Plasma and Brain Amino Acids in Walker 256 Carcinosarcoma-bearing Rats

Rudolf Krause, J. Howard James, Christopher Humphrey, and Josef E. Fischer

ABSTRACT

Decreased food intake (anorexia) and competition for nutrients by malignant tumors both contribute to depletion of the tumor-bearing host. Tumor-induced changes in metabolic patterns of the host are superimposed upon the state of malnutrition, which, as concerns amino acid metabolism, results in altered amino acid patterns in plasma and brain.

Plasma amino acid profiles of anorectic Walker 256 carcinosarcoma-bearing rats, non-tumor-bearing pair-fed rats, and non-tumor-bearing freely feeding control rats were determined in an attempt to differentiate between tumor-related changes in amino acid metabolism and the effects of malnutrition. Elevated plasma concentration of alanine and tyrosine and lowered plasma total tryptophan were noted in both tumor-bearing and pair-fed groups and were attributed to decreased food intake and malnutrition. Decreased plasma levels of serine, glycine, aspartate, and hydroxyproline were found in tumor-bearing rats only; these are presumably reflections of increased consumption of these amino acids by the tumor.

Brain amino acid concentrations were measured in all three groups of rats and discussed with reference to possible mechanisms in cancer anorexia. The results of previous studies suggested a relationship between the precursor amino acid tryptophan and the neurotransmitter serotonin and anorexia in tumor-bearing animals. Elevated concentrations of brain tyrosine in the present study might indicate some involvement of central catecholamine neurotransmitters in cancer anorexia.

INTRODUCTION

There is considerable evidence that protein metabolism is altered in growing malignant tissue, in that both the rate and pattern of protein synthesis are different in tumor cells. Synthesis of structural proteins is increased while the capacity to produce specialized proteins is often impaired or entirely lost. Host protein synthesis is also influenced by the tumor. Higher rates of tumor protein synthesis demand increased quantities of amino acids for incorporation into protein, the major source for which is supplied by the plasma free amino acid pool. The content of the plasma free amino acid pool is determined by dietary intake and by amino acids derived from proteolysis of muscle-lean body mass within the host. In addition, it is believed that amino acids from tumor protein breakdown may be used as precursors for the synthesis of new protein in tumor cells.

Tumor cells have the ability to concentrate amino acids from the plasma amino acid pool more efficiently than do normal cells, thus enabling them to compete successfully with the tissues of their host for available amino acids (17). During times of reduced dietary intake, resulting from anorexia, this competition may lead to depletion of the host. A similar type of competition exists in the metabolism of carbohydrates and fat, so that a rapidly growing malignant neoplasm may induce in the host a state of combined nitrogen and energy deficiency similar to that seen in malnutrition. If such quantitative changes in free plasma amino acids are superimposed upon tumor-related qualitative derangements of the normal plasma amino acid profile, the resulting amino acid pattern of the plasma in malignant disease may be expected to differ from that of malnutrition alone.

Reports of plasma amino acid patterns in tumor-bearing humans and animals are largely anecdotal and do not usually include complete amino acid profiles. Furthermore, adequate controls suffering from malnutrition alone have not usually been included.

In this study, we report experiments in which tumor-bearing rats have been compared not only with normal rats but also with animals without tumors but with the same degree of malnutrition as that of the tumor-bearing group, a situation achieved by careful pair-feeding.

This experimental design was deliberately chosen to allow the effects of the tumor upon amino acid patterns to be distinguished from the effect of malnutrition alone, acknowledging that this would not permit any conclusion to be drawn about the mechanism involved. In addition to studying plasma amino acid profiles, we have measured the concentration of amino acids within the brain. This aspect, which to the best of our knowledge has not been previously studied, was included because of the recent suggestions that changes in brain amino acid concentrations, acting through the agency of altered neurotransmitter synthesis, might influence feeding behavior (14, 15, 21, 25).

