Quetiapine affects neuropeptide Y and corticotropin-releasing hormone in cerebrospinal fluid from schizophrenia patients: relationship to depression and anxiety symptoms and to treatment response

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Abstract

Cumulative evidence indicates that neuropeptides play a role in the pathophysiology of schizophrenia. Early data showed increased neuropeptide Y (NPY) in cerebrospinal fluid (CSF) from schizophrenia patients and data from rodents show that antipsychotic drugs modulate NPY levels in and release from selected rat brain regions. In view of these findings we investigated whether the atypical antipsychotic quetiapine, originally used as an antipsychotic but subsequently shown to be efficient also in major depressive disorder and in both poles of bipolar disorder, would affect NPY-like immunoreactivity (-LI), and corticotropin-releasing hormone (CRH)-LI levels in CSF of schizophrenia patients. NPY-LI and CRH-LI in CSF were determined in 22 patients with schizophrenia. Lumbar puncture was performed at baseline and again after 4 wk of quetiapine treatment (600 mg/d). Patients were assessed with the Positive and Negative Syndrome Scale (PANSS) at baseline and at weekly intervals. Quetiapine treatment was associated with a significant increase in NPY-LI (p < 0.001) and decrease in CRH-LI (p < 0.01). Stepwise multiple regression analysis revealed that ΔNPY-LI and ΔCRH-LI levels predicted 63% (p < 0.001) of the variability of the ΔPANSS total score, ΔNPY-LI 42% (p < 0.05) of the ΔPANSS anxiety items (G2) and ΔCRH-LI 40% (p = 0.05) of the ΔPANSS depression items (G6). These results suggest that while quetiapine’s effects on monoamines are probably related to its antipsychotic properties, the modulation of NPY and CRH accounts for its antidepressant and anxiolytic effects and can be markers of response.

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Key words: CSF, neuropeptides, norquetiapine, quetiapine, schizophrenia.

Introduction

A number of studies have reviewed the roles of neuropeptide Y (NPY) and corticotropin-releasing hormone (CRH) in the central nervous system (CNS) physiology and pathophysiology and recently also as mediating the effects of drugs used in treatment of depression and schizophrenia (Eaton et al. 2007; Heilig, 2004; Holsboer & Ising, 2010; Mathé et al. 2007; Obuchowicz et al. 2004).

NPY is a highly conserved 36-residue peptide that was isolated from porcine brain (Tatemoto et al. 1982) and subsequently found in high concentrations and widely distributed in the mammalian central and peripheral nervous systems (Adrian et al. 1983; Allen et al. 1983). The effects of NPY are mediated by G-protein-coupled receptors, five of which have been cloned, namely, the Y1, Y2, Y4, Y5 and Y6 receptors (Larhammar & Salaneck, 2004; Michel et al. 1998). Of these, the Y1, Y2 and Y5 subtypes are predominant in the CNS, while Y4 is found in peripheral tissues and Y6 is non-functional in primates and most other...
mammalian species. The NPYergic system plays an important role in, e.g. the regulation of food intake (Stanley & Leibowitz, 1985), sexual behaviour (Kalra et al. 1988), information handling (Fuxe et al. 1990), cognition (Redrobe et al. 2006), learning and memory (Flood et al. 1987; Redrobe et al. 2004; Thorsell et al. 2000), sympathetic activity (Zukowska-Grojec & Wahlestedt, 1993) and sleep regulation (Antonijevic et al. 2000; Ehlers et al. 1997). Moreover, reduced expression of NPY in selected brain regions was found in genetic and environmental animal models of depression, in an animal model of post-traumatic stress disorder (PTSD), as well as in CSF from depressed patients and in post-mortem brains from depressed patients (Caberlotto & Hurd, 2001; Cohen et al. in press; Heilig, 2004; Husum & Mathé, 2002; Husum et al. 2006; Jimenez-Vasquez et al. 2000, 2001, 2007; Mathé et al. 1998; Widdowson et al. 1992). Consequently, impaired central NPY signalling may be involved in the pathophysiology of depression (Domschke et al. 2010; Karlsson et al. 2008; Mathé et al. 2007; Nikisch & Mathé, 2008; Nikisch et al. 2005), anxiety (Domschke et al. 2008; Heilig, 2004; Redrobe et al. 2003), schizophrenia (Beal et al. 1987; Berrettini et al. 1987; Peters et al. 1990; Widerlöv et al. 1988; Zech et al. 1986), alcoholism (Ciccocioppo et al. 2009; Cippitelli et al. in press; Mottagui-Tabar et al. 2005), and PTSD (Morgan et al. 2003; Seedat et al. 2003; Yehuda et al. 2006).

