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Plasma Tryptophan and Anorexia in Human Cancer*

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Abstract—*A correlation between anorexia and brain levels of serotonin and trytophan (TRP) has been reported in tumor-bearing animals. In the present investigation 45 patients with various types of cancer and 13 control subjects were studied. Prior to the study the patients had received no antineoplastic therapy and were unaware of the malignancy of their disease. Feeding behavior was investigated by means of a questionnaire in which the presence of anorexia (A), aversion to meat (MA), taste (TA) or smell (SA) alterations, nausea and/or vomiting (NV) and early satiety (ES) was assessed. Plasma levels of free TRP, the other neutral amino acids (NAA), albumin and non-esterified fatty acids (NEFA) were assayed. Plasma-free TRP was significantly increased in anorectic cancer patients. The free TRP/competing NAA ratio, which might better predict brain TRP levels, was significantly higher in patients with A, MA, TA, SA, NV and ES than in controls or in non-anorectic (NA) cancer patients. These findings seem to confirm that free TRP may play an important role in human cancer anorexia.*

INTRODUCTION

ANOREXIA is a common feature of cancer [1,2], and markedly contributes to progressive weight loss, advancing cachexia and decreased host resistance, thus indirectly favoring tumor aggressiveness and enhancing the morbidity and mortality of cancer [3,4].

The pathogenesis of anorexia occurring in the late stages of the disease is probably multifactorial, directly influenced by psychological, metabolic and therapeutic factors [5,6]. When anorexia is the presenting symptom of cancer, it must be more directly related to the presence of the tumor [7–9]. The mechanisms by which the feeding behavior is altered by the tumor are still unclear [10–14]. Several hypotheses have been advanced, each considering modifications in the mechanisms involved in the physiological regulation of hunger and satiety. These include involvement of the digestive tract, and thermostatic, glucostatic, lipostatic and hormonal regulation of appetite [7, 14, 15].

Changes in the concentration of plasma amino acids may also affect food intake [16, 17], by influencing the synthesis of brain neurotransmitters [18].

Although the catecholaminergic system has been shown to be related to food intake [19], serotonin and its precursor tryptophan (TRP) appear to be better candidates in the physiological regulation of feeding behavior [20, 21].

It has recently been proposed that an increase in brain serotonin synthesis may be, at least in part, responsible for the onset of cancer anorexia [22, 23]. This hypothesis emerged and was supported by the experimental evidence that brain TRP and serotonin turnover is increased in tumor-bearing animals [22]. Preliminary findings suggest that this hypothesis may also hold true in man [23, 24].

The present report deals with investigations carried out in patients with different types of cancer in whom anorexia and anorexia-related symptoms were carefully evaluated and compared with plasma levels of total and free TRP as well as with some biochemical indexes of the nutritional status. Particular attention was focused on serum albumin and non-esterified fatty acids (NEFA), as well as on plasma neutral amino acids (NAA). Albumin and NEFA, in fact, represent the major determinants of the unbound quota of plasma TRP [25, 26], whereas plasma NAA, by directly competing with free TRP for brain entry across the blood–brain barrier (BBB), may affect the availability of this amino acid for the cerebral synthesis of serotonin [27].

MATERIALS AND METHODS

Patients

The study comprised 45 patients (aged 34–80 yr, mean 64) with cancer in various sites and stages,

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and 13 healthy volunteer subjects (nine males and four females, aged 29–58 yr, mean 40). Diagnosis and other pertinent clinical data on the patients are given in Table 1. All patients had been referred to our department for diagnostic assessment. Most of the patients were then submitted to surgical exploration. The degree of tumor spread was established from pre-operative examination and findings at the operation. Patients with mechanical obstruction to food intake were not included in the study. Prior to the study patients received no antineoplastic treatment and were unaware of the malignancy of the disease.

Evaluation of anorexia

All patients were questioned regarding the presence of a subjective decrease in appetite. To better define anorexia, patients were then invited to report the presence of a series of symptoms, namely meat aversion (MA), taste (TA) and smell (SA) alteration, nausea and/or vomiting (NV) and early satiety (ES), all interfering with eating and possibly related to an altered central nervous system (CNS) regulation of feeding behavior. Another group of symptoms which might interfere with food intake without direct involvement of the hypothalamic regulation of feeding, namely chewing and swallowing problems, soreness or dryness in mouth, constipation, diarrhoea and psychological and/or socioeconomic problems, was also included in the questionnaire. Following this interview, patients reporting one or more of the symptoms included in the first series of symptoms were considered as 'anorectic'.