MATERIALS AND METHODS

Young female Sprague-Dawley rats (80 to 100 g) were kept individually in wire-bottomed cages at 20°C, and a 12-hr dark-light schedule was maintained. After base-line values were determined for daily food intake and growth for 3 consecutive days, groups of 6 animals were given injections of 10⁶ Walker 256 carcinosarcoma cells in the right thigh and permitted free access to rat chow (Purina laboratory chow) and water (tumor-bearing group). Two control groups of the same strain, sex, and weight were given injections of 0.9% NaCl solution. One group (freely feeding) was fed ad libitum while another group (pair-fed) received only the amount of diet consumed the previous day by its paired tumor-bearing rat, each animal in

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the pair-fed group being carefully matched by weight and age to a tumor-bearing rat. Food intake and body weight were estimated daily. Ten days after injection, when food intake of tumor-bearing rats had fallen significantly behind that of freely feeding rats for three days (Chart 1), all rats were stunned and decapitated. Blood was collected from the cervical wound into chilled, heparinized, and plasma was separated by centrifugation at 4°C. Brains were rapidly removed and frozen on dry ice, and plasma and brain were stored at —70°C until analysis. The tumors were excised in toto and weighed. Plasma and brain were stored at —70° until analysis. Immediately before analysis, each sample was passed through a Millipore filter (0.45-μm pore size) to remove particulate material. Amino acids were determined on 50 μl of each sample by a Beckman 121-MB automated amino acid analyzer using the 5-buffer, single-column lithium citrate system. In order to minimize analysis variability, plasma and brain samples from each animal were run alternately at the same recorder range setting of 0.5 absorbancy. Amino acid concentrations were reported as nmol/ml plasma or nmol/g, wet weight, of brain. All values were statistically analyzed by Student’s t test.

### RESULTS

All of the animals given injections of Walker 256 carcinosarcoma cells developed tumors of similar weight [9.7 ± 0.5 (S.D.) g]. None of the animals showed any evidence of bacterial infection.

Food intake in the tumor-bearing animals (and hence in the pair-fed group) was significantly less than that in the freely feeding controls (Table 1; Chart 1A). The tumor-bearing and

#### Table 1

<table>
<thead>
<tr>
<th>Time (days) after tumor transplant</th>
<th>Control (freely feeding)</th>
<th>Tumor-bearing</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–3</td>
<td>12.0 ± 0.7^a</td>
<td>11.4 ± 0.4</td>
<td>—5.0 (NS)^b</td>
</tr>
<tr>
<td>4–6</td>
<td>12.7 ± 1.5</td>
<td>10.2 ± 0.6</td>
<td>—19.7^c</td>
</tr>
<tr>
<td>7–9</td>
<td>11.9 ± 1.6</td>
<td>8.6 ± 0.4</td>
<td>—27.7^c</td>
</tr>
</tbody>
</table>

^a Mean ± S.D.  
^b NS, not significant.  
^c p < 0.01.

#### Table 2

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Freely feeding (FF)</th>
<th>p (FF) vs. TB</th>
<th>Tumor-bearing (TB)</th>
<th>p (TB) vs. FF</th>
<th>Pair-fed (PF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taurine</td>
<td>280 ± 29^d</td>
<td></td>
<td>213 ± 21</td>
<td>273 ± 34</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>41 ± 3</td>
<td></td>
<td>41 ± 3</td>
<td>41 ± 3</td>
<td></td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>49 ± 5</td>
<td>&lt;0.05</td>
<td>49 ± 5</td>
<td>49 ± 5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Threonine</td>
<td>265 ± 10</td>
<td></td>
<td>265 ± 10</td>
<td>265 ± 10</td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>330 ± 3</td>
<td>&lt;0.05</td>
<td>330 ± 3</td>
<td>330 ± 3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Asparagine</td>
<td>87 ± 5</td>
<td>&lt;0.05</td>
<td>87 ± 5</td>
<td>87 ± 5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>147 ± 9</td>
<td></td>
<td>147 ± 9</td>
<td>147 ± 9</td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td>228 ± 12</td>
<td>&lt;0.02</td>
<td>228 ± 12</td>
<td>228 ± 12</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Glycine</td>
<td>431 ± 5</td>
<td>&lt;0.01</td>
<td>431 ± 5</td>
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<td>&lt;0.01</td>
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<tr>
<td>Alanine</td>
<td>386 ± 25</td>
<td></td>
<td>386 ± 25</td>
<td>386 ± 25</td>
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</tr>
<tr>
<td>Cysteine</td>
<td>46 ± 1</td>
<td>&lt;0.01</td>
<td>46 ± 1</td>
<td>46 ± 1</td>
<td>&lt;0.01</td>
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<tr>
<td>Methionine</td>
<td>54 ± 2</td>
<td>&lt;0.01</td>
<td>54 ± 2</td>
<td>54 ± 2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>64 ± 1</td>
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<td>64 ± 1</td>
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<tr>
<td>Ornithine</td>
<td>42 ± 2</td>
<td>&lt;0.01</td>
<td>42 ± 2</td>
<td>42 ± 2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lysine</td>
<td>477 ± 37</td>
<td>&lt;0.01</td>
<td>477 ± 37</td>
<td>477 ± 37</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Histidine</td>
<td>70 ± 3</td>
<td>&lt;0.01</td>
<td>70 ± 3</td>
<td>70 ± 3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Arginine</td>
<td>194 ± 11</td>
<td>&lt;0.01</td>
<td>194 ± 11</td>
<td>194 ± 11</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>α-Amino adipic acid</td>
<td>26 ± 2</td>
<td></td>
<td>26 ± 2</td>
<td>26 ± 2</td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>128 ± 11</td>
<td>&lt;0.01</td>
<td>128 ± 11</td>
<td>128 ± 11</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