Interactions between the CNS CRH, noradrenergic and the hypothalamic-pituitary-adrenal (HPA) axis systems are critical in promoting adaptive responses to stress and fear. Malfunction of these stress regulatory systems plays a major role in the aetiology of disorders like depression and PTSD (Strohle & Holsboer, 2003; Yehuda et al. 2006), CRH dysregulation has been observed in depression, anxiety and PTSD (Claes, 2004; Kascikow et al. 2001; Mathé et al. 2007; Nikisch & Mathé, 2008; Nikisch et al. 2005). It has been proposed that NPY and CRH play opposing roles in regulation of anxiety disorders (Heilig, 2004; Shekhar et al. 2005; Thorsell, 2010; Thorsell et al. 2006; Valdez & Koob, 2004).

Thus, NPY in addition to being an antidepressant is also a potent anxiolytic, whereas CRH is anxiogenic; and the balance between these two peptides may exert important influences on behavioural regulation. NPY has been proposed as an endogenous buffer against the stressor-induced release of CRH (Heilig et al. 1994). Recent studies have shown that direct injection of NPY in the basolateral nucleus of the amygdala (BLA) prior to a CRH agonist significantly blocks the development of avoidance behaviour in the two-floor choice test, a modified version of the conditioned-place avoidance paradigm (Sajdyk et al. 2006). An opposing effect of CRH and NPY on GABAergic transmission has been observed in the bed nucleus of stria terminalis (BNST) which may also contribute to stress and anxiety outcomes (Kash & Winder, 2006). Increased emotionality and anxiety may result from a dysregulation of the allostatic balance between CRH and NPY systems, particularly in the amygdala.

Interestingly, antidepressant treatments, e.g. antidepressants and ECT, normalize decreased NPY and increase CRH levels in the CSF of depressed patients (Nikisch & Mathé, 2008; Nikisch et al. 2005), supporting the inverse relationship between these peptides in the CNS.

It has been proposed that the efficacy of quetiapine in schizophrenia and its mood-stabilizing properties in depression and mania are mediated through a combination of dopamine D2 receptor and serotonin (5-HT2A) receptor antagonism, with the active metabolite N-desalkylquetiapine (norquetiapine) having similar activity at D2, but greater activity at 5-HT2A receptors than the parent drug (Goldstein et al. 2007; Jensen et al. 2008). In addition, its efficacy in depression was proposed to be due to the high affinity and potent inhibitory effects of norquetiapine on the norepinephrine transporter (Goldstein et al. 2007). Expanding on these results, in view of the cumulative data strongly indicating that neuromediators play a role in schizophrenia and depression and constitute one of the mechanisms of action of treatment modalities for these disorders, we explored our working hypothesis that the antidepressive effects of quetiapine, in similarity to mechanisms of action of other antidepressants, will be a consequence of its effect on NPY and CRH, and tested this assumption by treating a group of schizophrenia patients with 600 mg quetiapine daily for 4 wk and measuring NPY and CRH in their CSF.

Materials and methods

Subject selection and assessment

Relationships between the clinical outcome and CSF quetiapine, norquetiapine, CSF 5-HIAA, HVA, MHPG (Nikisch et al. 2010a, b), and the effect of quetiapine on ABCB1 genetics (Nikisch et al. 2011) and of dopamine D2 receptor occupancy (Nikisch et al. 2010a, b) in schizophrenia patients treated with quetiapine were reported. Briefly, 22 patients (eight females) with a schizophrenic episode, aged 18–55 yr [mean ± s.d. (standard deviation) age 35.9 ± 7.4 yr] and mean
duration of illness of 20.3 ± 24.8 months participated in the study (Table 1). During the study all patients were inpatients at the Department of Psychiatry and Psychotherapy, Fulda, Germany. After the study they were all treated as outpatients. Upon entry into the study, patients were evaluated using the Structured Clinical Interview confirmed diagnoses for DSM-IV, and met criteria for schizophrenia paranoid type \( (n = 22) \). The Structured Clinical Interview was performed by two trained raters who independently determined the diagnosis which was confirmed by a third psychiatrist, blind to the previous evaluations. Inter-rater reliability was very high \( (\rho = 0.95, p < 0.001) \). A ≥20% reduction in Positive and Negative Syndrome Scale (PANSS) total scale score (Kay et al. 1987) from baseline to week 4 was defined a priori as response, while lack of such a change was defined as non-response (Fleischhacker & Kemmler, 2007). The symptom severity threshold for response comprised a score of ≤3 (mild) on each of the following two PANSS single items: anxiety (G2) and depression (G6).

