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The relationships between the availability of *L*-tryptophan to the brain, the spontaneous HPA-axis activity, and the HPA-axis responses to dexamethasone in depressed patients

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Summary. The present study was conducted in order to investigate the negative relationships between measures of hypothalamic-pituitary-adrenal (HPA)-axis activity and the availability of L-tryptophan (L-TRP) to the brain in depressive patients. To this end we measured the following: plasma total L-TRP, the ratio of L-TRP to the sum of competing amino acids (CAA), free urinary cortisol (UFC) excretion in 24 hr urine samples, and the 8 a.m. postdexamethasone cortisol levels. We found that the availability of L-TRP to the brain was significantly negatively correlated with the postdexamethasone cortisol values. Cortisol non-suppressors averaged significantly lower L-TRP and L-TRP/CAA values compared to suppressors. No significant relationship was established between the availability of L-TRP and UFC excretion. It is concluded that the availability of L-TRP is related to the actual alterations in cortisol induced by dexamethasone rather than with the spontaneous baseline HPA-axis activity.

Keywords: L-tryptophan – Depression – DST – Free urinary cortisol

Introduction

The hypothalamic-pituitary-adrenal (HPA)-axis function and the ratio of circulating L-tryptophan (L-TRP) to the sum of amino acids known to compete for the same cerebral uptake mechanism (CAA) have been of interest to many psychiatric investigators in the field of depressive disorder.

The L-TRP/CAA ratio has been found to be decreased in major depression [1, 2]. There is evidence that this ratio is an index of the availability of L-TRP to the brain and hence for the serotonin synthesis in the brain [3, 4].

In a notable proportion of major depressed patients, disturbances in the dexamethasone suppression test (DST) were found [5, 6]. In the same patients a

moderately elevated basal urinary free cortisol (UFC) excretion was observed [7, 8].

Serotonin has a dual effect on the regulation of the HPA-axis: it stimulates the secretion of corticotropin-releasing-factor (CRF) [9] and it is a necessary link in the inhibiting effects of corticosteroids on CRF and adrenocorticotropin hormone (ACTH) [10].

Maes et al. [2] observed that both circulating L-TRP levels and the L-TRP/CAA ratio were significantly negatively correlated to the postdexamethasone cortisol values, particularly those at 8.00 a.m. This relationship could be explained on several grounds: 1. Glucocorticoid hypersecretion (for which an abnormal DST could be an index) induces the hepatic L-TRP pyrrolase activity and this in turn could result in lower concentrations of circulating L-TRP in the serum [11]. 2. The deficiency of L-TRP in the serum attenuates the enhancing effects of serotonin on the negative feedback of corticosteroids on hypothalamic CRF and pituitary ACTH [2]. 3. HPA-axis and the availability of L-TRP are affected independently but in parallel by other variables putatively disordered in major depressives.

This prospective study has been undertaken in order to investigate if the putative decrements in the availability of L-TRP during major depression are related either to spontaneous HPA-axis hyperfunction or to the actual HPA-axis responses to dexamethasone administration.

Methods

Patients

The subjects were 50 consecutively admitted depressive inpatients. The study period extended from August 1987 till April 1988. Patients were assigned to the following DSM-III [12] subgroups: minor depression including dysthymic disorder (300.40) and adjustment disorder with depressive mood (300.00); major depression without melancholia (296.22, 296.32) and major depression with melancholia and/or psychotic features (296.23, 296.33, 296.24, 296.34). The severity of illness was measured with the Hamilton Depression Rating Scale. Bipolar patients were not allowed to enter this study. Patients who had taken lithium or MAOIs the year before hospitalization were excluded. Patients with substance use disorders (abuse or dependence) were also excluded. Eighteen patients were drug free for at least 1 month before admission into hospital. The others had taken benzodiazepines and/or antidepressants. After admission 26 patients did not receive any medication at all. The others were on benzodiazepines (<lorazepam 6 mg/day). Carroll's [5] exclusionary criteria were applied to avoid false-positive and false-negative test results in the DST. Medical, laboratory (blood and urine analysis, liver and kidney function tests, sedimentation rate and leukocytes) and neurological investigations showed no major pathologies.

