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There are numerous reports of abnormalities in the plasma amino acid pattern in uraemic patients (Gulyassy et al, 1968; Giordano et al, 1970; Young & Parsons, 1970; Condon & Asatoor, 1971). It is also known that uraemic patients lose amino acids during peritoneal dialysis and haemodialysis (Rubini & Gordon, 1968; Young & Parsons, 1969; Norée et al, 1971). This may contribute to the protein depletion with hypoproteinaemia and muscle wasting which occur in such patients. Recent studies have shown that the concentration of several free amino acids is considerably higher in the cells than in the intracellular fluid (Munro, 1970). The largest pool of intracellular free amino acids is in the skeletal muscle tissue. By determining free amino acid concentrations in muscle tissue it should be possible to extend our knowledge of amino acid metabolism under normal and pathological conditions.

In the present study we determined free amino acids in muscle biopsy specimens from normal subjects and from patients with chronic uraemia, treated with intermittent peritoneal dialysis with and without administration of essential amino acids (+ histidine) intravenously.

MATERIAL AND METHODS

Fifteen normal healthy volunteers and 4 patients with chronic uraemia were studied. The uraemic patients were treated with intermittent peritoneal dialysis for 22 hours twice a week. During the period of investigation the patients were kept on a controlled diet, providing 60 g protein and 2400 kCal a day. After at least one week on the diet muscle biopsies were performed before and at the end of a 22-hour peritoneal dialysis, each time after an overnight fast. One week later, the patients were studied in a similar way except that the eight essential amino acids (2.2 g N) in the proportions recommended by Rose (1949) + His (0.46 g N) were infused intravenously during the last 4 hours of dialysis (Norée et al, 1971).*

*The amino acid solution was kindly provided by AB Astra, Södertälje, Sweden
At the time of each muscle biopsy a plasma sample was collected for electrolyte and amino acid determination. The free amino acid concentration in plasma was also studied before, immediately after and 30 min after the end of 6 additional dialyses in the same patients.

The control subjects, who were on a free diet, were studied after an overnight fast. Muscle biopsies were performed in 12 of the subjects.

Muscle tissue was obtained by needle biopsy from the quadriceps femoris muscle (Bergström, 1962).

The material was divided into three pieces, one (30-50 mg) for determination of free amino acids and two (10-20 mg) for water and chloride determination. The tissue for amino acid determination was homogenized and protein was precipitated with 4% sulphosalicylic acid. The supernatant was analysed for free amino acids according to a modified Stein and Moore technique using a lithium buffer system (Kedemberg, 1971). Plasma samples obtained simultaneously were precipitated with 6% sulphosalicylic acid and analysed as above. Water was determined by weighing before and after drying at 90°C. Neutral fat was extracted with petroleum ether; chloride was determined by electrometric titration. The calculation of extra- and intracellular water in muscle tissue was based on the chloride method (Bergström et al, 1971).

If the extracellular concentration and the tissue content is known, the concentration in the intracellular water of the substance in question can be calculated. This was done for each of the amino acids, assuming that the extracellular concentration was equal to the plasma concentration.

RESULTS

1. Normal subjects (Figure 1)

The majority of the amino acids had a much higher concentration in intracellular water than in plasma. The concentration gradient was especially high for Gln, Glu, and Tau. Exceptions were Cys and some of the essential amino acids, notably Val, Leu, Ile, and Phe, which had approximately the same concentration in intracellular water as in plasma.

2. Uraemic patients

Before dialysis (Figure 2) In plasma all the essential amino acids, except Phe and Met, were significantly decreased compared with the normal subjects. Met was increased as were a number of non-essential amino acids. In muscle tissue the intracellular concentrations of practically all the amino acids were increased or showed a tendency to an increase compared to the normal subjects. Exceptions were Thr which was decreased (p<0.05), Val, and Tyr. The ratio Phe/Tyr was significantly increased in plasma as well as in muscle tissue (p<0.001).
The effect of dialysis without amino acid supply (Figure 3). The concentration of most of the amino acids decreased considerably in plasma. Gln and Gly tended to increase. Gln, Arg, Asn, and Phe were unchanged. In plasma collected 30 min after the end of dialysis, the concentrations of the different amino acids were unchanged compared with the concentrations in plasma at the end of dialysis. Exceptions were Cys and Met, which increased after 30 min (p<0.05). In muscle tissue the intracellular concentration of several amino acids tended to decrease after dialysis. For Val this change was almost significant (p<0.05).
Figure 2. Concentration of plasma and intracellular free amino acids in uraemic patients before dialysis (plasma: n = 13; muscle: n = 7). The mean concentrations of amino acids in the normal subjects in Figures 2-5 are shown for comparison.

\[ x = p<0.001 \quad x = p<0.01 \quad x = p<0.05 \quad (x) = p<0.1 \]