All patients had normal laboratory and physical examinations and were free of alcohol or drug abuse for at least 3 months. Eight patients had previously failed to respond to one or more second-generation antipsychotic medication trials. Fourteen patients were drug-naïve in their first episode. At baseline, participants had to meet criteria for a schizophrenic disorder (paranoid type) with symptoms of at least ≥60 on PANSS total scale score. Written informed consent was obtained and the protocol was approved by the Ethics Committee of the Johann-Wolfgang-University of Frankfurt a.M., Germany, according to the 1975 Declaration of Helsinki.

Inclusion criteria were: age between 18 and 55 yr, diagnosis of schizophrenia by DSM-IV, clinical indication for medication adjustment/change with second-generation antipsychotic drugs. Exclusion criteria were: a history of a manic or hypomanic episode, any other DSM-IV Axis I diagnosis, substance or alcohol dependence at enrolment, significant suicide risk and medical illness and, last, pregnancy or lactation.

### Study design

At baseline, weeks 1, 2, and 3, and at the end of the study an extensive physical and laboratory check up, including haematology, clinical chemistry toxicological urine tests, 12-lead electroencephalography recordings (EEG), and electrocardiogram (ECG) were performed.

Collections of samples and assay techniques have been described previously (Nikisch et al. 2010a,b, 2011).

### CSF

**CSF quetiapine and norquetiapine measurements**

Samples were collected, stored at −20°C and the determinations performed by Bioanalytical Systems (USA) using liquid-liquid extraction, followed by reversed-phase liquid chromatography separation and electrospray ionization-tandem mass spectrometry detection (LC/MS-MS) (Davis et al. 2010) as previously described (Nikisch et al. 2010a,b, 2011). Quantitation ranges for each analyte were from <0.7 to 2000 ng/ml. Between-day precision and accuracy (coefficient of variation) was less than 6% (6.4%) for quetiapine, and less than 9.4% (and 5.9%) for norquetiapine.

**CSF NPY-LI and CRH-LI measurement**

Lumbar puncture was performed on days 0 and 28; thus, each subject served as his/her own control. A total of 26 ml CSF was collected in 12 aliquots and the ninth aliquot was selected for analyses. Lumbar puncture was performed with patients in the lateral

### Table 1. Baseline demographic characteristics of subjects with schizophrenia disorder

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>Total samples (n = 22)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yr)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22–29</td>
<td>7 (32)</td>
<td></td>
</tr>
<tr>
<td>32–39</td>
<td>9 (41)</td>
<td></td>
</tr>
<tr>
<td>41–49</td>
<td>6 (27)</td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14 (64)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>8 (36)</td>
<td></td>
</tr>
<tr>
<td><strong>Psychiatric history</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final diagnosis: schizophrenia (paranoid type)</td>
<td>22 (100)</td>
<td></td>
</tr>
<tr>
<td>Drug-naïve, in first episode</td>
<td>14 (64)</td>
<td></td>
</tr>
<tr>
<td>Hospitalized at baseline</td>
<td>8 (36)</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Duration of illness (month)</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>5</td>
<td>1–60</td>
</tr>
<tr>
<td>Female</td>
<td>25</td>
<td>2–72</td>
</tr>
<tr>
<td>Baseline medications</td>
<td></td>
<td>(%)</td>
</tr>
<tr>
<td>Risperidone</td>
<td>4</td>
<td>(50)</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>3</td>
<td>(38)</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>1</td>
<td>(12)</td>
</tr>
</tbody>
</table>
decubitus position at 09:30 hours after maintaining bed rest and fasting from 23:00 hours the preceding night. After centrifugation, the supernatants were immediately frozen in 2-ml aliquots in sialinized tubes and stored at −80 °C until assayed.

NPY-LI and CRH-LI were determined as previously described (Husum & Mathé, 2002; Nikisch & Mathé, 2008; Nikisch et al. 2005).

