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Plasma tryptophan and tyrosine ratios to competing amino acids in relation to antidepressant response to citalopram and maprotiline

A preliminary study

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Abstract. Pretreatment plasma ratios of tryptophan (Trp) and tyrosine (Tyr) to other large neutral amino acids were determined in 27 depressed patients who completed a double-blind trial of citalopram, a selective serotonin uptake inhibitor, against maprotiline, a selective noradrenaline uptake inhibitor.

The Trp ratio and the Tyr ratio were decreased in the total patient sample as compared with healthy controls. Plasma Tyr ratio was normal in the endogenous, but significantly decreased in the non-endogenous depressives. There was no significant relationship between the plasma Trp ratio and the probenecid-induced accumulation of 5-HIAA in the CSF, or between the plasma Tyr ratio and HVA level in CSF, whereas the CSF level of MHPG correlated significantly with the plasma Tyr ratio.

There was a significantly positive correlation between the Trp ratio, the Tyr ratio, their sum and the final Hamilton depression score in 14 patients treated with citalopram; on the whole, this association was evident also in the endogenous and non-endogenous subgroups. In 13 patients on maprotiline there was a significantly positive correlation between the plasma Tyr ratio and the percent reduction of Hamilton depression score; this association was poor in the endogenous, whereas a trend towards a correlation remained in the non-endogenous subgroup. The results suggest that the plasma Trp and Tyr ratios may be determinants of clinical improvement in depressed patients to treatment with citalopram and maprotiline. However, further studies are needed on larger patient samples to allow a firm conclusion.

Key words: Depressive disorder – Tryptophan ratio – Tyrosine ratio – Response prediction – Citalopram – Maprotiline

The serotonin and noradrenaline hypotheses of the affective disorders have stimulated a large number of trials on depressed patients during the past 2 decades. However, this research has been complicated for some methodological reasons. One confusing factor comes from the lack of pharmacologically "clean" drugs since the first generation tricyclic antidepressants like imipramine and amitriptyline generally affect more than one neurotransmitter in the brain. This difficulty has been minimized with the development of drugs like citalopram and maprotiline, which selectively inhibit the reuptake of neuronal serotonin and noradrenaline, respectively (Hyttel 1982).

A second complicating factor has been the lack of suitable and convenient methods for the evaluation of the degree of the suspected brain deficit of serotonin and noradrenaline in the depressives. Some studies have estimated the monoamine metabolite levels in urine or cerebrospinal fluid specimens. Another possible indicator of brain amine syntheses could be the availability from plasma to brain of the precursor amino acids tryptophan (Trp) and tyrosine (Tyr).

In laboratory animals brain serotonin synthesis parallels brain Trp concentration (Fernstrom and Wurtman 1972), which varies directly with plasma Trp concentration and inversely with the sum of the plasma concentrations of the other large neutral amino acids (LNAA), i.e. valine, isoleucine, leucine, Tyr and phenylalanine (Fernstrom et al. 1973), that compete with Trp for their common transport carrier at the blood-brain barrier (Pardridge 1977; Yuwiler et al. 1977). Other basic studies have found supporting evidence, in that the rate of formation of brain noradrenaline is determined by the brain concentration of Tyr (Gibson and Wurtman 1977), which is reflected in the plasma by the molar ratio of Tyr to its competing amino acids, notably valine, isoleucine, leucine, phenylalanine and Trp (Fernstrom and Faller 1978).

Whether the plasma ratios Trp/LNAA and Tyr/LNAA are correlated with brain serotonin and noradrenaline syntheses in man also is not clear. However, these pretreatment plasma amino acid ratios have been found to correlate with the clinical response in depressed patients to treatment with L-tryptophan (Møller et al. 1980), imipramine (Møller et al. 1981), amitriptyline (Møller et al. 1983), and nortriptyline (Møller et al. 1985). The present study reports on the relationship between the plasma Trp and Tyr ratios and the

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antidepressant response in patients treated with maprotiline or citalopram.

