SSRI augmentation of antipsychotic alters expression of GABA_A receptor and related genes in PMC of schizophrenia patients

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Abstract
Clinical studies have shown that negative symptoms of schizophrenia unresponsive to antipsychotic given alone can improve after augmentation with SSRI antidepressant. Laboratory investigations into the mechanism of this synergism showed that co-administration of SSRI and antipsychotic produces changes in GABA_A receptor and related systems, which differ from the effects of each drug alone. To examine the clinical relevance of these findings, the current study examined the effects of SSRI augmentation treatment on GABA_A receptor and related systems in schizophrenia patients. Schizophrenia patients with high levels of negative symptoms unresponsive to antipsychotic treatment received add-on fluvoxamine (100 mg/d). Blood was taken before and 1, 3 and 6 wk after adding fluvoxamine and peripheral mononuclear cells (PMC) isolated. RNA encoding for GABA_A β3, 5-HT_2A, and 5-HT_7 receptors, PKC_β2, and brain-derived neurotrophic factor (BDNF) was assayed with real-time RT-PCR. Plasma BDNF protein was assayed using ELISA. Clinical symptoms were assessed with validated rating scales. We found significant increase in mRNA encoding for GABA_A β3 and 5-HT_2A, 5-HT_7 receptors and BDNF and a reduction in PKC_β2 mRNA. Plasma BDNF protein concentrations were increased. There were significant correlations among the genes. Clinical symptoms improved significantly. mRNA expression of PKC_β2, 5-HT_2A and 5-HT_7 showed significant associations with clinical symptoms. Combined SSRI + antipsychotic treatment is associated with changes in GABA_A receptor and in related signalling systems in patients. These changes may be part of the mechanism of clinically effective drug action and may prove to be biomarkers of pharmacological response.

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Introduction
There is considerable evidence that combination of SSRI and antipsychotic can ameliorate negative symptoms of schizophrenia unresponsive to antipsychotic alone (Falkai et al. 2006; Rummel et al. 2005; Silver & Nassar, 1992) and improve resistant symptoms in obsessive compulsive and affective disorders (Bloch et al. 2006; Bobo & Shelton, 2009). More specifically, three controlled studies in chronic schizophrenia patients with persistent negative symptoms showed that adding fluvoxamine to ongoing antipsychotic treatment improved negative symptoms when compared to add-on placebo (Silver & Nassar, 1992; Silver et al. 2000; Silver, 2003) and the non-SSRI antidepressant, maprotiline (Silver & Shmugliakov, 1998). The
improvement included core primary negative symptoms (Silver et al. 2003a) and could be detected after 2 wk of augmentation treatment (Silver et al. 2003c).

The synergistic action cannot be explained by the known mechanisms of the individual drugs.

In previous studies our group (Chertkow et al. 2006; Danovich et al. 2010; Silver et al. 2009) identified changes in the GABA system and related pathways in localized regions of the rat brain following treatment with the SSRI, fluvoxamine combined with haloperidol (Hal + Flu), but not with each drug alone. In rat frontal cortex changes unique to the combined treatment included altered subcellular distribution of GABA\(_\alpha 3\) receptor with decreased levels of the receptor in the cytosolic fraction, and increased levels in the membrane compartment, and altered regulation of molecular signalling pathways that modulate GABA\(_\alpha\) receptor function, including protein kinase C (PKC) and extracellular signal-regulated kinase-2 (ERK2) (Danovich et al. 2010). In primary cortical cell culture, short-term treatment (15 min) with Hal + Flu combination increased GABA\(_\alpha 3\) subunit phosphorylation levels via a PKC-dependent pathway (Danovich et al. 2010). Significantly, many of these unique changes were also observed with clozapine, an atypical antipsychotic drug also effective for negative symptoms of schizophrenia.

