Neuropeptide Y and corticotropin-releasing hormone in CSF mark response to antidepressive treatment with citalopram

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Abstract

Neuropeptides appear to play a role in the pathophysiology of depression and electroconvulsive treatment and lithium affect these compounds in human cerebrospinal fluid (CSF) and rodent brain. Consequently, we investigated whether long-term treatment with the selective serotonin reuptake inhibitor (SSRI) citalopram (Cit) would also affect neuropeptides in CSF of depressed patients. Changes in CSF monoamine metabolites were also explored. CSF concentrations of corticotropin-releasing hormone (CRH)-like immunoreactivity (LI), neuropeptide Y (NPY)-LI, and Cit were determined in 21 patients with major depression. Lumbar puncture was performed in the morning at baseline and was repeated after at least 4 wk of Cit treatment (40 mg/d). The severity of depression was assessed by the Hamilton Rating Scale for Depression (HAM-D). Cit treatment was associated with a significant increase in NPY-LI and decrease in CRH-LI. An evaluation of the relationship between changes in concentrations of NPY-LI, CRH-LI, and the clinical response showed significant correlations between these parameters. Significant NPY and CRH changes in CSF following treatment as well as correlations to changes in HAMD support the hypothesis that these two peptides play a role in affective disorders and are markers of therapeutic response.

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Key words: Cerebrospinal fluid, citalopram, corticotropin-releasing hormone, major depression, neuropeptide Y.

Introduction

Investigations of monoamines in cerebrospinal fluid (CSF) from depressed patients have often yielded inconclusive results and interpretation of data may be obscured by methodological and analytical variations (Nordin et al., 1992) as well as the fact monoamine metabolites from different brain regions contribute unevenly to their CSF concentrations (Ågren et al., 1986). The effects of tricyclic antidepressants (Scheinin, 1985) as well as of the selective serotonin reuptake inhibitors (SSRIs) on 5-hydroxyindoleacetic acid (5-HIAA), 3-methoxy-4-hydroxyphenylglycol (MHPG) and homovanillic acid (HVA) have not been consistent (Aberg-Wistedt et al., 1985; Bjerkenstedt et al., 1985; Mårtensson et al., 1989; Potter et al., 1985). Studies examining the selectivity of clomipramine, zimeldine, citalopram (Cit), and fluoxetine in CSF have found only a partial selectivity for serotonin (Mårtensson et al., 1991; Scheinin, 1985). Moreover, it has been difficult to separate the effects of treatments from those of the vicissitudes of the disorder being treated.

Dysfunction of the serotonin and noradrenaline systems has been implicated in the pathophysiology of major depression (Goodwin and Jamison, 1990; Harro and Oreland, 2001). Previous investigators have proposed that tricyclics, SSRIs and monoamine inhibitors as well as electroconvulsive treatment (ECT)
exert their therapeutic effects by altering the availability of monoamines or receptor sensitivity, resulting in a net increase in post-synaptic neuronal activity (Asberg et al., 1984; Nordin et al., 1992). These hypotheses were based on investigations both of animal models and analyses of CSF concentrations of 5-HIAA, MHPG, and HVA, the major metabolites of serotonin, noradrenaline, and dopamine respectively.

While dysfunction of a monoaminergic system may constitute a sufficient cause of and account for the pathophysiology of depression, there is no evidence that it is also a necessary event. Consistent with such reasoning are the findings that lithium and antiepileptic drugs act predominantly via other mechanisms, such as effects on G-proteins, myo-inositol phosphatase, phosphoinositol biphosphohate cycle (PIP2), voltage-sensitive Na channels, etc. Moreover, cumulative evidence points to the role of other classes of compounds, e.g. neuropeptides, in affective disorders. Thus, several investigators (Banki et al., 1987; Gold et al., 1988) have proposed that dysphoric hyperarousal in major depression reflects hypersecretion of corticotropin-releasing hormone (CRH). One study of fluoxetine effects on CSF CRH in depressed patients (De Bellis et al., 1993) suggested that its therapeutic efficacy may be related to decrease in CRH synthesis in the central nervous system (CNS). ECT, probably the most effective antidepressant treatment, was also found to decrease CSF CRH (Nemeroff et al., 1991).

Another peptide of interest in this context is neuropeptide Y (NPY). It is widely but unevenly distributed in brain regions and, compared with other neuropeptides, its concentrations in the CNS are high. In animals, NPY has been repeatedly shown to possess antidepressant and anxiolytic properties (Heilig and Widerlöv, 1995; Husum et al., 2000; Mathé, 1999). It also increases motor activity and feeding behaviour. Decreased NPY concentrations in brain from suicide victims (Widdowson et al., 1992) and in CSF from patients diagnosed with major depression, compared to controls, have been reported (Heilig et al., 2004; Widerlöv et al., 1988).