Statistical analysis

Comparisons between quetiapine responders and non-responders were analysed by Fisher’s exact test for contingency tables (qualitative variable: sex) or by using two-tailed probability t tests for paired samples for independent samples (quantitative variables: age; PANSS total scale score with the subscales positive, negative, and general psychopathology; PANSS single items: anxiety (G2), depression (G6), CSF neuropeptide levels and quetiapine and norquetiapine levels in CSF).

Two-way repeated-measures analysis of variance (ANOVA) was also used with one with-subjects factor (time: CSF neuropeptide data before and after week 4 of quetiapine treatment) and one between-subjects factor (group: responders vs. non-responders).

For reasons of non-normal distributions, Spearman’s rank-order correlations (\(r_{pb}\)) were used to determine the possible relationship between the changes in the PANSS total scale score (treatment week 4 – baseline; \(\Delta\)PANSS total scale score with the subscale scores; \(\Delta\)PANSS positive, \(\Delta\)PANSS negative, and \(\Delta\)PANSS general psychopathology) and \(\Delta\)PANSS single items: AG2 and \(\Delta\)G6 and changes in CSF neuropeptide levels. Pearson’s product-moment correlation coefficients (\(r\)) were calculated to examine the possible relationship between the changes in the CSF NPY-LI and CRH-LI levels and the NPY/CRH-LI and CRH/NPY-LI ratios (treatment week 4 – baseline; \(\Delta\)NPY-LI and \(\Delta\)CRH-LI, and NPY/CRH-LI, CRH/NPY-LI ratios).

To determine which variables might predict maximum changes in PANSS total scale score with the subscale scores positive, negative and general psychopathology and PANSS single items: anxiety (G2) and depression (G6) and CSF quetiapine and norquetiapine levels, as dependent variables, we performed multiple linear regression analyses entering demographic variables (e.g. age, sex, duration of illness, and number of previous episodes), and CSF NPY-LI, CRH-LI levels with their ratios, as independent values using the general linear model (GLM) procedure of SPSS (SPSS Inc., USA).

For all ANOVA procedures, the F value was corrected by adjusting the degrees of freedom by Greenhouse–Geisser epsilon (\(\varepsilon\)), if the sphericity test (Mauchly W test) was significant, indicating heterogeneity of covariances (Huynh–Feldt correction). When multiple comparisons were made, the corrected p values were also calculated for multiple testing using Bonferroni’s test for equal variance or Dunnett’s T3 for non-equal variance.

The diagnostic performance of optimal CSF NPY-LI and CRH-LI levels for response was assessed by means of ROC (receiver-operating characteristics) curve analysis (Kronig et al. 1995) with computation of the area under the ROC curve (AUC), sensitivity, and specificity. The statistical significance of the association between response and CSF NPY-LI and CRH-LI levels, respectively, was tested by \(\chi^2\) analysis.

Statistical significance was set at \(\alpha < 0.05\) (two-tailed tests). Data analysis was performed using SPSS v. 19 (SPSS Inc.).

Results

Clinical effectiveness of quetiapine

Four weeks of treatment with quetiapine resulted in significant improvement in clinical symptoms as measured by change from baseline (Table 2).

Twelve patients were considered to be responders, as their PANSS total score decreased by \(\geq 20\%\). In order to validate the above-mentioned repeated-measures ANOVA method, this analysis was performed for the PANNS scores controlling for sex. As predicted, the interaction between PANNS change and the responder category was strong (\(F_{1,21} = 38.3, p < 0.001\)), and there was no sex \(\times\) response interaction.

Effects of quetiapine on NPY-LI and CRH-LI levels and their ratios in CSF

After 4 wk of quetiapine treatment, the CSF level 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. Using a two-way repeated-measures ANOVA, there was a significant interaction with the responder and non-responder category in NPY-LI and CRH-LI; the responders compared to non-responders, showed a larger increase in NPY-LI (\(F_{1,21} = 9.5, p < 0.001\)) and a decrease in CRH-LI (\(F_{1,21} = 5.2, p < 0.05\)).