There is increasing evidence that the GABA system is abnormal in schizophrenia (Lewis et al. 2005) so it was logical to examine the clinical relevance of these laboratory findings. A first step was to determine whether patients treated with SSRI + antipsychotic combination show changes in the GABA system. We chose to study the expression of mRNAs of genes encoding for the GABA\(_\alpha 3\) receptor and for proteins related to modulation of the GABA system in peripheral mononuclear cells (PMC) of schizophrenia patients during augmentation treatment. PMC and other blood products are readily accessible and have been widely investigated in schizophrenia patients as they may reflect molecular processes in the central nervous system (CNS) (Gladkevich et al. 2004; Liew et al. 2006). Human PMC express a range of GABA\(_\alpha\) receptor subunits (Alam et al. 2006) and although their functions and relationships with brain processes are unclear, demonstration of treatment-related changes would provide a ‘proof of concept’ and support their potential clinical relevance. The GABA\(_\alpha 3\) subunit was chosen as our previous study (Chertkow et al. 2006) showed that GABA\(_\alpha 3\) was significantly altered in rat frontal cortex following chronic treatment with haloperidol + fluvoxamine and that this drug combination regulated expression of GABA\(_\alpha 3\) receptor subunit and PKC\(\beta 2\) genes and proteins differently than each drug alone. Published evidence indicates that phosphorylation is an important mechanism of GABA\(_\alpha 3\) receptor regulation, particularly serine phosphorylation of the \(\beta 3\) subunit by PKC\(\beta 2\) (Brandon et al. 1999, 2000).

In the present study we added the SSRI, fluvoxamine to steady ongoing antipsychotic treatment in chronic schizophrenia patients with persistent negative symptoms, a population similar to that in our previous controlled studies (Silver & Nassar, 1992; Silver & Shmugliakov, 1998; Silver et al. 2000). We collected blood samples at baseline, before addition of fluvoxamine, and after 1, 3 and 6 wk of combined treatment. The PMC were isolated from the blood, mRNA extracted and assayed using real-time reverse transcriptase–polymerase chain reaction (RT–PCR). Plasma brain-derived neurotrophic (BDNF) protein was assayed using ELISA. To minimize the influence of inter-subject variability we used a ‘within-subject’ design where changes from baseline at each time-point were calculated for each individual.

Methods

Subjects

Eighteen patients were recruited into the study; for 14 patients, complete data was available and these are reported here. They included 11 men and three women whose mean age was 35.00 (S.D. = 10.33) yr, mean education 9.93 (S.D. = 1.591) yr, age at first hospitalization 30.36 (S.D. = 10.89) yr and mean number of hospitalizations 5.94 (S.D. = 6.08). The diagnosis of schizophrenia was made using DSM-IV criteria and included paranoid (n = 10), residual (n = 2), disorganized (n = 1) and unspecified (n = 1) subtypes. All were in-patients with mean hospitalization duration prior to study of 12.6 (range 7.0–50.4) months, and on continuous antipsychotic treatment since admission. To ensure clinical and medication stability, inclusion criteria required that the medication type and dose be unchanged for at least 2 wk prior to study onset. The mean period of unchanged antipsychotic medication prior to study entry was 26.34 (range 3–116) wk. Seven patients received atypical antipsychotics (risperidone: n = 3, dose range 3–6 mg/d; olanzapine: n = 1, 20 mg/d; ziprazidine: n = 3, dose range 600–700 mg/d), the rest, typical antipsychotics.

All patients provided written informed consent for participation in the trial after receiving a full explanation of the study procedures. The Institutional Review Board of Sha’ar Menashe Mental Health Center, Israel approved the study that was carried out...
in accordance with the Declaration of Helsinki, and is registered with the clinical trials register (http://ClinicalTrials.govID NCT00645580).

**Procedure**

Fluvoxamine (100 mg/d) was added to antipsychotic treatment, which remained steady.

Clinical assessments used the Scale for Assessment of Negative Symptoms (SANS) for negative symptoms, Simpson–Angus Scale (SAS) for extrapyramidal side-effects, Assessment of Involuntary Movement Scale (AIMS) for involuntary movements and the Calgary Depression Rating Scale (CDRS) for depressive symptoms. Enquiry was made from patients and treating clinicians to detect emergent side-effects. A cognitive battery was also administered and the findings will be presented separately.

The clinical and cognitive assessments tools are described and referenced elsewhere (Silver et al. 2003c).