In line with human data, animal models of depression, both genetic and environmental, have reproducibly demonstrated lower concentrations of NPY in the hippocampus (Husum et al., 2002; Husum and Mathé, 2002; Jiménez et al., 2000; Jiménez-Vasquez et al., 2001; Mathé et al., 1998). With regard to antidepressant treatment modalities, antidepressant drugs have been claimed to increase NPY, and also to have no effects or even decrease NPY (Bellmann and Sperk, 1993; Berrettini et al., 1987; Heilig et al., 1988). In contrast, electroconvulsive stimuli (ECS) consistently increase both NPY protein and expression in rodents and ECT elevates NPY-like immunoreactivity (LI) in the CSF of depressed patients (Husum et al., 2000; Jiménez et al., 2000; Mathé, 1999; Mathé et al., 1996, 1998; Stenfors et al., 1989, 1992, 1994; Zachrisson et al., 1995a). Similarly, lithium also increases NPY protein in selected rat brain regions (Husum et al., 2000; Mathé et al., 1990a, 1994; Zachrisson et al., 1995b).

Citalopram (Cit) is a widely used and well-tolerated SSRI and is recommended for long-term treatment of depression (Baumann, 1992; Luo and Richardson, 1993; Montgomery et al., 1993). It exhibits highly selective serotonin reuptake-inhibiting properties at the presynaptic nerve terminals. The affinity is low for a variety of receptors, e.g. 5-HT1B, 5-HT2B, adrenergic α1, α2, β1, and β2, dopaminergic, and muscarinic (Hyttel, 1982).

Recently, relationships between the clinical outcome and plasma S-Cit, CSF S-Cit, CSF S-Cit-PROP, CSF 5-HIAA (Nikisch et al., 2004), and the effect of Cit on the HPA axis (Nikisch et al., In Press) in depressive patients treated with Cit were reported.

Two CSF samples – one immediately before and the other after the completed treatment – were obtained from all patients, each subject serving as his/her own control. This design made it possible to correlate pre- to post-treatment changes in biochemical variables as well as to correlate those to changes in depression.

Method

Subjects

The subjects were 21 in-patients (nine men, 12 women), age 35.9 ± 9.4 yr (mean ± s.d.; range 19–55 yr), at the Department of Psychiatry and Psychotherapy, Klinikum Fulda, Germany. All patients were diagnosed with a major depression according to DSM-IV criteria (APA, 1994) and then assessed at weekly intervals during treatment by an expert clinician using the Hamilton Rating Scale for Depression (HAMD; Hamilton, 1960). Response was defined as a ≥50% decrease in HAMD score after a 4-wk treatment with Cit [responders vs. non-responders (partial responders)]. All subjects were free of serious medical disorders by history, physical and laboratory examinations. Written informed consent was obtained after explanation of the purpose and design of the study. The protocol was approved by the Ethics Committee of the Philipps University of Marburg, Germany, according to the 1975 Declaration of Helsinki.
Treatment

The patients were free from drugs that could possibly interfere with CNS amine metabolites for at least 7 d prior to the study. Exclusion criteria included ECT within 4 wk prior to the study, ongoing lithium treatment and previous participation in a Citalopram clinical trail. Medications preceding entry into the study are shown in Table 1. All patients received active treatment with Citalopram, starting with 20 mg/d, and from day 5 onwards 40 mg/d at breakfast. No other drugs were given during that period.

Procedures

Physical examination, routine blood, toxicologic urine screening, and electrocardiograms (ECG) were evaluated at baseline and after 4 wk on active treatment. CSF samples were obtained by lumbar puncture (LP) during the last 3 d of the placebo observation period and again after at least a 4-wk medication trial. Plasma samples for determination of Citalopram were drawn immediately prior to the LP. After anticoagulation (20 IU heparin/ml), the blood samples were centrifuged for 10 min at 4000 g at 4 °C and stored frozen at −80 °C until assayed. LPs were performed in the lateral decubitus position between 08:00 and 09:00 hours, after overnight bed rest and fasting. A total of 30 ml of CSF was collected in 12 separate aliquots, and the ninth aliquot (4 ml) was selected for analysis because there is a cisternal-to-lumbar gradient for 5-HIAA (Siever et al., 1975). After centrifugation, the samples were immediately frozen in 2 ml aliquots in silanized tubes and stored at −80 °C until analysed.