^d Mean ± S.E.  
^P versus FF, p < 0.01.
pair-fed rats gained less weight than those in the freely feeding group (Chart 1B).

**Plasma Amino Acids (Table 2; Chart 2).** A comparison of the plasma amino acid patterns of the freely feeding and pair-fed rats shows that the only significant differences which can be related to a reduced food intake are a higher concentration of plasma tyrosine and a lower concentration of plasma tryptophan. Similar changes were noted in the tumor-bearing rats, and it is reasonable to suggest that these were also of dietary origin. The plasma concentration of alanine was also raised in tumor-bearing and pair-fed rats as compared with the freely feeding group, but the differences did not attain statistical significance.

The tumor-bearing rats also showed a plasma amino acid pattern which differed significantly from that of the pair-fed group and was therefore presumably tumor related. Table 2 shows that the tumor-bearing rats differed from their pair-fed controls in having significantly reduced plasma concentrations of aspartate (—28 ± 7%, p < 0.02), hydroxyproline (—44 ± 7%, p < 0.02), asparagine (—28 ± 8%, p < 0.05), glycine (—30 ± 7%, p < 0.02), citrulline (—43 ± 4%, p < 0.01), and arginine (—35 ± 8%, p < 0.02).

**Brain Amino Acids (Table 3; Chart 3).** Concentrations of amino acids in the brains of pair-fed and freely feeding rats differed significantly only with respect to increased concentrations of leucine and tyrosine in the pair-fed rats (17 ± 6%, p < 0.05; 65 ± 22%, p < 0.05, respectively) and a decreased concentration of tryptophan (33 ± 5%, p < 0.01). The in-
increased brain concentration of tyrosine in the pair-fed rats reflects a similar change in the plasma concentration of tyrosine in these animals, but this is not the case for leucine and tryptophan. Furthermore, the tumor-bearing rats which had even lower concentrations of plasma tryptophan had relatively high brain concentrations of this amino acid. This apparently anomalous finding is explained by the fact that the tumor-bearing rats had a 3-fold increase in plasma free tryptophan despite a decrease in the total tryptophan concentration. This was due in part to a 250% increase in the concentration of plasma free fatty acids which compete with tryptophan for binding to albumin and in part to a 25% reduction in albumin concentration in the tumor-bearing rats (21).

When compared with their pair-fed controls, the tumor-bearing rats showed significantly reduced brain concentrations of taurine (−8 ± 2%, p < 0.02), citrulline (−38 ± 5%, p < 0.05), ornithine (−34 ± 5%, p < 0.01), and arginine (−31 ± 5%, p < 0.02).

**DISCUSSION**

These experiments show that rats bearing transplanted Walker 256 carcinosarcomas have different amino acid patterns in both plasma and brain when compared with normal control rats. The use of a second control group whose dietary intake was matched to that of the tumor-bearing group enables us to assert that some of these amino acid changes are most probably secondary to reduced food intake, while others are probably tumor related.

Whether such changes are due to the tumor, to malnutrition, or to a combination of those 2 circumstances, the actual mechanisms involved may be complex and include such factors as digestion, absorption, transport, and subsequent metabolic handling. Some of these factors are outside the scope of this paper, but others may be discussed with some benefit.

The impact of protein and calorie deprivation upon plasma amino acid concentrations is well documented (3, 4, 12, 18). Badger and Tumbleson (3, 4) showed that severe protein malnutrition in animals produced a reduction in plasma concentrations of threonine, phenylalanine, tyrosine, and the branched-chain amino acids while concentrations of alanine and methionine were elevated. Energy deprivation produced similar but less marked changes with the exception of alanine which remained grossly elevated. In these experiments, the changes in plasma amino acids were also partially reflected in the free amino acid pool within the brain, as in malnourished animals which have lower plasma and brain tryptophan concentrations (11, 14).