**Isolation of PMC**

Blood samples (40 ml) were collected in EDTA-coated tubes at baseline, before addition of fluvoxamine, and at 1, 3 and 6 wk.

Blood was diluted with PBS and gently mixed. PMC were isolated according to Histopaque protocol (Sigma, USA). After addition of Histopaque (Sigma) reagent, the tubes were centrifuged (400 g, 30 min), the interphase was transferred to a new tube, mixed with 100 ml PBS and centrifuged (400 g, 10 min). The supernatant was discarded and the pellet containing the PMC was washed three times with PBS (400 g, 10 min).

**Real-time RT–PCR**

Extraction of total RNA from PMC was performed using an RNA purification kit according to the manufacturer’s protocol (Norgen Biotek Corp., Canada).

Two micrograms of total RNA were denatured and reverse-transcribed using random hexanucleotides (0.5 μg/μl) as previously described (Chertkow et al. 2006).

Real-time quantitative assessment was performed using LightCycler with FastStart DNA Master SYBR Green I ready-to-use PCR mix kits according to the manufacturer’s protocol (Roche Diagnostics, Germany). The sequences of the primers are described in Table 1. The results were analysed in real-time on the provided program of LightCycler. Normalizing to the housekeeping gene *PPIB-rRNA* and comparing to control values assessed the relative expression level of a given mRNA.

### Table 1. Human primers sequences and conditions for quantitative real-time RT–PCR

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Oligonucleotide sequence (5′–3′)</th>
<th>Experimental conditions (temperature in °C, time in s)</th>
<th>Acquisition temp./product, Tm</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPIB</td>
<td>F: 5′-AAGAGCATCTACCGGT-3′</td>
<td>Denaturation 95 (15)</td>
<td>Annealing 65 (10)</td>
</tr>
<tr>
<td>GABA_A/3</td>
<td>R: 5′-GTTTATCCCCGGCTGC-3′</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HT_A</td>
<td>F: 5′-TCTTTCAGCTTCTCCCTCA-3′</td>
<td>95 (15)</td>
<td>65 (10)</td>
</tr>
<tr>
<td>5-HT_7</td>
<td>R: 5′-TGCAAGACTTCTCGGTCAAT-3′</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PKC/2</td>
<td>F: 5′-GAAGAGTGCTGCCAAAACACA-3′</td>
<td>95 (15)</td>
<td>65 (10)</td>
</tr>
<tr>
<td>BDNF</td>
<td>R: 5′-GGTGGCTGTCTTTCTCTAC-3′</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All templates were initially denatured for 10 min at 95 °C. Amplification was done for 35 cycles. In order to receive melting temperatures of the products, melting curve analysis was done by continuous acquisition from 65 °C to 95 °C with temperature transition rate of 0.1 °C/s.
Plasma BDNF protein

BDNF protein concentration in plasma was assayed using BDNF Emax ImmunoAssay system (Promega, USA), designed for the sensitive and specific detection of BDNF in an antibody sandwich format, according to the manufacturer’s protocol.

Statistical analysis

The values for PMC and plasma elements were expressed as a proportion of the baseline value for that individual. To compare changes over time we used a mixed-effects linear regression model. This analysis accommodated missing values at some time-points, without the need for imputation. Not all blood measures were available for all patients so for consistency, the data presented includes the 14 patients in whom results on all biochemical substances were available. Values >3 times baseline value (one value for GABA<sub>β3</sub> at week 3 and two values for PKC<sub>β</sub>2 at week 3, one value for BDNF mRNA at week 3 and one value at week 6) which were probably due to a measurement error were excluded from the analyses reported in the text. Change in clinical symptoms between the times was analysed with the mixed model. Correlations used spearman’s ρ. Stata version 10 (StataCorp, USA) and SPSS version 11.5 (SPSS Inc., USA) were the programs used.