Citalopram plasma and CSF concentrations were assessed in Prilly-Lausanne, Switzerland (Nikisch et al., 2004). S- and R-enantiomers of Citalopram were determined in plasma by a recently developed chiral reverse-phase liquid chromatography assay with a lower quantitation limit of 5 ng/ml for each enantiomer and intra- and inter-assay coefficients of variation of 2.9–8.6% (Kosel et al., 1998). However, only Citalopram (S-Cito + R-Cito) concentrations in CSF will be reported here.

CSF concentrations of 5-HIAA and HVA were determined by reverse-phase high-performance liquid chromatography (rp-HPLC), and NPY-LI and CRH-LI by radioimmunoassay, as previously described (Little et al., 1999; Mathé et al., 1990b, 1996; Stenfors et al., 1994).

Demographic and clinical data were analysed using SPSS11 software (SPSS Inc., Chicago, IL, USA). All results are expressed as mean ± S.D. Baseline and post-treatment CSF 5-HIAA, HVA, NPY-LI and CRH-LI and HAMD scores were compared using two-tailed probability t tests for paired samples. Pearson’s correlations and linear regression models were used to determine relationship among CSF measures. Non-parametric tests were used for calculations in relationship with HAMD (Spearman correlation coefficient). Repeated-measures analysis of variance (ANOVA) was also used for statistical evaluation of different patients subgroups (responders vs. non-responders).

Results

Effects of Citalopram on NPY-LI and CRH-LI in CSF

After 4 wk of Citalopram treatment, the CSF concentration of NPY-LI was significantly higher while CRH-LI was significantly lower compared to baseline (Table 2). Splitting the analysis according to response, the same significance pattern was seen (Table 3). Using a repeated-measurements ANOVA model (comparing before vs. after Citalopram treatment and controlling for sex, there was a significant interaction with the responder/ non-responder category in the CRH-LI change

### Table 1. Medication before entering the Citalopram study

<table>
<thead>
<tr>
<th>Previous medication</th>
<th>Average dosage (mg/d)</th>
<th>Responder (n=11)</th>
<th>Non-responder (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitriptyline</td>
<td>225</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Clomipramine</td>
<td>150</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Doxepine</td>
<td>250</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mirtazapine</td>
<td>45</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>60</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Reboxetine</td>
<td>12</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Sertraline</td>
<td>150</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>275</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table 2. CSF concentrations of CRH-LI and NPY-LI (pmol/l ± S.D.) before and after treatment with Citalopram in 21 patients with major depression

<table>
<thead>
<tr>
<th></th>
<th>All patients (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td>CRH-LI</td>
<td>27.1±9.4</td>
</tr>
<tr>
<td>NPY-LI</td>
<td>34.1±12.6</td>
</tr>
</tbody>
</table>

CRH-LI, Corticotropin-releasing hormone-like immunoreactivity; NPY-LI, neuropeptide Y-like immunoreactivity.
Table 3. CSF concentrations of CRH-LI and NPY-LI (pmol/l ± S.D.) before and after treatment with citalopram in 21 patients with major depression; responders vs. non-responders

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After treatment</th>
<th>Change (%)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders (n = 11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRH-LI</td>
<td>29.2 ± 9.9</td>
<td>8.8 ± 9.1</td>
<td>−74</td>
<td>−14.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NPY-LI</td>
<td>32.8 ± 13.7</td>
<td>42.5 ± 13.4</td>
<td>+36</td>
<td>3.70</td>
<td>0.004</td>
</tr>
<tr>
<td>Non-responders (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRH-LI</td>
<td>24.8 ± 8.8</td>
<td>10.2 ± 8.0</td>
<td>−60</td>
<td>−9.51</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NPY-LI</td>
<td>35.5 ± 11.8</td>
<td>40.0 ± 11.2</td>
<td>+15</td>
<td>5.14</td>
<td>0.006</td>
</tr>
</tbody>
</table>

CRH-LI, Corticotropin-releasing hormone-like immunoreactivity; NPY-LI, neuropeptide Y-like immunoreactivity.

Clinical effects of Cit treatment

Cit significantly decreased the mean HAMD scores, from 24.8 ± 2.9 to 14.1 ± 5.3 (t = 11.33, d.f. = 20, p = 0.0001). In 11 patients the HAMD score dropped by 50% or more. These patients were considered responders. The weekly mean changes in HAMD scores for all the patients and for the responders respectively non-responders are shown in Figure 1. In order to validate the above-mentioned repeated-measures ANOVA method, this analysis was carried out for the HAMD scores controlling for sex. As predicted, the interaction between HAMD change and the responder category was strong [F(1, 20) = 38.30, p < 0.0001], and there was no sex × response interaction. The significant changes (Δ) in NPY-LI, CRH-LI, 5-HIAA, and HVA induced by Cit were associated with a reduction in ΔHAMD score on day 28 relative to baseline. Comparisons between their importance in predicting the ΔHAMD scores in univariate analyses showed that ΔNPY explained 33% of the variance in ΔHAMD (p = 0.0061); ΔCRH explained 29% (p = 0.0111).