NPY-LI and CRH-LI levels were significantly correlated before \(r = 0.651, p < 0.01; t_{21} = 5.3, p < 0.01\) and after quetiapine treatment \(r = −0.887, p < 0.001\);
Table 2. Outcome measurement before and after treatment with quetiapine in 22 patients with schizophrenia disorder

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>Baseline Mean ± S.D.</th>
<th>Post-treatment Mean ± S.D.</th>
<th>p value</th>
</tr>
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<tbody>
<tr>
<td>Positive and Negative Syndrome Scale</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PANSS positive subscore</td>
<td>38.4 ± 8.3</td>
<td>19.2 ± 8.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PANSS negative subscore</td>
<td>21.5 ± 9.9</td>
<td>10.1 ± 5.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PANSS general psychopathology subscore</td>
<td>33.9 ± 10.8</td>
<td>21.5 ± 9.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PANSS total score</td>
<td>94.0 ± 13.5</td>
<td>50.8 ± 16.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PANSS (G2)</td>
<td>6.9 ± 1.2</td>
<td>4.1 ± 0.6</td>
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</tr>
<tr>
<td>PANSS (G6)</td>
<td>6.5 ± 1.4</td>
<td>4.4 ± 0.5</td>
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<td>NPY-LI (pmol/l)</td>
<td>30.7 ± 9.3</td>
<td>65.8 ± 19.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRH-LI (pmol/l)</td>
<td>13.6 ± 1.8</td>
<td>7.7 ± 1.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NPY/CRH-LI</td>
<td>2.4 ± 1.0</td>
<td>9.7 ± 2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRH/NPY-LI</td>
<td>0.6 ± 0.3</td>
<td>0.2 ± 0.1</td>
<td>&lt;0.01</td>
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s.d., Standard deviation; p value, t tests (two-tailed) for paired samples; NPY-LI, neuropeptide Y-like immunoreactivity; CRH-LI, corticotropin-releasing hormone-like immunoreactivity.

Fig. 1. Individual cerebrospinal fluid (CSF) levels (pmol/l) of NPY-LI and CRH-LI in 22 schizophrenia patients treated with 600 mg/d quetiapine for 28 d.

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<tr>
<td>NPY/CRH-LI</td>
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<td>&lt;0.001</td>
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<td>0.2 ± 0.1</td>
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s.d., Standard deviation; p value, t tests (two-tailed) for paired samples; NPY-LI, neuropeptide Y-like immunoreactivity; CRH-LI, corticotropin-releasing hormone-like immunoreactivity.

Fig. 1. Individual cerebrospinal fluid (CSF) levels (pmol/l) of NPY-LI and CRH-LI in 22 schizophrenia patients treated with 600 mg/d quetiapine for 28 d.

The baseline and post-treatment NPY-LI/CRH-LI and CRH-LI/NPY-LI ratios are displayed in Table 2 and show significant correlation with the decreased baseline NPY-LI/CRH-LI ratio and the increased ratio after treatment (NPY-LI/CRH-LI ratio: $r=0.835$, $p=0.001$; $t_{21}=9.3$, $p=0.001$). On the other hand, the baseline and post-treatment CRH-LI/NPY-LI ratios showed significant correlation with the increased baseline CRH-LI/NPY-LI ratio and the decrease post-treatment (CRH-LI/NPY-LI ratio $r=0.655$, $p=0.01$; $t_{21}=4.8$, $p=0.01$).

Treatment response and NPY-LI and CRH-LI CSF levels

For CSF ΔNPY-LI and ΔCRH-LI thresholds of 29.19 and −6.15 pmol/l were associated with a sensitivity of 82% and 77% and a specificity of 72% and 68% (Fig. 2). Results of $\chi^2$ tests with Pearson corrections to compare responder and non-responder thresholds were significant ($p=0.018$ and $p=0.035$, respectively).

Furthermore, the ΔNPY-LI and ΔCRH-LI levels predicted 63% of the variability of the ΔPANSS total score ($F_{6,14}=8.5$, $p<0.001$), ΔNPY-LI predicted 42% of the ΔPANSS anxiety items ($F_{6,14}=3.7$, $p<0.05$) and
CRH-LI predicted 40% of the PANSS depression items ($F_{(6,21)} = 3.5, p < 0.05$). These results demonstrate that the NPY and CRH CSF levels are significant determinants of clinical symptoms of depression and anxiety.