Results

PMC measures

Changes in gene products during treatment are shown in Figs 1 and 2. PMC levels of mRNA encoding for GABA<sub>β3</sub> increased significantly during treatment (mixed model for time: β = 0.0836, p = 0.001). The increase was significantly different from baseline at week 6 (p = 0.007). Increased expression was also observed for mRNA encoding for 5-HT<sub>2A</sub> receptor (mixed model: β = 0.0492, p = 0.017; difference from baseline was significant at week 6, p = 0.017) and 5-HT<sub>7</sub> receptor [mixed model: β = 0.0594, p = 0.010; difference from...
baseline was significant at week 3 \((p = 0.037)\) and week 6 \((p = 0.029)\) and mRNA coding for BDNF [mixed model: \(\beta = 0.1098, p < 0.001\); difference from baseline at week 3 \((p = 0.007)\) and week 6 \((p < 0.001)\)]. PMC expression of PKC\(\beta\)2 mRNA decreased significantly over time [mixed model: \(\beta = -0.0849, p = 0.003\); difference from baseline was significant at week 3 \((p = 0.001)\) and week 6 \((p = 0.001)\)]. Plasma concentration of BDNF increased during treatment (mixed model: \(\beta = 0.0646, p = 0.001\); difference from baseline was significant at week 6 \((p = 0.001)\)).

### Correlations between gene products

We looked for evidence of associations between individual genes as they may indicate systematic changes (Table 2). After 3 wk of combined treatment,
expression of mRNA for 5-HT$_2A$ showed significant correlation with that for GABA$_A$$\beta$3 ($r = 0.855$, $p < 0.01$) and for PKC$_{b2}$ ($r = -0.70$, $p < 0.01$) at week 3. Expression of mRNA for 5-HT$_2A$ at week 6 showed significant correlation with that for GABA$_A$$\beta$3 ($r = 0.60$, $p < 0.05$) and 5-HT$_7$ ($r = 0.86$, $p < 0.01$) at week 3. PMC levels of mRNA encoding for BDNF at 6 wk showed significant correlations with 5-HT$_2A$ at week 1 ($r = 0.64$, $p < 0.05$), week 3 ($r = 0.76$, $p < 0.01$) and week 6 ($r = 0.58$, $p < 0.01$) and with levels of 5-HT$_7$ mRNA at week 3 ($r = 0.62$, $p < 0.05$). Levels of mRNA encoding for BDNF at week 1 showed significant correlation with levels of mRNA encoding for GABA$_A$$\beta$3 ($r = 0.58$, $p < 0.05$) at week 1, and for 5-HT$_7$ at week 1 ($r = 0.82$, $p < 0.01$) and week 6 ($r = 0.69$, $p < 0.01$).

There was no significant association between PMC levels of mRNA encoding for BDNF and the concentration of BDNF protein in the plasma at any time-point.

The presence of these associations indicates that the changes observed in PMC are not disconnected responses of individual genes.

Clinical symptoms

As expected from the selection criteria, the patients showed high levels of negative and positive symptom scores and these improved significantly with treatment (Table 3). By selection, levels of depressive, extrapyramidal and involuntary movement symptoms, which can confound diagnosis of primary negative symptoms, were low. There were no changes in extrapyramidal or involuntary movement side-effects during treatment, and no other treatment-emergent side-effects were noted (Table 3).

There were significant correlations (Table 4, Fig. 3) between mRNA encoding for PKC$_{b2}$ after 1 wk of treatment and positive symptoms at baseline ($r = -0.79$, $p < 0.01$), week 3 ($r = -0.64$, $p < 0.05$) and week 6 ($r = -0.66$, $p < 0.01$) and with negative symptoms at week 6 ($r = -0.71$, $p < 0.01$). Levels of mRNA encoding for GABA$_A$$\beta$3 ($r = 0.85$, $p < 0.01$), 5-HT$_{1A}$ ($r = 0.64$, $p < 0.05$) and 5-HT$_7$ ($r = 0.59$, $p < 0.05$) at week 3 showed significant correlations with positive symptom score at endpoint.