Materials and methods

The study took place at the Deltaziekenhuis, Rotterdam Mental Hospital in Holland, and included 29 female inpatients with a major depressive disorder according to the DSM-III (American Psychiatric Association 1980), and with a pretreatment score of at least 18 on the Hamilton depression rating scale (HDRS) items 1–17 (Hamilton 1960). Excluded from the study were patients who did not give their informed consent, patients below 18 and above 65 years of age, pregnant patients, patients with serious concomitant somatic diseases (heart, liver, kidney), patients with an organic cerebral syndrome, schizophrenics or patients with a paranoid psychosis, alcoholics or patients addicted to narcotics, patients with epilepsy, and patients having received MAO-inhibitors within the last 3 weeks.

Prior to the test period the patients were assessed by means of the Newcastle diagnostic rating scale (Carney et al. 1965), which classified the patients in an endogenous and a non-endogenous (including diagnostically doubtful patients) group. After a wash-out period of at least 7 days, in which benzodiazepines were allowed as a sedative, the patients were treated under double-blind conditions from Day 1 for 4 weeks with either 40 mg citalopram or 75 mg maprotiline administered as single doses at 10 p.m. If no therapeutic effect was evident on Day 14, as globally evaluated, the doses were increased to 60 mg citalopram or 150 mg maprotiline from Day 14. Eleven out of 14 patients in the citalopram group and 10 out of 13 patients in the maprotiline group received the larger doses, which were maintained throughout the study. A further two patients in the maprotiline group did not complete the study; one patient committed suicide by drowning on Day 5, and the other patient took an overdose of the test drug on Day 9, and the drug code had to be broken. During the test period Hamilton ratings were performed on Day 14 and Day 28. The vast majority of patients received at least one benzodiazepine preparation every day during the entire test period. Nine patients also received chloral hydrate as a hypnotic.

Blood samples for the determination of plasma amino acids by ion-exchange chromatography (Møller 1977) were collected after at least 3 wash-out days at 8 a.m. after a 12-h fast. Thirty-nine drug-free female members of the staff at the Sct. Hans Mental Hospital, Roskilde, Denmark served as healthy control subjects for plasma amino acids. Serotonin in whole blood and platelet-rich plasma was measured fluorometrically following deproteinization and reverse phase liquid chromatography. In the wash-out period 22 patients remained in bed and took orally 1 g probenecid at 8, 9, 10, 11 a.m. and noon, and then had a lumbar puncture performed at 1 p.m. The first 5 ml of cerebrospinal fluid (CSF) was used at the clinical laboratory. The next 10 ml of CSF was stored at −20° C until assayed for 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) by means of liquid chromatography and fluorescence detection. Serotonin and monoamine metabolites were determined at the Deltaziekenhuis (H.J.L.-Y.).

Results were evaluated by means of 2-tailed *t*-test, by linear regression analysis, or by 2-tailed non-parametric tests described by Siegel (1956). *P*-values exceeding 0.05 were regarded as not significant.

Table 1. Biochemical variables in controls and depressed patients. Results are presented as the mean ± 1 SD

	n	Mean age (years)	Age range	Trp/LNAA	Tyr/LNAA
A					
Controls	39	40	19–65	0.090 ±0.016	0.122 ±0.021
All depressives	29	53	30–64	0.070 ±0.013	0.110 ±0.019
P		<0.001[a]	–	<0.001	<0.02
B					
Endogenous	16	53	33–64	0.069 ±0.014	0.115 ±0.020
Non-endogenous	13	53	30–64	0.071 ±0.012	0.105 ±0.015[b]
P		–	–	NS	NS

	Serotonin		CSF concentration (nmol/l)		
	Whole blood (nmol/ml)	Platelets (amol/platelet)	MHPG	HVA	5-HIAA
C					
All depressives	1.1 ±0.6 (*n*=29)	4.0 ±2.2 (*n*=29)	52 ±17 (*n*=22)	385 ±188 (*n*=22)	213 ±104 (*n*=22)

[a] By the *U* test
[b] Significantly decreased as compared with controls ($P<0.005$)