Figure 1. Weekly changes in HAMD total score in depressed in-patients treated with citalopram. Twenty-one patients were diagnosed with major depression according to the DSM-IV criteria and then assessed at weekly intervals during citalopram treatment using the Hamilton Rating Scale for Depression (HAMD). No other medications were allowed during the study. (a) All patients (–●–); (b) shows the change in HAMD separately for responders (–□–) and non-responders (–●–).
**Plasma and CSF concentrations of R-Cit and S-Cit**

After 4 wk Cit treatment, the patients mean steady-state plasma and CSF concentrations of Cit [mean ± S.D. (range)] were 66 ± 23 (36–112) ng/ml, and 31 ± 10 (19–52) ng/ml respectively. The corresponding values of the other parameters were: plasma S-Cit 21 ± 10 (10–39) ng/ml, CSF S-Cit 10 ± 4 (6–17) ng/ml, plasma R-Cit 44 ± 14 (26–76) ng/ml, and CSF R-Cit 21 ± 6 (13–35) ng/ml.

**Discussion**

Cit treatment was associated with a significant CRH-LI decrease and NPY-LI increase in CSF from patients with major depression. Cit affects the NPY system in the rat brain (Husum et al., 2000) and, to our knowledge, this is the first report that it also affects NPY and CRH in human CSF. The effects of Cit would, thus, appear to be similar to those of ECT and ECS as well as lithium in human CSF and animal brain regions (Mathe, 1999; Mathe et al., 1994, 1996, 1998; Stenfors et al., 1989, 1992, 1994).

Involvement of CRH and the hyperactivity of the HPA axis in pre- and post-natal stress, anxiety and depression have been extensively documented and reviewed (cf. Muller et al., 2002, 2004; Strohle and Holsboer, 2003). Thus, elevated CRH has been reported in CSF from depressed patients and in rat brain following stress (Hatalski et al., 2000; Nemeroff et al., 1991). Moreover, CRH given intracerebroventricularly (i.c.v.) results in similar endocrine and behavioural responses as does exposure to stress (Koob et al., 1993), and according to growing evidence also leads to hippocampal atrophy (Brunson et al., 2001), currently considered to be a hallmark of both chronic stress in animals and major depression in humans. Our findings are also of interest in view of the CRH-NPY interactions. The two peptides appear to have opposite effects, that is, NPY counteracts the deleterious effects of CRH (Britton et al., 2000; Ehlers et al., 1997; Heilig et al., 1994; Kask et al., 2002; Sheriff et al., 2001). Thus, the CRH decrease following Cit treatment and the correlation between changes in CRH and the HAM-D scale are in line with and extend the above-cited reports on the role of CRH in affective disorders.

NPY concentrations increased markedly following Cit treatment and there was a strong positive correlation between the rise in NPY and clinical improvement. This indicates that the rise in NPY is primarily due to the change in patients’ clinical status and not to the action of Cit per se, although part of the change may be secondary to the effects of Cit. The findings are consistent with the previously reported NPY increase in depressed patients following ECT (Mathe et al., 1996) and the effects of other antidepressant treatment modalities described above. The increase in NPY correlating to improvement in the clinical condition strengthens the experimentally obtained antidepressant and anxiolytic effects of NPY observed in rodents. Thus, NPY given i.c.v. to rats, both healthy and the ‘depressed’ Flinders Sensitive Line strain, as well as to mice, has antidepressant and anxiolytic effects as measured in the Forsolt swim test and elevated plus maze (Mathé and Gruber, 2004; Husum et al., 2000; Redrobe et al., 2002).

In conclusion, our results show that a 4-wk Cit treatment markedly increases NPY and decreases CRH in CSF from depressed patients and that these changes are strongly correlated to the clinical improvement. The findings are consistent with previous human and animal data and support the hypotheses that NPY and CRH play a role in affective disorders and are affected by antidepressive treatment.

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**Statement of Interest**

Bayer Vital GmbH who provided partial financial support for this study has marketed citalopram in Germany, but not in Switzerland. P.B. and H.A. are on advisory boards of Lundbeck, a company which has both citalopram and escitalopram marketed in Switzerland and Sweden, but which did not sponsor this study.

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