Patients treated with atypical antipsychotics did not differ from those treated with typical antipsychotics in clinical symptom levels or response, side-effects, PMC measures or in the relationships between them.
Discussion

To our knowledge, this is the first report showing that SSRI augmentation treatment can regulate expression of GABA<sub>A</sub> receptor subunits in PMC cells of schizophrenia patients. It is consistent with evidence that SSRIs can increase GABA levels peripherally and in the brain, including in plasma of depressed patients (Kuçükibrahimöglu et al. 2009), occipital cortex of chronically treated depressed patients and acutely treated healthy individuals (Bhagwagar et al. 2004; Sanacora et al. 2002) and in CSF of rats (Goren et al. 2007). Increased GABA<sub>A</sub> receptor expression has been reported in post-mortem brains of schizophrenia patients, particularly in the hippocampal formation, anterior cingulate cortex and prefrontal cortex (Benes et al. 1996; Dean et al. 1999).

The reduction in PKC mRNA expression by augmentation treatment was of interest since overactivity of PKC can impair PFC function (Arnsten, 2009), a region closely linked with negative symptoms (Weinberger et al. 1988), and impair working memory (Birnbaum et al. 2004), a putative core dysfunction in schizophrenia (Silver et al. 2003b). PKC abnormalities have been implicated in schizophrenia pathology and response to psychotropics (Arnsten, 2009).

PKC is involved in phosphorylation of many proteins and may thus be related to changes in clinical features additional to the target symptoms. While in this, as in previous studies (Silver & Nassar, 1992; Silver et al. 2000; Silver & Shmugliakov, 1998) we found good tolerability and no evidence of emergent side-effects, the possibility that PKC and other PMC changes may be related to side-effects warrants investigation.

The observed correlations between changes in PKC<sub>B2</sub> and in GABA<sub>A</sub>β3 and 5-HT<sub>1A</sub> are consistent with the known interactions between GABAergic and serotonergic systems (Carlsson et al. 2001) and the central role of PKC in regulation of GABA<sub>A</sub> and 5-HT<sub>1A</sub> receptor activity in the brain, including receptor trafficking (Bhattacharyya et al. 2002; Brandon et al. 2000). Healthy individuals show an inverse relationship between 5-HT<sub>2A</sub> receptor availability and PKC activity in the PFC (Shelton et al. 2009) so one interpretation of our findings is that augmentation treatment ‘normalizes’ abnormal relations between these systems in illness (Carlsson et al. 2001). Reports that expression of PMC mRNA for the receptor for activated C-kinase 1 (Rack1), which is associated with PKC<sub>B2</sub> and PKC-mediated GABA<sub>A</sub> receptor phosphorylation (Feng et al. 2001; Song & Messing, 2005), is reduced in schizophrenia patients treated

Table 4. Correlations between gene products and clinical symptoms

<table>
<thead>
<tr>
<th>Substance</th>
<th>Treatment duration</th>
<th>Negative symptoms</th>
<th>Positive symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>Week 3</td>
</tr>
<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;β3</td>
<td>Week 1</td>
<td>0.077</td>
<td>0.284</td>
</tr>
<tr>
<td></td>
<td>Week 3</td>
<td>−0.027</td>
<td>−0.155</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>−0.154</td>
<td>−0.096</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;1A&lt;/sub&gt;</td>
<td>Week 1</td>
<td>0.169</td>
<td>0.211</td>
</tr>
<tr>
<td></td>
<td>Week 3</td>
<td>−0.200</td>
<td>−0.231</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>0.068</td>
<td>0.097</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;7&lt;/sub&gt;</td>
<td>Week 1</td>
<td>−0.046</td>
<td>0.189</td>
</tr>
<tr>
<td></td>
<td>Week 3</td>
<td>−0.196</td>
<td>−0.180</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>0.037</td>
<td>0.194</td>
</tr>
<tr>
<td>PKC&lt;sub&gt;B2&lt;/sub&gt;</td>
<td>Week 1</td>
<td>−0.573</td>
<td>−0.336</td>
</tr>
<tr>
<td></td>
<td>Week 3</td>
<td>−0.309</td>
<td>−0.351</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>−0.297</td>
<td>−0.369</td>
</tr>
<tr>
<td>BDNF</td>
<td>Week 1</td>
<td>−0.253</td>
<td>−0.039</td>
</tr>
<tr>
<td></td>
<td>Week 3</td>
<td>−0.566*</td>
<td>−0.270</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>−0.401</td>
<td>−0.363</td>
</tr>
<tr>
<td>Plasma BDNF</td>
<td>Week 1</td>
<td>0.152</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Week 3</td>
<td>−0.024</td>
<td>−0.266</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>−0.248</td>
<td>−0.282</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01.
with fluvoxamine augmentation (Chertkow et al. 2007) is consistent with this.