Nutritional status

Weight loss, skin tests, total lymphocyte count and serum albumin, transferrin and cholinesterase levels were used to assess nutritional status in each patient.

Biochemistry

Fasting blood samples were collected in all patients for the determination of plasma total and free TRP, NAA and serum NEFA.

Tryptophan determination

Plasma total TRP was determined by the spectrophotofluorimetric method described by Denckla and Dewey [28], as revised by Bloxam and Warren [29]. The same method was used to estimate free TRP in an ultrafiltrate obtained from 2 ml of plasma, centrifuged at 800 ***g*** in an Amicon 224-CF-50 for 45 min at room temperature. All values were corrected for reagent blank. This method showed a recovery of 96% for total, and 88% for free TRP.

Amino acid determination

Blood was drawn into heparinized tubes and then immediately centrifuged at 3000 rpm for 15 min. Thirty milligrams of solid sulfosalicylic acid were then added to plasma for deproteinization. The samples were centrifuged at 5000 rpm for 20 min. Hydroxyproline was added as internal stan-

Table 1. *Patients' characteristics*

Cancer		Sex		Age (yr)	Tumor spread*				Weight loss†
	No.	M	F	Range (mean)	1	2	3	Anorexia	>10%
Lung CA	12	10	2	34–75 (60.2)	7		5	9/12	10/12
Breast CA	8		8	45–70 (61.3)	1		7	8/8	5/8
N.H. lymphoma	4	3	1	47–72 (58.5)	3		1	1/4	1/4
G. intestinal CA	3	3		39–72 (59.7)	1	1	1	2/3	2/3
Thyroid CA	2	1	1	65–68 (66.5)	1	1		1/2	1/2
Cutaneous CA	2		2	68–80 (74.0)	2			0/2	0/2
Melanoma	1		1	53			1	0/1	0/1
Naso-pharynx CA	1	1		68			1	1/1	1/1
Parotid CA	1	1		75			1	1/1	1/1
Pleural mesothelioma	1	1		60		1		1/1	1/1
Ovarian CA	1		1	56			1	1/1	1/1
Uterin leiomyoma	1		1	69			1	0/1	0/1
Pancreatic CA	1		1	77			1	1/1	1/1
Renal CA	1	1		58			1	0/1	1/1
Bladder CA	1		1	64	1			1/1	1/1
Unknown origin	5	3	2	58–72 (64.8)			5	3/5	2/5
Total	45	22	23	34–80 (62.5)	16	3	26	30/45	27/45

* 1 = local, 2 = regional nodes, 3 = generalized.

† With respect to the usual body weight.

dard to the supernatant to reach a final concentration of 0.50 μmol/ml. The samples were then filtered through Whatman No. 1 paper and stored at −70°C until use. One hundred microliters were analyzed in a Carlo Erba automatic analyzer, model 3A28, using lithium buffers to separate glutamine and asparagine. NEFA were determined colorimetrically [30]. Albumin was measured using the bromcresol green method as described by Doumas *et al.* [31].

Statistical analysis

Student's *t* test for unpaired data and linear regression analysis were used for statistical analysis of the results.

RESULTS

Evaluation of anorexia

The results of the patients' interview are reported in Table 2. Thirty-one of the 45 patients examined reported the presence of a subjective decrease in appetite. The anorexia was confirmed in 30 patients by the results of the questionnaire. In only one patient was the referred decrease in appetite not associated with any of the other symptoms included in the questionnaire. In the anorectic patients meat aversion was present in 50%, taste alteration in 70%, smell alteration in 46%, nausea and/or vomiting in 53% and early satiety in 70% of cases.

Nutritional status

Twenty-seven of the 45 patients (60%) reported a weight loss of more than 10% of their usual body weight in a mean time of 4.12 ± 3.15 months. Nevertheless, none of the patients lost more than 20% of their usual body weight. The incidence of weight loss was significantly higher in anorectic (23/30, i.e. 76.6%) than in non-anorectic (4/15, i.e. 26.6%) patients ($P < 0.05$). Weight loss was not correlated with skin test reactivity or total lymphocyte count, whereas serum albumin, transferrin and cholinesterase levels, whilst remaining in the normal range, were significantly lower in patients who lost weight than in those who did not (Table 3).