Procedures

A baseline 24 hr urine collection started at 10 p.m. five days after admission of the patients into hospital. During the 24 hr collection they were kept in a state of rest (in bed or in an armchair). The urine collection was considered complete if the creatinin excretion was

more than 0.7 g/day or the urine volume more than 0.7 L/day. Dexamethasone (1 mg, orally) was administered three days later (day 8 after admission) at 11 p.m. The next day blood samples were taken at 8 a.m. after an overnight fasting for the determination of cortisol and dexamethasone. Three days later fasting serum samples were taken at 8 a.m. for the determination of the amino acids.

The amino acid determinations were made by liquid chromatography. The ratio between the L-TRP value and the sum of the five competing amino acids was calculated and multiplied by 100. Free urinary cortisol and postdexamethasone cortisol values were determined using a commercial enzymo-immuno-assay kit. Methods, inter-assay coefficients of variation are reported elsewhere [2].

Statistics

The independency of classification systems was verified by X^2-test. Relationships between variables were analyzed by means of Pearson's product moment, point-biserial correlation calculation, multiple regression and canonical correlation analysis. Student's *t*-test, the one-way analysis of variance (ANOVA), the factorial ANOVA the analysis of covariance (ANCOVA), the linear discriminant analysis (LDA) and the MANOVA were applied to the clinical and biological data. For the multiple a posteriori comparisons among treatment means Fisher's least significant difference was used. Each biochemical measure was tested for normality of distribution by means of the Kolmogorov-Smirnov test. If the results were not normally distributed transformations were used. The significance was set at $\alpha = 0.05$ (two tailed).

Results

Table 1 summarizes the demographic data of the patients. There were no significant differences in sex ratio between the DSM-III subgroups. The severity of illness increased from: minor → simple major → major depression with associated features. There were no significant differences in drug state among the depressive categories.

All amino acid data, the L-TRP/CAA ratio, dexamethasone, HDRS and age were normally or lognormally distributed. The 8 a.m. postdexamethasone cortisol values approached a Gaussian distribution after logarithmic transformation.

The drug state of the patients (i.e. use of benzodiazepines the day prior to testing, length of drug free period before admission to hospital) was not related to any of the biological data (L-TRP, L-TRP/CAA ratio, dexamethasone, 8 a.m. postdexamethasone cortisol, UFC). So, we have no evidence of major effects of the drug state on our results.

Creatinin excretion and 24 hr urinary output explained together $\pm 20\%$ of the variance in the UFC values. Therefore, the UFC data were assessed as the residual values (UFC_R) which have partialled out the relative effects of both above mentioned variables (by means of regression analysis).

Table 2 lists the postdexamethasone cortisol, dexamethasone, UFC, L-TRP and L-TRP/CAA values. Patients with melancholia exhibited significantly

Table 1. Demographic data of the 50 depressed inpatients

DSM-III category	Index	Age		Sex ratio	HDRS[1]	BZ	DF
		Mean (±1SD)	Range	Men/Women	Mean (±1SD)	Yes/No	Yes/No
Minor depression	md	41.0 (±15.9)	22–75	6/9	15.3 (±2.0)	8/7	7/8
Major depression without melancholia (296.X2)	MD − M	43.4 (±14.5)	18–77	10/12	21.4 (±4.0)	11/11	7/15
Major depression with melancholia or with psychotic features (296.X3, 296.X4)	MD + M	52.2 (±14.6)	28–75	6/7	27.4 (±3.0)	7/6	4/9

HDRS Hamilton Depression Rating Scale, *BZ* use of benzodiazepines (< 6 mg lorazepam/day) the day before blood sampling, *DF* length of drug free period (more than 1 month drug free or not) before admission into hospital.

[1] All HDRS scores are significantly different from each other, ANOVA, $F = 48$, $df = 2/49$, $p < 10^{-4}$.

