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Platelet serotonin 5-HT_{2A} receptor binding in patients with carcinoid tumor

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Background: As carcinoid tumors produce and secrete serotonin, various serotonin markers in blood, plasma and urine have been used as diagnostic tools, and quantification of the urinary excretion of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) is the method most frequently used. *Methods*: [^{3}H]lysergic acid diethylamide ([^{3}H]LSD) binding to the platelet serotonin 5-HT_{2A} receptor was investigated in nine patients with carcinoid tumors. The possible effect of serotonin-rich food on the receptor binding was also investigated. *Results*: B_{max} for [^{3}H]LSD binding was significantly lower in the carcinoid group than in the control group (mean $\pm$ SD: 17.6 ± 1.3 vs. 23.9 ± 5.2 fmol/mg protein; $p = 0.007$). K_d for [^{3}H]LSD binding was significantly higher in the carcinoid group than in the control group (median: 1.14 vs. 0.71 nmol/L; $p = 0.03$). B_{max} was inversely related to the urinary 5-HIAA excretion, but the correlation did not reach statistical significance ($r_s = -0.57$; $p = 0.14$). Intake of five bananas per day for one week had no effect on B_{max} or K_d in healthy volunteers. *Conclusions*: The results are consistent with a down-regulation of the 5-HT_{2A} receptor as a response to the high serotonin levels found in patients with carcinoid tumors. Intake of serotonin-rich food does not affect the receptor characteristics. Further studies are needed to determine whether the platelet 5-HT_{2A} receptor status can be used as a supplement to urinary 5-HIAA and other biochemical variables in carcinoid tumors.

Key words: Carcinoid; 5-hydroxyindoleacetic acid; lysergic acid diethylamide; platelet; serotonin; tumor markers

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INTRODUCTION

Carcinoid tumors are neuroendocrine tumors derived from enterochrommafin cells. These cells are able to produce serotonin, and when a carcinoid tumor develops, excessive amounts of serotonin are usually secreted. In order to diagnose carcinoid tumors, various serotonin markers have been used, with urinary excretion of the serotonin metabolite 5-hydroxyindoleacetic

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acid (5-HIAA) being the one most frequently used [1]. However, the urinary 5-HIAA levels may be within the reference interval in approximately 30% of patients with carcinoid tumors [2, 3]. In addition, urinary 5-HIAA levels may be enhanced in healthy subjects after consumption of serotonin-rich food such as bananas and some other fruits, and also after intake of several drugs [3]. Thus, the predictive value of urinary 5-HIAA is relatively low [4]. Other markers of serotonin secretion are the urinary [3] and the platelet [5–7] serotonin content. A major limitation of urinary serotonin is that it is predominantly produced from the precursor 5-hydroxytryptophan (5-HTP) in the renal tubular cells. Therefore, it may only be of value in the detection of the subgroup of carcinoid tumors producing 5-HTP. On the other hand, 5-HTP is only slightly increased by a serotonin-rich diet [8]. A disadvantage of the platelet serotonin content method is that it is dependent on the extent of serotonin uptake from plasma by active transport. It is, however, not influenced by the dietary intake of serotonin [8].

The most important biochemical marker for screening of carcinoid tumors is held to be chromogranin A in plasma [1, 9]. This method has a sensitivity of 80–100% in patients with differentiated tumors. However, in a recent study [10], chromogranin A alone was found to have a relatively low diagnostic value. Moreover, as the plasma levels of chromogranin A are increased in other neuroendocrine tumors as well, the method might be less specific than urinary 5-HIAA for the diagnosis of carcinoid tumors [9]. In addition, chromogranin A cannot be used to detect an enhanced serotonin production.

The production and secretion of serotonin in carcinoid tumors is related to the primary site of the tumor. Midgut carcinoids primarily produce serotonin. In contrast, foregut carcinoids predominantly produce other substances such as catecholamines, histamine and 5-HTP, and hindgut carcinoids usually do not produce serotonin [1, 2]. Instead, in patients with hindgut carcinoids, increased plasma levels of neuropeptide Y, substance P and vasoactive intestinal peptide can be noticed. A carcinoid syndrome with flushing, diarrhea, bronchial wheezing, and heart manifestations may arise after massive release of serotonin from a tumor, usually after liver metastases have developed from a midgut tumor [1]. In these cases, urinary 5-HIAA is a useful marker. In contrast, platelet and urinary serotonin, and plasma chromogranin A can be more sensitive markers of tumors with a lower serotonin production [5, 7].