**Relationship between drug and NPY-LI and CRH-LI CSF levels**

Quetiapine treatment was associated with a $127\pm 52\%$ increase in NPY-LI and a $-41\pm 15\%$ decrease in CRH-LI. In the CSF (Fig. 3a), quetiapine levels showed significant correlation with the increase in ΔNPY-LI ($p = 0.001$). Significant negative correlation (Fig. 3b) was also found between CSF norquetiapine levels and the decrease in ΔCRH-LI ($p = 0.01$). On the other hand, neither plasma quetiapine nor norquetiapine correlated significantly with increased ΔNPY-LI and decreased ΔCRH-LI levels.

Stepwise multiple regression analysis revealed that ΔNPY-LI predicted CSF quetiapine levels, explaining 62% of the variability ($F_{(6,21)} = 8.3, p < 0.001$) and ΔCRH-LI predicted CSF norquetiapine levels, explaining 48% of the variability ($F_{(6,21)} = 4.3, p < 0.01$).

**Discussion**

To the best of our knowledge, this is the first study to investigate the effects of quetiapine on NPY-LI and CRH-LI levels in the CSF of schizophrenia patients. The relationships between CSF levels of quetiapine and norquetiapine, changes in NPY-LI and CRH-LI CSF levels, and the treatment response were studied.

The first major finding of our study is the marked increase in NPY-LI levels in CSF following quetiapine CSF ΔNPY-LI levels (pmol/l) (Fig. 3). Significant positive correlation ($r = 0.888; p = 0.0001$) was found between quetiapine CSF levels (ng/ml) and ΔNPY-LI levels (pmol/l) in 22 patients treated with quetiapine.

CSF ΔCRH-LI levels (pmol/l) (Fig. 3) showed significant negative correlation ($r = 0.577; p = 0.01$) between CSF norquetiapine levels (ng/ml) and ΔCRH-LI levels (pmol/l). These results indicate that quetiapine treatment is associated with a marked increase in NPY-LI and a decrease in CRH-LI levels in CSF.
treatment and the strong correlation between the rise in NPY-LI and clinical improvement.

Only a few studies have been published on the potential role of NPY in schizophrenia and their results differ. In a post-mortem study (Frederiksen et al. 1991), hypothalamic NPY was unchanged and temporal cortex NPY was decreased in both neuroleptic-treated and untreated schizophrenics, which suggested that antipsychotic therapy could not explain the reduced temporal NPY. Reduced NPY has been reported in the cerebral cortex of cognitively impaired schizophrenics (Gabriel et al. 1996). Beal and co-workers (Beal et al. 1987) did not observe significant differences in amygdala NPY between normal controls and schizophrenia patients. Increased NPY levels in CSF from drug-free chronic schizophrenia patients and after withdrawal of haloperidol maintenance therapy were reported (Peters et al. 1990). The authors suggested elevated NPY as indicating a vulnerability to schizophrenia. They found a negative relationship between NPY and brain atrophy, a positive correlation between positive symptoms and NPY in stable patients and a positive correlation between negative symptoms and NPY in relapers after haloperidol withdrawal. In contrast, other studies did not find significant CSF NPY differences between the drug-free schizophrenics and schizophrenics treated with neuroleptics (Berrettini et al. 1987; Widerlöv et al. 1988). In view of the small number of patients studied, confounding post-mortem factors, differences in study designs, and NPY measurements, no definite conclusions can be drawn from these studies.

NPY and dopamine have been shown to interact reciprocally in rat brain (Heilig & Widerlöv, 1990; Kerkerian et al. 1998). This has been shown in brain regions relevant to the pathophysiology of schizophrenia, such as the prefrontal cortex and nucleus accumbens (Csernansky & Bardgett, 1998; Lewis & Moghaddam, 2006). Chronic treatment with antipsychotic drugs regulates NPY-LI transmission (Obuchowicz et al. 2004). Antipsychotic drugs such as olanzapine, clozapine, haloperidol and risperidone as well as d-amphetamine also affect extracellular levels of NPY-LI in the ventral striatum (Gruber et al. 2006; Huang et al. 2006). Interestingly, NPY-Y₁ receptor activation was found to be crucial for the behavioural effects of amphetamine and the dopamine agonist apomorphine. The ability of Y₁ antagonists to modulate these behavioural responses implies that Y₁ receptors may be involved in the mediation of psychosis and reinforcement (Kask & Harro, 2000).