Table 2. Results of the measurements of postdexamethasone cortisol values, the dexamethasone levels, urinary free cortisol, *L*-TRP and the *L*-TRP/CAA ratio

Index	Postdexamethasone cortisol ($\mu g/dL$)	Dexamethasone (ng/mL)	UFC (μg/24 hr)	*L*-TRP[1] (μ mole/*L*)	*L*-TRP/CAA[1] $\times 100$
md	1.47 ($\pm$1.57)	3.23 ($\pm$1.10)	117.2 ($\pm$53.9)	57.5 ($\pm$9.8)	10.25 ($\pm$1.57)
MD − M	4.11 ($\pm$5.52)	2.93 ($\pm$1.42)	144.5 ($\pm$123.1)	57.0 ($\pm$9.7)	9.65 ($\pm$1.47)
MD + M	5.55 ($\pm$6.17)[2]	3.09 ($\pm$1.46)	188.9 ($\pm$173.2)	50.1 ($\pm$12.6)	8.41 ($\pm$1.78)[3]

md/MD $\pm$ M: for legends see Table 1.
All results are expressed as mean ($\pm$1SD).
[1] Means are unweighted, i.e., male and female means are assigned equal weights.
[2] Significantly different from md, ANOVA on logarithmic transformations, $F = 3.16$, $df = 2/49$, $p = 0.050$.
[3] Significantly different from md and MD − M, factorial (sex as second factor) ANOVA, $F = 5.49$, $df = 2/49$, $p < 0.01$, LSD = 1.14.

Table 3. Results of the uni- and multivariate regression analysis: postdexamethasone cortisol levels/independent variables

Independent variables	Pearson's correlations with the postdexamethasone cortisol values		Multiple regression[1]	
	coefficients	*p* value	*F* statistic	*p* values
L-TRP	−0.31	0.046	NS	—
L-TRP/CAA ratio	−0.40	0.008	10.9	0.002
UFC_R	0.48	0.002	14.0	0.0009
Dexamethasone	−0.52	0.0006	16.1	0.0005
HDRS	0.25	0.104	NS	—
Age	0.34	0.026	NS	—
Sex (♂ = 1, ♀ = 0)	−0.01	0.924	NS	—

NS non significant.

[1] Up to 57% of the variance in the postdexamethasone cortisol values is explained by the regression on the *L*-TRP/CAA ratio, UFC_R and dexamethasone ($F = 16.6$, $df = 3/41$, $p < 10^{-4}$).

higher postdexamethasone cortisol compared to minor depressives. Dexamethasone and UFC did not differ between the diagnostic subcategories. The L-TRP/CAA ratio was significantly lower in the most severely depressed patients.

Table 3 displays the uni- and multivariate regression analysis results of postdexamethasone cortisol on the listed independent variables. The postdexamethasone cortisol values correlated significantly negatively with L-TRP, L-TRP/CAA ratio and dexamethasone values. Significant positive relationships were established for UFC_R and age. Up to 57% of the variance in the postdexamethasone cortisol data was explained by the multiple regression on L-TRP/CAA, UFC_R and dexamethasone. UFC_R and dexamethasone explained together 44% of the variance in these cortisol values.

A canonical correlation analysis was carried out in order to determine the maximum relationship between the cortisol data (postdexamethasone cortisol and UFC_R), and L-TRP, L-TRP/CAA, dexamethasone, age, HDRS and sex. Table 4 summarizes the results. One significant canonical variate was extracted. This first canonical relationship was between the postdexamethasone cortisol values, and L-TRP, L-TRP/CAA, dexamethasone and age.

Table 5 shows the dexamethasone, UFC_R, L-TRP and L-TRP/CAA data in DST non-suppressors and suppressors. Non-suppressors were characterized by decreased dexamethasone, L-TRP and L-TRP/CAA values and by increased UFC_R excretion ranges. Non-suppressors and suppressors were significantly discriminated by implementing these variables in a LDA ($F = 68$, $p < 10^{-5}$) or MANOVA (Wilk's Lambda = 12, $p < 10^{-5}$). The HDRS score and age did not differ between the two groups.