Results

Mean age of patients and controls differed markedly (Table 1). However, because no significant correlation was found in the control sample between age and plasma ratio Trp/LNAA ($r=-0.14$) or plasma ratio Tyr/LNAA ($r=0.16$), or between the specified variables in the patient sample ($r=0.08$ and $r=0.30$, respectively), age has not been taken into account in the study of amino acid ratios. The mean pretreatment plasma ratio Trp/LNAA of the total patient sample was significantly lower than the mean of the controls ($P<0.001$), as was the plasma ratio Tyr/LNAA ($P<0.02$; Table 1 A). The group of 16 endogenous depressives was comparable with the group of 13 non-endogenous (including two doubtful) depressives regarding the pretreatment plasma Trp ratio and plasma Tyr ratio (Table 1 B). The plasma Tyr ratio was significantly decreased in the non-endogenous depressives ($P<0.005$) but not in the endogenous group as compared with the controls. There was no significant correlation between the pretreatment plasma Trp concentration and whole blood serotonin ($r=-0.08$; $n=29$) or platelet serotonin ($r=0.02$).

Prior to active treatment 22 patients had a lumbar puncture performed for the estimation of the probenecid-induced accumulation of monoamine metabolites in the CSF

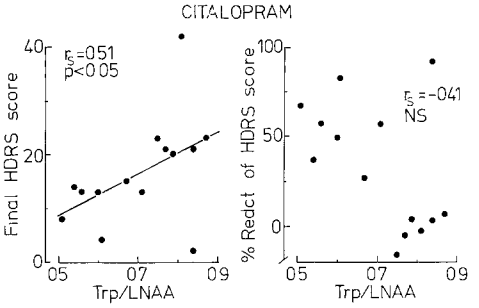

Fig. 1. Relation between the pretreatment plasma ratio Trp/LNAA and the final Hamilton depression rating scale (HDRS) score (*left*) or percent reduction of HDRS score (*right*) of 14 patients treated with citalopram for 4 weeks. r_s = the Spearman rank correlation coefficient

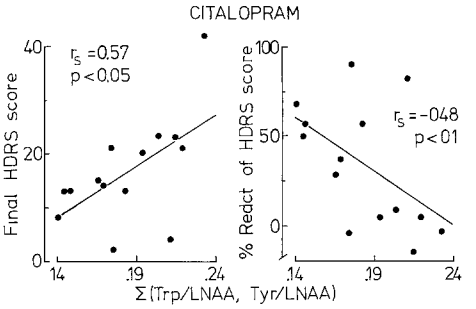

Fig. 2. Relation between the sum of pretreatment plasma ratios Trp/LNAA and Tyr/LNAA and the final Hamilton depression rating scale (HDRS) score (*left*) or percent reduction of HDRS score (*right*) of 14 patients treated with citalopram for 4 weeks. r_s = the Spearman rank correlation coefficient

(Table 1 C). Neither the pretreatment plasma Trp concentration nor the plasma ratio Trp/LNAA was significantly correlated with the accumulated 5-HIAA in CSF ($r = -0.28$ and -0.34, respectively). Plasma Tyr concentration was not significantly correlated with the CSF level of MHPG ($r = 0.29$) or HVA ($r = 0.09$), whereas the plasma ratio Tyr/LNAA showed a significant correlation with MHPG in CSF ($r = 0.53$, $P < 0.02$), but not with HVA in CSF ($r = 0.29$, NS). Results on the associations between CSF monoamine metabolite levels and diagnostic variables and therapeutic response will be reported separately.

In 27 patients who completed the trial there was no significant correlation between the pretreatment plasma ratio Trp/LNAA and the HDRS score on Day 0 ($r_s = 0.28$) or the percent reduction of HDRS score on Day 28 ($r_s = -0.25$). In addition, the pretreatment plasma ratio Tyr/LNAA was not significantly associated with the specified clinical evaluations ($r_s = 0.09$ and $r_s = 0.00$, respectively).