As reflected in this overview, all serotonin markers have some advantages and some drawbacks, and none of them is useful in all settings. Therefore, we investigated another putative serotonin activity marker, the platelet serotonin 5-HT_{2A} receptor, in patients with carcinoid tumors. This receptor would be expected to be down-regulated as a result of excessive concentrations of circulating serotonin [11]. The aim of the present study was to investigate whether patients with carcinoid tumors differ from healthy controls with respect to platelet 5-HT_{2A} receptor characteristics, and to explore whether a possible effect on the platelet 5-HT_{2A} receptor status was correlated to the 24-h urinary 5-HIAA excretion.

MATERIALS AND METHODS

Nine patients with carcinoid tumors were included in the study after giving their informed consent. Of these, seven had midgut carcinoids (five in the ileum and two in the cecum), one had a foregut tumor and one had a hindgut tumor. The patients' ages and the gender distribution are presented in Table I. Six subjects were treated with octreotide and interferon α-2b. Six individuals had liver metastases and had been treated with hepatic artery embolization.

Two of the patients were followed longitudinally with a total of six samples from each individual during a 2-year period. One of these patients was a 57-year old male who had had a diagnosis of carcinoid tumor for three years before the first sampling. The other was a 49-year old female who had had the diagnosis for one year before the first sampling. Both were treated with octreotide and interferon α-2b continuously during the sampling period.

As a control group, 25 age-matched subjects recruited from the community were included in the study after giving their informed consent. These subjects were considered healthy as assessed by medical history, physical examination, and routine blood chemistry tests. Their age and the gender distribution are presented in Table I.

TABLE I. Subject characteristics and B_{max} and K_d for [^{3}H]LSD binding to platelet 5-HT_{2A} receptors from patients with carcinoid tumor (n = 9) and healthy controls (n = 25).

	Carcinoid group	Control group	p-value
No. of females/males	4/5	18/7	–
Age (years), mean ± SD	63.7 ± 9.2	63.8 ± 11.2	–
B_{max} (fmol/mg protein), mean ± SD	17.6 ± 1.3	23.9 ± 5.2	0.007
K_d (nmol/L), median (25–75 percentile)	1.14 (0.88–1.75)	0.71 (0.57–1.16)	0.03

To study the influence of serotonin-rich food, five additional healthy subjects were included in a crossover study. In phase A, the subjects ate five bananas daily for a week before the sample was obtained. In phase B, they did not eat bananas the week before the sample was obtained. The subjects were randomized to start with either phase A or phase B.

The study was approved by the Ethics Committee at the University of Umeå.

Venous blood samples for the study of platelet 5-HT_{2A} receptors were taken with a 20-gauge needle and collected into polyethylene tubes containing 1.6 mg ethylenediamidetetraacetate (EDTA) per ml blood. Total blood volume obtained was 37.5 ml. Platelet 5-HT_{2A} receptor binding characteristics were analysed by binding of the radioligand [^{3}H]lysergic acid diethylamide ([^{3}H]LSD) to platelet membranes. The method used for this assay has been described in detail previously [12]. In brief, platelet-rich plasma was obtained by centrifugation at 180 g for 15 min at 20°C. The platelet pellet was then obtained by centrifugation at 1200 g for 10 min at 10°C and stored frozen at −70°C until use. On the day of the experiment, the platelet pellet was resuspended in hypotonic Tris-buffer, homogenized and centrifuged at 30 000 g for 15 min, washed, homogenized, centrifuged once more and suspended in the incubation buffer. Thereafter, aliquots of the preparation were incubated in triplicate for 4 h at 37°C with seven concentrations of [^{3}H]LSD (Du Pont N.E.N., Boston, MA, USA) ranging from 0.25 to 2.5 nM. Nonspecific binding was assessed in the presence of 300 nM spiperone (Sigma, St. Louis, MO, USA).