Table 4. Results of canonical correlation analysis between cortisol data and other independent variables

Variables	Loadings	
	CV_1	CV_2
y: postdexamethasone cortisol	0.97	−0.24
UFC_R	0.25	−0.97
x: L-TRP	−0.40	0.14
L-TRP/CAA	−0.58	−0.34
Dexamethasone	−0.71	0.03
HDRS	0.31	−0.33
Age	0.44	−0.19
Sex	−0.00	0.19
correlation coefficient	0.77	0.25
explained variance for *y*/*x*	29%	3%

y indicates the dependent variables, *x* independent variables, *CV* canonical variates. The significant loadings are underlined (>0.4).

Discussion

In accordance with the work of Maes et al. [2] we found that the postdexamethasone cortisol values and the L-TRP availability were respectively increased and decreased in the most severely depressed patients. We did not observe any differences in UFC excretion among the DSM-III depressive categories. This concurs with the observations of Berger et al. [13] who described normal corticosteroid excretion rates during major depression. Carroll et al. [7], and Stokes et al. [8], on the other hand, found significantly increased UFC in major depressives as compared with healthy controls or minor depressives.

The results of our study showing that the L-TRP disposition and UFC excretion are not significantly and negatively correlated, do not support the hypothesis that the decrements in L-TRP in patients with major depression or with DST non-suppression, are induced by endogenous corticosteroid overdrive. Subsequently, the induction of liver L-TRP pyrrolase by corticosteroids [11] is not a likely cause for the lowered L-TRP availability in those patients. Reductions in L-TRP disposition could be determined by a variety of other intervening variables, e.g. gastro-intestinal malabsorption of L-TRP, the effects of sympathetic hyperactivity, increased extraction of circulating L-TRP by the brain or by peripheral organs.

We were able to consolidate our earlier report [11] linking a decreased availability of L-TRP to disturbances in the DST results.

Table 5. Differences in dexamethasone levels, urinary free cortisol (UFC), *L*-TRP and *L*-TRP/CAA ratio between cortisol suppressors and non-suppressors

Cortisol response (DST) at 8a.m. cut off value 3.5 $\mu g/dL$	Dexamethasone (ng/mL)	UFC_R (μg/24hr)[1]	*L*-TRP (μ mole/*L*)[1]	*L*-TRP/CAA ratio[1] × 100
non-suppressors	2.27 (±1.16)[2]	0.463 (±0.750)[3]	49.0 (±8.2)[4]	8.29 (±1.52)[5]
suppressors	3.45 (±1.22)	−0.231 (±0.425)	58.9 (±9.9)	10.05 (±1.72)

All the data are expressed as mean (±1SD).

[1] Means are unweighted, i.e., male and female means are assigned equal weights.

[2] Significantly different from suppressors, *t*-test, $t = 3.059$, $p = 0.003$.

[3] Significantly different from suppressors, *t*-test, $t = 3.162$, $p = 0.002$.

[4] Significantly different from suppressors, $F_4 = 10.73$, $F_5 = 10.99$, all factorial (sex as second factor) ANOVA, $df = 1/49$, $p < 0.05$.

We found that the L-TRP/CAA ratio, the spontaneous HPA-axis activity (i.e. UFC excretion) and the dexamethasone levels are cumulative in explaining the variance in the postdexamethasone cortisol values. In other words, the availability of L-TRP explains a part of the variability in the postdexamethasone cortisol values (i.e. 12–16%) that is not attributable to the variability in the spontaneous HPA-axis activity, the bioavailability of the test substance, age or the severity of illness.

As a result, we may conclude that the availability of L-TRP to the brain is related to the actual alterations in cortisol induced by dexamethasone rather than with the spontaneous HPA-axis activity. In other words, the first raised hypothesis (q.v. Introduction) explaining the relation between the DST results and the availability of L-TRP is not supported by the results of this study. Further research should focus on the underlying pathophysiological mechanisms (q.v. hypothesis 2 or 3) which could explain the negative correlations between the cortisol escape from suppression by dexamethasone and the availability of L-TRP.

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