The effect of amino acid administration during the last 4 hours of dialysis (Figures 4 and 5) Amino acid administration increased the concentration in plasma of the essential amino acids and His. Decreases were observed in Gly, Pro, and Asn. Thirty minutes after the end of dialysis and amino acid administration, the plasma concentrations of Val, Gly, Phe, and Met tended to decrease, while Pro increased markedly. In muscle tissue the intracellular concentrations of Val, Met, and Phe were significantly increased. The other essential amino acids also tended to increase. The concentration
of Arg and Orn decreased. The Phe/Tyr-ratio showed a further increase after the amino acid administration due to marked accumulation of Phe and unchanged Tyr concentration.

DISCUSSION

The results of the studies in normal subjects confirm animal studies, which indicate that the intracellular pool of amino acids is much larger than the extracellular (Munro, 1970; Adibi, 1971). Zachmann et al (1966) studied the concentration of free amino acids in human muscle tissue obtained at operations. His values are in fair agreement with those obtained by us. The
concentration gradients for some of the amino acids across the cell membrane are very high. This is, however, not the case with the branched essential amino acids and Phe and Cys.

Uraemia is a metabolic disorder, known to influence amino acid metabolism. The intracellular concentration of amino acids has, however, not been studied in uraemic patients before. The finding of significantly increased intracellular concentrations for most amino acids in uraemia before dialysis may indicate that the patients were in a state of increased protein catabolism.
It is known from animal experiments (Sidransky & Verney, 1970; Munro, 1970; Adibi, 1971) and from studies in man (own unpublished observations) that severe protein catabolism results in an increase in the concentration of several free amino acids in muscle tissue. The concentration of amino acids in erythrocytes has been found to be increased in spite of low plasma concentrations in patients with protein malnutrition (Björnesjö et al, 1969; Levy & Barkin, 1971). In our material from uraemic patients, high intracellular concentrations together with low plasma concentrations were found especially for some of the essential amino acids, indicating a change in membrane transport or permeability.

The finding of an increased ratio of Phe/Tyr in muscle as well as in plasma is of special interest since this has earlier been observed in protein malnutrition (Snyderman et al, 1963; Padilla et al, 1971). Thus, the increased concentration of several amino acids in muscle tissue as well as the increased ratio of Phe/Tyr may be considered as non-specific signs of a catabolic state.

Earlier studies from our group indicate that His seems to be essential for patients with severe uraemia and, thus, cannot be synthesised from other nitrogen sources (Josephson et al, 1970; Bergström et al, 1970; Fürst, 1972). The low plasma His concentration in the uraemic patients before dialysis may be a sign of impaired His metabolism. However, the intracellular concentration in muscle was normal.

The known fact that dialysis treatment extracts a much larger amount of
amino acids from the organism than can be accounted for by a decrease in
plasma concentration, raises the question whether the intracellular free
amino acid pool is reduced by the dialysis treatment. This was the case in
the present study, although the decrease in concentration for most of the
amino acids was only small and non-significant. The fact that the intracellu-
lar amino acid pool in the muscle was only slightly reduced (by approximately
3 mMol/l intracellular water adding all the amino acids determined) as a re-
result of dialysis treatment, may indicate that the size of the intracellular
amino acid pool was kept up by continuous breakdown of muscle protein.

When essential amino acids + His were administered during the last hours
of dialysis, higher intracellular concentrations of essential amino acids were
observed than after dialysis without amino acid administration. This indicates
that part of the amino acids administered intravenously were transported into
the muscle cells. This could be of benefit in stimulating protein synthesis.
Studies with $^{15}$N by our group indicate that administration of essential amino
acids in connection with dialysis treatment stimulates protein synthesis in
muscle tissue (Fürst et al, 1970).

Although Phe, which is a constituent of the amino acid solution, increased
remarkably in intracellular water, the Tyr concentration remain unchanged,
ie the Phe/Tyr-ratio increased further. This observation could indicate a
block in the synthesis of Tyr from Phe, possibly by inhibition of Phe-hydroxy-
lase. That such an abnormality could exist in uraemia was pointed out by

CONCLUSIONS

Patients with chronic uraemia treated with intermittent peritoneal dialysis
exhibit abnormalities of the intracellular amino acid pattern, which are con-
sistent with an increased protein catabolism. The intracellular concentration
of a number of amino acids tended to decrease as a result of a 22-hour peri-
toneal dialysis. Intravenous administration of essential amino acids during
the last 4 hours of dialysis prevented the loss of essential amino acids from
the muscle tissue. The higher Phe/Tyr-ratio found in the uraemic patients
before treatment was further increased, when amino acids were administered
during dialysis, indicating a block in the synthesis of Tyr from Phe in uraemia.

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