Table 2. Incidence of symptoms that might interfere with eating

Symptom	No. of patients reporting this symptom	% of the patients examined
Decreased appetite	31	69
Meat aversion	15	33
Altered taste	21	47
Altered smell	14	31
Nausea and/or vomiting	16	35
Early satiety	21	47
Chewing problems	14	31
Dryness in mouth	17	38
Soreness in mouth	9	20
Swallowing problems	4	9
Constipation	12	27
Diarrhea	3	7
Psychosocial	3	9
Socioeconomic	—	—

Total and free tryptophan

Plasma total TRP levels in cancer patients without anorexia were comparable to those in control subjects (Table 4). Conversely, total TRP in anorectic patients was significantly lower than in control subjects and non-anorectic patients ($P < 0.02$ and $P < 0.05$ respectively). Mean free TRP level in all cancer patients was higher than in control subjects. Plasma-free TRP levels in both non-anorectic and anorectic patients were signi-

Table 3. Evaluation of the nutritional status of the 45 cancer patients studied

	Normal range	Weight loss > 10%	Weight loss < 10%
No. of cases		27	18
Total lymphocyte count (/mm^3)	1200–3000	1993 ± 1096	1632 ± 709
Serum albumin (g/dl)	3.5–5.5	3.35 ± 0.77†	3.99 ± 0.91
Serum transferrine (mg/dl)	200–400	264 ± 58*	323 ± 90
Serum cholinesterase (U/l)	3000–9000	4769 ± 1866*	6666±2936
Skin test positivity		48%	50%

Results as mean ± S.D. Statistical significance of difference (*$P < 0.02$ and †$P < 0.05$) reflects significance between patients who lost more than 10% vs patients who lost less than 10% of the usual body weight.

Table 4. Behavior of the examined determinants of brain tryptophan levels

No. of cases	Controls (13)	Non-anorectics (15)	Anorectics (30)
Plasma total TRP (μmol/dl)	4.83 ± 1.30	4.55 ± 1.11	3.89 ± 0.91*†
Plasma free TRP (μmold/dl)	0.42 ± 0.08	0.61 ± 0.26	0.86 ± 0.48*
Sum of plasma NAA (μmol/dl)	62.4 ± 21.8	74.3 ± 19.4	69.2 ± 18.1
Plasma free TRP/Σ NAA	0.0069 ± 0.0018	0.0085 ± 0.0038	0.0125 ± 0.0062*†
Serum albumin (gm/dl)	4.45 ± 0.56	4.04 ± 0.90	3.52 ± 0.48*†
Serum NEFA (mVal/l)	0.34 ± 0.17	0.46 ± 0.12	0.51 ± 0.19*

Results as mean ± S.D.
Significantly different from *controls (minimal significance $P < 0.02$) and †non-anorectic cancer patients (minimal significance $P < 0.05$).

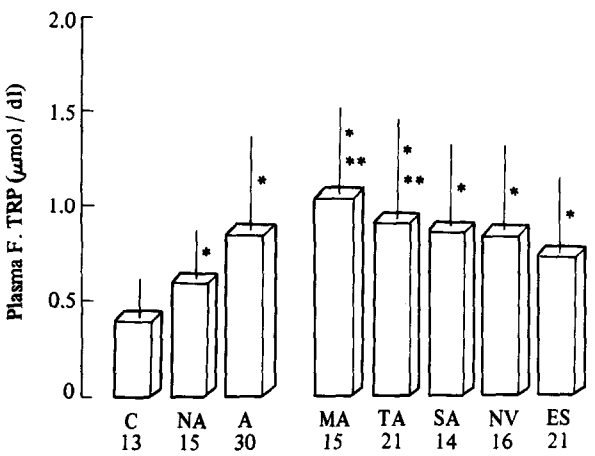

*Fig. 1. Plasma free tryptophan (F.TRP) levels (μmol/dl, mean ± S.D.) in control subjects (C), non-anorectic cancer patients (NA), anorectic cancer patients (A) and in patients referring meat aversion (MA), taste (TA) and smell (SA) alteration, nausea and/or vomiting (NV) and early satiety (ES). * Significantly different from C. ** Significantly different from NA.*

ficantly different from controls ($P < 0.02$ and $P < 0.01$ respectively). Plasma-free TRP levels were higher in anorectic than in non-anorectic patients, the difference being at the limit of statistical significance ($0.05 < P < 0.10$). In patients presenting MA and TA, free TRP levels were significantly higher than those in control subjects ($P < 0.001$) and in patients without anorexia ($P < 0.05$). Free TRP levels in patients with SA, NV and ES were significantly higher than in control subjects (minimal significance, $P < 0.01$) but were not significantly different from those in non-anorectic patients (Fig. 1).