The citalopram group ($n = 14$) and the maprotiline group ($n = 13$) were comparable with respect to age (51 ± 11 and 56 ± 8 years), pretreatment level of plasma ratio Trp/LNAA (0.071 ± 0.012 and 0.070 ± 0.016), of ratio Tyr/LNAA (0.114 ± 0.023 and 0.106 ± 0.014), and of HDRS score on Day 0 (25 ± 6 and 26 ± 5, respectively).

In the citalopram group there was a significant correlation between the pretreatment plasma ratio Trp/LNAA and the HDRS score on Day 28 ($r_s = 0.51$, $P < 0.05$; Fig. 1) and between ratio Tyr/LNAA and the HDRS score on Day 28 ($r_s = 0.56$, $P < 0.05$). While the pretreatment plasma ratios Trp/LNAA and Tyr/LNAA were not mutually correlated, the sum of the two specified amino acid ratios was significantly correlated with the HDRS score on Day 28 ($r_s = 0.57$, $P < 0.05$) and showed a trend towards an inverse correlation with the percent reduction of HDRS score on Day 28 ($r_s = -0.48$, $P < 0.1$; Fig. 2).

The citalopram group comprised eight endogenous and six non-endogenous depressives as classified by the Newcastle scale. In the endogenous group the sum of ratios Trp/LNAA and Tyr/LNAA showed a slight correlation with final HDRS score ($r_s = 0.55$, NS) and percent reduction of HDRS score ($r_s = -0.52$, NS), while the correlation was stronger in the non-endogenous group ($r_s = 0.93$, $P < 0.05$, and $r_s = -0.89$, $P < 0.1$, respectively).

In the maprotiline group the pretreatment plasma ratio Trp/LNAA correlated significantly with the HDRS score on Day 0 ($r_s = 0.61$, $P < 0.05$), but poorly with the HDRS score on Day 28 ($r_s = 0.37$, NS) and with percent reduction

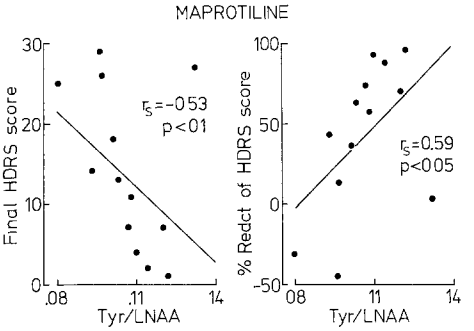

Fig. 3. Relation between the pretreatment plasma ratio Tyr/LNAA and the final Hamilton depression rating scale (HDRS) score (*left*) or percent reduction of HDRS score (*right*) of 13 patients treated with maprotiline for 4 weeks. r_s = the Spearman rank correlation coefficient

of HDRS score ($r_s = -0.27$, NS). The pretreatment plasma ratio Tyr/LNAA showed no significant relationship with the HDRS score on Day 0 ($r_s = 0.18$), whereas this ratio correlated significantly with the percent reduction of HDRS score on Day 28 ($r_s = 0.59$, $P < 0.05$) and showed a trend towards an inverse correlation with the HDRS score on Day 28 ($r_s = -0.53$, $P < 0.1$; Fig. 3).

The maprotiline group comprised eight endogenous and five non-endogenous (including one doubtful) depressives. There was no correlation between pretreatment plasma ratio Tyr/LNAA and percent reduction of HDRS score in the endogenous group ($r_s = 0.19$) but a trend towards a correlation in the non-endogenous group ($r_s = 0.9$, $P < 0.1$). In the former group the optimum relationships were found between plasma ratio Trp/LNAA and final HDRS score ($r_s = 0.53$, NS) and percent reduction of HDRS score ($r_s = -0.50$, NS).

Discussion

This study has shown a significant relationship between the pretreatment plasma amino acid pattern and the clinical response in depressed patients to treatment with citalopram or maprotiline. The differential antidepressant response within the two treatment groups was related neither to the steady-state serum levels of the respective drugs nor to the type of depression (to be published).