NPY levels increased markedly following quetiapine treatment and there was a strong positive correlation between the rise in NPY and clinical improvement. This indicates that the rise in NPY may be due both to the change in the patient’s clinical status and to the direct effects of quetiapine. Our findings are consistent with the previously reported NPY increase in depressed patients following ECT (Mathe´, et al. 1996; Nikisch & Mathe´, 2008) 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, ECS, lithium, citalopram, fluoxetine, all increase NPY expression in selected brain regions of control rats and ‘depressed’/stressed rats, indicating that both treatment and pathology play a role. NPY given intracerebroventricularly (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 Porsolt swim test and elevated plus maze (Husum & Mathe´, 2002; Husum et al. 2000; Jimenez-Vasquez et al. 2000, 2007; Mathe´ & Gruber, 2004; Redrobe et al. 2002).

The second major finding is that the CSF CRH-LI levels decreased markedly following quetiapine and there was a strong impact between the decreases in CRH-LI and clinical improvement.

An association between psychosocial stress exposure and schizophrenia is well documented (Brown, 2009; Walker et al. 2008). By governing a cascade of hormonal events, the HPA axis mediates the biological response to stress and the regulatory feedback inhibition to the brain. In response to several stressors, CRH is released from the paraventricular nucleus of the hypothalamus. Interestingly, the administration of corticosteroids for therapeutic reasons is associated with an increased risk for psychosis (Walker et al. 2008). A major outcome at the level of the hypothalamus is that CRH production is not dampened, and the HPA axis continues to be overactive (Walker et al. 2008). The functional disconnectivity between hippocampus and hypothalamus is believed to prominently contribute to many behavioural and pathophysiological sequelae of chronic stress in all diagnostic categories (Bernstein et al. 2007; Holsboer, 2003; Raison & Miller, 2003; Sapolsky, 2002). Although typically associated with affective disorders, there is compelling evidence for hyperactivity of the HPA axis with hypercortisolism also in schizophrenia (Brown, 2009; Pfennig et al. 2005; Ryan et al. 2004; Walker et al. 2008). Post-mortem studies have shown that the density of hippocampal glucocorticoid receptor mRNA is greatly reduced in schizophrenia (Webster et al. 2002) indicating that this pathological mechanism is active in
that disorder. An increased baseline cortisol secretion can be measured in patients with schizophrenia not only as a result of antipsychotic medication, but also in first-episode, drug-naive patients (Afzal & Thakore, 2009; Ryan et al. 2004; Walker et al. 2008). Early work has shown that the level of CRH in the CSF is slightly but significantly higher in schizophrenia patients than in control subjects (Banki et al. 1987). CRH levels are not significantly related to severity of psychosis and rise further when haloperidol is withdrawn or re-placed by placebo (Forman et al. 1994).

The design of this study had some limitations. First, the number of patients was relatively small, limiting statistical power, especially as some correlations are weak. Second, since there was no untreated control patient group, it cannot be ruled out that factors other than quetiapine treatment, such as the natural course of the disease, account for the increased NPY-LI and decreased CRH-LI levels.

In conclusion, quetiapine markedly increased NPY and decreased CRH in CSF from schizophrenia patients leading to a strong negative correlation between NPY and CRH. These effects were strongly correlated to the clinical improvement, in particular to the decrease in depression and anxiety PANSS items. These results imply that while effects of quetiapine on monoamines are probably related to its antipsychotic properties, the modulation of NPY and CRH accounts for its antidepressant and anxiolytic effects. Since psychiatric diagnoses are phenotypical, i.e. clusters of symptoms of various durations, and there are hardly any pathognomonic signs, our approach to correlate changes in specific symptoms, to biological markers and effects of drugs would seem to be a fruitful strategy to elucidate the neurobiology of specific symptoms independently of the underlying psychiatric diagnoses.

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Statement of Interest

Pierre Baumann has received honoraria for teaching CME courses from AstraZeneca and other pharmaceutical companies marketing psychotropic drugs in Switzerland.

References


Jiménez-Vasquez PA, Díaz-Cabiale Z, Caberoñito L, Bellido I, et al. (2007). Electroconvulsive stimuli selectively affect behavior and neuropeptide Y (NPY) and NPY Y1 receptor gene expressions in hippocampus and hypothalamus of Flinders Sensitive Line rat model of depression. European Neuropsychopharmacology 17, 298–308.


Redrobe JP, Dumont Y, Quirion R (2002). Neuropeptide Y (NPY) and depression: from animal studies to the human condition. Life Sciences 71, 2921–2937.