Ratio free tryptophan/sum of neutral amino acids

Both in anorectic and non anorectic patients, the molar sum of the other large NAA (valine, leucine, isoleucine, tyrosine, phenylalanine and methionine) which compete with free TRP for the transport through the BBB, was not significantly different from that in control subjects (Table 4). The free TRP/ΣNAA ratio in non-anorectic cancer patients was not different from that in control subjects, whereas it was significantly higher in patients with cancer anorexia than in control subjects or in non-anorectic patients ($P < 0.005$ and $P < 0.05$ respectively) (Table 4). The ratio was significantly higher in patients with MA, TA, SA and NV than in controls or in patients without anorexia (minimal significance $P < 0.025$). In patients presenting ES the ratio was significantly increased only with respect to control subjects ($P < 0.02$) (Fig. 2).

Albumin and NEFA

Non-anorectic cancer patients showed similar serum albumin values to control subjects (Table 4), whereas in anorectic patients values were significantly reduced compared to control subjects or to non-anorectic patients ($P < 0.001$ and $P < 0.01$ respectively). Serum NEFA levels were higher in cancer patients than in control subjects, though the difference was significant only between anorectic patients and control subjects ($P < 0.02$) (Table 4). Linear regression analysis was used to assess the possible correlation between free TRP, albumin and NEFA. A statistical significant inverse correlation was found only between free TRP and albumin in the anorectic patients ($P < 0.05$).

DISCUSSION

Tumor, anorexia and cachexia frequently represent a triad in which the exact relationship between the three factors is not fully understood [10–14].

Once the anorexia–cachexia syndrome is present in cancer patients, most therapeutic attempts to improve life expectancy are usually unsuccessful [3,4]. This could be due either to tumor status

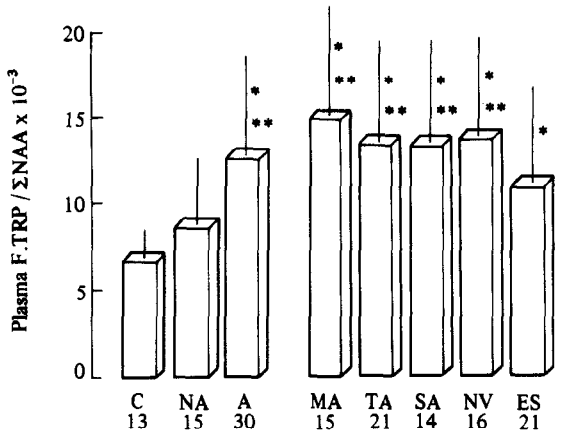

*Fig. 2. Plasma molar ratio of free tryptophan (F.TRP) as compared with the sum of neutral amino acids (ΣNAA) in control subjects (C), non-anorectic cancer patients (NA), anorectic cancer patients (A) and in patients referring meat aversion (MA), taste (TA) and smell (SA) alteration, nausea and/or vomiting (NV) and early satiety (ES). * Significantly different from C. ** Significantly different from NA.*

and/or to severe malnutrition.

Discussion of anorexia in cancer is made difficult by the concomitant influence of several determinants. Indeed, the clinical picture of cancer anorexia is affected by either variables in the tumor or host as well as by the effect of the therapeutic measures [5, 6, 8]. This is particularly true in the anorexia–cachexia syndrome occurring in the late stages of the disease. When the anorexia is the presenting symptom of cancer, it is probably dependent upon the presence of the tumor [7–9].

Several hypotheses have been advanced to explain how the tumor may alter the physiological mechanisms normally regulating feeding behavior [7, 15]. That upon which attention has been focused over the last few years concerns the involvement of the central regulation of feeding in cancer anorexia [22, 23].