Plasma ratio Trp/LNAA was significantly decreased in the total patient sample and in either of the diagnostic sub-

groups as compared with the healthy controls. However, these findings may be influenced in part by the different mean age of the groups, since there was a slight age-related reduction in the plasma Trp ratio in the control group who was younger than the patient group. One study has previously found a decreased plasma Trp ratio in depressives (Joseph et al. 1984), while others have not (Møller et al. 1980, 1981, 1983; Dunlop et al. 1983). In a preliminary study DeMyer et al. (1981) found more severe depressions to be associated with lower plasma Trp ratios, but this relationship was not replicated in a follow-up study (Dunlop et al. 1983). In the present patient sample, there was also no significant association between plasma Trp ratio and severity of depression.

In addition, the plasma ratio Tyr/LNAA was significantly decreased in the total patient sample, although this difference was attributed to a low level in the non-endogenous group, whereas the level in the endogenous group was normal. There is no ready explanation for the differences in the plasma amino acid ratios between the groups.

Because of the selectivity of citalopram on serotonin reuptake, it could be anticipated that the plasma Trp ratio alone would be correlated with the antidepressant response to citalopram, as previously observed with L-tryptophan (Møller et al. 1980) and amitriptyline (Møller et al. 1983). However, the clinical response to citalopram was related at least as closely to the sum of the plasma ratios Trp/LNAA and Tyr/LNAA, showing a significant correlation with final HDRS score and a trend towards an inverse correlation with percent reduction of HDRS score, as to either of the two plasma ratios alone. On the whole, this response relationship with amino acid ratios was sustained in the endogenous and non-endogenous subgroups. These findings suggest that the antidepressant effect of citalopram could be related not only to a facilitation of serotoninergic function but may involve noradrenergic mechanisms as well.

Depressives with a subnormal plasma Tyr ratio might be expected to benefit more from treatment with maprotiline than patients with a supernormal Tyr ratio, as observed on imipramine (Møller et al. 1981) and nortriptyline (Møller et al. 1985). Nevertheless, the percent reduction of HDRS score in patients on maprotiline was directly and significantly correlated with the pretreatment plasma Tyr ratio. However, the significance of this relationship is questioned by the virtual lack of association between plasma Tyr ratio and clinical response in the endogenously depressed patients. Instead, in this subgroup there emerged a slight, albeit non-significant, correlation between the plasma Trp ratio and the clinical response, indicating that serotoninergic mechanisms could possibly be involved in the antidepressant action of maprotiline.

During the wash-out period the plasma Trp concentration showed no association with serotonin level in platelets or whole blood, in agreement with findings in controls and schizophrenics (Freedman et al. 1981). More unexpectedly, not only did the plasma ratio Trp/LNAA fail to correlate positively and significantly with the probenecid induced accumulation of 5-HIAA in the CSF, but the coefficient was negative. This result might change if the 5-HIAA levels in CSF were corrected according to the CSF probenecid concentrations (van Praag and Korf 1974; Faull et al. 1981). On the other hand, the findings accord with the lack of correlation between plasma ratio Trp/LNAA and basal 5-HIAA concentration in CSF of healthy subjects as reported by Hagenfeldt et al (1984). The failure of the plasma Tyr ratio to correlate with the probenecid induced accumulation of HVA in CSF of the depressed patients also parallels the findings of Hagenfeldt et al. (1984). In opposition to the present findings, these authors found a positive and significant correlation between plasma Tyr concentration and MHPG level in the CSF. Hagenfeldt et al. did not report on the association between the plasma ratio Tyr/LNAA and MHPG level in CSF, which in the present study were positively and significantly correlated.

In conclusion, the clinical response in depressed patients to citalopram or maprotiline was significantly associated with the pretreatment levels of plasma ratio Trp/LNAA and Tyr/LNAA, which possibly reflect the synthesis in brain of serotonin and noradrenaline. Although the patient samples were small, the results are in consistency with previous findings suggesting that biochemical characteristics of depressed patients may influence the therapeutic response to antidepressive treatment.

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