In our experiments, the tumor-bearing and pair-fed rats consumed smaller amounts of a balanced diet than did the freely feeding controls. This presumably resulted in moderate protein and energy deprivation. Of the changes in plasma amino acid concentrations which we have observed in the tumor-bearing rats, only the decreased tryptophan and increased alanine and possibly tyrosine (30) are explicable on the basis of malnutrition. Alanine is a preferential gluconeogenic amino acid (13, 31) and is synthesized in abundance when glycolysis is increased as in trauma (1), sepsis (24), or cancer (16) and also in the catabolic state associated with starvation (13, 26). The tendency toward higher plasma concentrations of alanine in the tumor-bearing rats may reflect the high rate of gluconeogenesis in malignant disease (16).

Differences in plasma concentrations of glycine and serine between tumor-bearing and pair-fed rats might reflect the known capacity of Walker 256 tumors to utilize these amino acids in vitro. Addition of glycine and serine to a standard medium increases the growth rate of the tumors (27). Although speculative, the lower concentrations of arginine and aspartate in the tumor-bearing rats may also be due to tumor consumption since those amino acids have been reported as being readily incorporated into growing malignant cells (17). Plasma concentrations of hydroxyproline were also significantly reduced in the tumor-bearing rats. This amino acid is present in plasma in 3 forms, free, peptide, and protein bound, and its primary source is breakdown of collagen. Approximately 80% of total hydroxyproline is bound (7). At the present time, data on factors affecting this binding are not available. Since hydroxyproline is measured after precipitation of proteins, the actual amount of total hydroxyproline present in the plasma is undetermined.

Within the brain, concentrations of taurine, citrulline, and arginine were also lower in the tumor-bearing rats. This was probably due to secondary decreases in plasma concentrations of these amino acids. Although there was no difference in the plasma concentrations of ornithine between tumor-bearing and pair-fed animals, the brain concentration was significantly lower in tumor-bearing rats. This remains unexplained.

The relatively high brain concentrations of tryptophan in the tumor-bearing rats are of particular interest. That this has occurred despite a reduction in the concentration of plasma total tryptophan is, we believe, due to an increased concentration of that part of the total tryptophan not bound to albumin (21). Transport of tryptophan across the blood-brain barrier is a matter of some controversy (9, 15, 32). In addition to the role of plasma free tryptophan (5, 9, 29), consideration must be given to the molar ratio of tryptophan to that of the competing neutral amino acids (valine, leucine, isoleucine, phenylalanine, tyrosine, and methionine) because they share a common carrier system (15, 20). In addition, increased blood-brain transport may influence brain tryptophan uptake as has been demonstrated by James et al. (19) in experimental hepatic failure. In previous experiments, we found no evidence to suggest altered competition between tryptophan and the other neutral amino acids. Furthermore, the brain uptake index for tryptophan in the tumor-bearing rats was not different from that in the control groups. Thus, we incline to the view that here the brain concentration of tryptophan is determined under these circumstances to a large extent by the concentration of plasma free tryptophan.

In these studies, we demonstrate increased serotonin turnover secondary to the higher brain concentrations of tryptophan in tumor-bearing animals. Electrophysiological and neuropharmacological studies have suggested that serotoninergic mechanism may be involved in regulation of food intake, with increased serotoninergic activity producing hypophagia, while a decrease in activity is followed by hyperphagia (6, 22). Although many hypotheses have been advanced to explain the cause of anorexia in cancer, concepts which have included both central and peripheral mechanisms, its precise etiology remains unclear (33). Anorexia may be present in an early stage of cancer, even when the tumor is still barely detectable.
(28), suggesting a central mechanism. In our previous studies, we hypothesized that tumor anorexia might be a consequence of this altered serotonin activity in cancer.

Because catecholamines may also play a role in feeding regulation (2, 23), the brain concentrations of the catecholamine precursor tyrosine in the tumor-bearing rats may also be of relevance, particularly since some have recently related tyrosine concentration to brain norepinephrine.

To what extent the observed alterations in plasma and brain amino acid concentrations are tumor specific is an open question, but one which needs answering. Such specific changes may lead to a better understanding of tumor metabolism and its influence upon the tumor-bearing host.

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