Initially, Whatman GF/F filters (Whatman, Maidstone, Kent, England) were used for filtration. However, when we changed to a new filter batch before the samples from the subjects eating bananas were analyzed, we observed that the new GF/F filters had completely different filtration properties. Therefore, we were forced to change to Whatman GF/C filters, prewashed in a 0.3% solution of polyethylenimine. Owing to the change of filters, the B_{max} values generally became lower than before. Therefore, the results from the subjects eating bananas cannot be compared with those of the carcinoid group or the control group.

The radioactivity trapped by the filters was determined by liquid scintillation spectroscopy. Total bound [^{3}H]LSD did not exceed 2% of the total radioactivity. The protein concentrations were measured as described elsewhere [13, 14]. Values for B_{max} (describing the maximum binding capacity, or "the number of" receptors) and K_d (a variable inversely related to the affinity of the radioligand to the receptor) in the [^{3}H]LSD binding experiments were determined by least squares linear regression analysis of Scatchard plots. When the same filter type was used, the inter-day coefficient of variation for the method was 7.7% for B_{max} and 11.7% for K_d.

In the patients with carcinoid tumor, 24-h urine samples for the quantification of the excretion of 5-HIAA were collected in close connection to the blood sampling for analysis of the platelet 5-HT_{2A} receptor binding. Urinary 5-HIAA was analyzed by a method published in detail elsewhere [15, 16]. In brief, after filtration, the urine samples were injected directly on a reversed phase column with tributhyl phosphate as liquid phase. The analysis was carried out using high-pressure liquid chromatography (HPLC) with a fluorescence detector. Hydroxyindolepropionic acid was used as the internal standard.

Student's *t*-test was used for statistical comparisons between patients and controls. The paired version was used to compare the two phases in the subjects eating bananas. As K_d has been found to be log normally

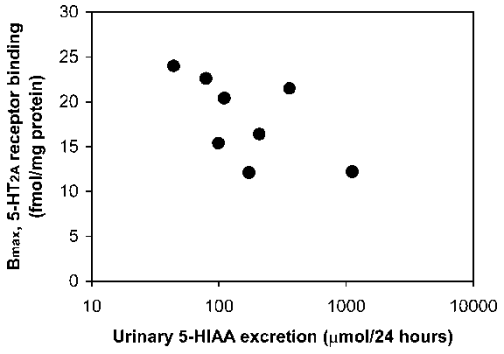

FIG. 1. Relationship between urinary 5-hydroxyindoleacetic acid (5-HIAA) excretion and B_{max} for [^{3}H]LSD binding to platelet serotonin 5-HT_{2A} receptors in eight patients with carcinoid tumor (one of the nine subjects is excluded owing to a missing 5-HIAA value). For clarity, the scale on the abscissa is logarithmic. Although there was a trend towards a negative correlation ($r_s = -0.57$), the value did not reach statistical significance ($p = 0.14$).

distributed, the K_d values were log transformed prior to the inclusion in the analysis. Because it was found that the distribution of 5-HIAA values was skewed, Spearman's rank correlation test was used to evaluate the correlations between B_{max}, K_d and the urinary 5-HIAA excretion.

RESULTS

B_{max} and K_d values for [^{3}H]LSD binding to 5-HT_{2A} receptors in the subjects with carcinoid tumor and the controls are presented in Table I. B_{max} was significantly lower ($p = 0.007$) and K_d was significantly higher ($p = 0.03$) in the carcinoid group than in the control group. In the eight patients from whom 5-HIAA values were available, B_{max} was inversely related to urinary 5-HIAA excretion (Fig. 1), but the correlation did not reach statistical significance ($r_s = -0.57$; $p = 0.14$). There was no significant correlation between K_d and 5-HIAA ($r_s = -0.05$; $p = 0.91$). In the two subjects followed longitudinally with a total of six samples from each, no significant correlations were found between 5-HIAA and B_{max} ($r_s = -0.14$; $p = 0.79$ and $r_s = -0.14$; $p = 0.79$, respectively), or between 5-HIAA and K_d ($r_s = 0.26$; $p = 0.62$ and $r_s = -0.60$; $p = 0.21$, respectively).

Intake of five bananas per day for one week had no significant effect on B_{max} or K_d in healthy volunteers (Table II).