The hypothalamus represents the anatomical location of 'hunger' and 'satiety' centres [29, 32]. Experimental evidence demonstrates that the central catecholaminergic system is involved in the hypothalamic regulation of feeding [19]. The factors linking central catecholaminergic activity to food consumption have not yet been fully defined. Changes in plasma amino acid levels, by affecting the brain availability of neurotransmitter amino acid precursors, may control food intake [18]. However, since the activity of tyrosine hydroxylase, the enzyme regulating the rate of catecholamine synthesis, is also influenced by the end products of the reaction, plasma amino acids are unlikely to regulate the activity of feeding centers exclusively through the catecholaminergic system [23].

It has been suggested that the serotoninergic system may influence catecholamine activity, thus behaving as an intermediary in the regulation of food intake [23]. Serotonin synthesis is regulated exclusively by the brain availability of its precursor TRP, as the enzyme converting TRP to serotonin is not saturated at normal brain TRP concentration [18, 20, 33].

A number of theoretical and experimental findings support the role played by serotonin in the regulation of feeding. The hypothalamus contains a large number of serotoninergic fibers [34]. Pharmacologically induced variations in brain serotonin levels or serotoninergic activity result in fluctuation of appetite [35–37].

As already mentioned, brain synthesis of serotonin is directly dependent upon the cerebral concentration of its precursor. Brain TRP levels are determined mainly by three factors: (i) plasma-free TRP (i.e. the quota unbound to albumin) [38, 39]; (ii) the molar ratio of free TRP as compared with the other NAA in plasma that compete with TRP for brain entry [20]; and (iii) the activity of the common transport system across the BBB [27].

The hypothesis that TRP and serotonin may be involved in cancer anorexia is appealing. There is experimental evidence that in tumor-bearing animals the onset of anorexia is correlated with high brain TRP and serotonin levels [22]. The present study seems to support this hypothesis, suggesting that also in man an enhanced serotonin synthesis may contribute to cancer anorexia.

We are confident that anorexia in our patients was related primarily to the presence of the tumor. In fact, the influence of emotional, therapeutic and nutritional factors was excluded, by including in the study only patients who were not aware of the malignancy of their disease, had not received any specific treatment and were not severely malnourished. Moreover, we purposely looked for the presence of symptoms, frequently complained of by anorectic tumor-bearing patients [15], that might suggest a primitive involvement of central neurotransmission. This is the case of modifications in taste and smell (including meat aversion), nausea, vomiting and early satiety. Those patients reporting at least one of the above symptoms were arbitrarily defined as 'anorectic'. According to this criterion, 69% of the patients were considered as anorectic. Interestingly, this rate was close to that in patients spontaneously reporting decreased appetite.

Results emerging from the present study support the role played by the serotoninergic system in cancer-related anorexia. If, in fact, plasma free TRP is considered as one of the determinants of brain TRP levels, it may be supposed, from the increased concentration of free TRP in plasma, that brain serotonin synthesis is increased. Indeed, in the present series of patients with cancer anorex-

ia significantly elevated levels of plasma-free TRP were found. Brain TRP may be better predicted taking into account the plasma concentration of the other NAA which compete with free TRP for the common transport system across the BBB. This is expressed by the ratio free TRP/ΣNAA, which was very closely correlated with the presence of anorexia in our patients.

The rise of free TRP in the anorectic cancer patients may be due to the fact that under physiological conditions about 90% of TRP is bound to plasma albumin [25] and that plasma NEFA, as well as a number of drugs, displaces TRP from albumin by competing for the same binding sites [26]. Significantly, negative correlation between free TRP and albumin levels was found in the plasma of anorectic patients, whereas no correlation was observed between free TRP and NEFA plasma levels. According to these results, the reduction of albumin binding sites would appear *per se* to be responsible for the rise in circulating free TRP levels.

The reduction in serum albumin cannot be considered as indicative of malnutrition. However, it may reflect the presence of a catabolic state. Hypoalbuminemia-inducing substances produced by the tumor itself have also been suggested [40].

Whether anorexia causes a reduction in albumin and a consequent rise in free TRP, or whether free TRP increases primarily for, as yet, unexplained reasons, inducing anorexia and thus decreasing albumin, cannot be established from the data presented. The increased levels of free TRP might, in any case, lead to the onset or worsening of anorexia.

If the hypothesis of an altered serotoninergic activity in cancer anorexia is even partially correct, the clinical implications are far reaching. Indeed, cancer anorexia can be potentially improved by pharmacologically modulating the central serotoninergic activity or by reducing brain availability of TRP. This may be achieved by plasma manipulation of either free TRP and/or of the factors influencing its penetration into the brain.

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