DISCUSSION

The principal finding in this study is that patients with carcinoid tumors had lower B_{max} for [^{3}H]LSD binding to 5-HT_{2A} receptors than healthy controls. The uneven gender distribution is unlikely to have influenced the results, as we have previously shown that there are no gender differences in [^{3}H]LSD binding characteristics [11]. A reduction in B_{max} means that fewer receptors are available for the radioligand. Thus, most likely, elevated serotonin levels in plasma and/or in the platelets have caused a down-regulation of the number of functional receptors. Whereas B_{max} was reduced in the carcinoid group, K_d was enhanced. As

TABLE II. Effect of eating five bananas daily in a week on B_{max} and K_d for [^{3}H]LSD binding to platelet serotonin 5-HT_{2A} receptors.

Subject number	Gender, age (years)	B_{max} (fmol/mg protein), usual diet	B_{max} (fmol/mg protein), usual diet plus bananas	K_d (nmol/L), usual diet	K_d (nmol/L), usual diet plus bananas
1.	Male, 51	11.2	12.5	0.51	0.50
2.	Male, 53	11.1	17.3	0.73	0.61
3.	Male, 57	9.7	7.9	0.58	0.48
4.	Male, 62	12.0	9.3	0.63	0.54
5.	Male, 59	10.2	6.2	0.63	0.20
Mean ± SD (for B_{max})		10.8 ± 0.9	10.6 ± 4.4*		
Median (for K_d)				0.63	0.50†

*$p = 0.91$ vs. usual diet.
†$p = 0.17$ vs. usual diet.

the affinity of [^{3}H]LSD to the receptor decreases when the K_d value increases, it is tempting to suggest that also the K_d finding is a result of a compensatory action at the 5-HT_{2A} receptor.

Midgut carcinoids predominantly produce serotonin, whereas foregut and hindgut tumors mainly produce other substances. These differences would be expected to affect the 5-HT_{2A} receptor status also, but owing to the low number of subjects with non-midgut tumors, it was not possible to perform a meaningful comparison between these groups. The existence of liver metastases is also associated with higher plasma serotonin levels, thus possibly affecting the 5-HT_{2A} receptor status. However, because only three subjects did not have liver metastases, we would nevertheless be unable to detect any differences in 5-HT_{2A} receptor status between these groups.

The negative correlation between urinary 5-HIAA and B_{max} for [^{3}H]LSD binding to platelet 5-HT_{2A} receptors did not reach statistical significance. The lack of a significant correlation is most likely caused by a type II error, as only eight subjects were included in this analysis. A negative correlation would be expected from a mechanistic point of view, given the predicted effects of high serotonin levels on urinary 5-HIAA excretion and on platelet [^{3}H]LSD binding. In the two subjects followed longitudinally, the correlations were also negative, although the correlation coefficients were closer to zero than for the cross-sectional analysis. However, in the longitudinal analyses, even fewer samples were included, thus decreasing the robustness of the correlation coefficient.

Intake of five bananas daily for one week did not affect 5-HT_{2A} receptor status. It has previously been found that intake of bananas did not influence the urinary and platelet serotonin levels, but the urinary excretion of 5-HIAA was increased approximately threefold [3]. Thus, the present result adds further evidence to the assumption that ingested serotonin is converted to 5-HIAA by monoamine oxidase present in the gastrointestinal tract before it reaches systemic circulation, thereby not affecting serotonin markers other than 5-HIAA.

In conclusion, we found a decreased binding of [^{3}H]LSD to platelet 5-HT_{2A} receptors in patients with carcinoid tumor. This result is consistent with a down-regulation of the receptor as a response to high plasma and/or platelet serotonin levels. Intake of serotonin-rich food did not affect the receptor status. Consequently, it could be speculated that the platelet 5-HT_{2A} receptor status could be used as a supplement to urinary 5-HIAA and other biochemical parameters in carcinoid tumors. However, before the place of platelet 5-HT_{2A} receptor measurements can be adequately evaluated, studies including a large number of patients with various clinical characteristics as well as patients followed over an extended period of time have to be carried out. These studies should also include other markers than urinary 5-HIAA excretion, such as plasma chromogranin A and whole blood or platelet serotonin levels. Finally, studies on the longitudinal effect in patients effectively treated with either surgery or drug therapy also have to be performed.

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