The significant correlations between mRNA expression of PKCβ2, GABA_Aβ3 and 5-HT_2A and clinical symptoms support the hypothesis that these gene expression changes are linked to the clinical state. Of particular note was the strong association between change in PKCβ2 mRNA after 1 wk of treatment and negative symptoms at endpoint as it raises the possibility that it may be an early predictor of response.

Increased expression of 5-HT_2A and 5-HT_7 receptors in augmentation treatment is consistent with evidence that serotonergic receptors may be markers of schizophrenia (Arranz et al. 2003) and treatment response (Meltzer et al. 2003). Recent studies reported decreased 5-HT_2A receptor levels in the PFC and increased 5-HT_2A receptor levels in the caudate nucleus of antipsychotic-naive patients with first-episode schizophrenia (Dean et al. 1999; Rasmussen et al. 2010). 5-HT_7 receptor was reported to be reduced in PFC of schizophrenia patients, and unaffected by antipsychotic treatments in rat PFC (East et al. 2002). Serotonergic abnormalities in peripheral tissues include overexpression of 5-HT receptor and transporter mRNA in leukocytes of antipsychotic-free schizophrenia patients (Wang et al. 2010) and increase in platelet 5-HT_2A receptors in drug-free schizophrenia patients, which declined to normal level after antipsychotic treatment (Govitrapong et al. 2000). Padin and colleagues reported parallel changes in 5-HT_2A receptor expression in PFC and platelets of rats after olanzapine treatment (Padin et al. 2006), suggesting that peripheral changes reflect drug-induced changes in the brain.

SSRIs can up-regulate 5-HT_2A expression in PFC of depressed patients (Moresco et al. 2000) but we found no previous reports of 5-HT receptor response to SSRI treatment in PMC of schizophrenia patients. 5-HT_2A receptor gene polymorphisms have been linked to clozapine responsiveness (Arranz et al. 1995).

The increase in plasma BDNF protein levels and BDNF mRNA expression in PMC after augmentation is consistent with reports that antidepressants can increase blood BDNF levels in depressed patients (Sen et al. 2008; Shimizu et al. 2003). Low blood BDNF levels have been reported in depressed (Piccinni et al. 2008) and antipsychotic-naive first-episode schizophrenia (Jindal et al. 2010) patients, so BDNF may be a treatment target in both conditions, perhaps correcting disturbed neuronal transmission and synaptic plasticity (Angelucci et al. 2005). The strong correlations between expression of BDNF mRNA, and serotonergic (5-HT_2A, 5-HT_7) and GABA_A receptors observed in our study, are consistent with the purported role of BDNF in supporting synaptic integrity and plasticity.

Fig. 3. Relationship between PKC mRNA level after 1 wk of augmentation treatment and clinical symptoms at (a) endpoint and (b) baseline. Vertical axis represents the levels of clinical symptoms at (a) before and (b) 6 wk after addition of fluvoxamine. Horizontal axis represents PKCβ2 mRNA relative expression levels 1 wk after addition of fluvoxamine. Red points stand for negative symptom (SANS total) scores (r = −0.573, p = 0.07 for baseline; r = −0.706, p < 0.01 at endpoint); green points stand for positive symptom (SAPS total) scores (r = −0.790, p < 0.01 for baseline; r = −0.664, p < 0.05 at endpoint).
There was no significant correlation between changes in expression of BDNF mRNA in PMC and levels of BDNF protein in plasma. This is consistent with evidence that most of the BDNF present in serum and plasma is released from BDNF stores (Karege et al. 2005; Piccinni et al. 2008). The main peripheral BDNF stores in humans are platelets and circulating BDNF levels in plasma are 100-fold lower than in serum (Yamamoto & Gurney, 1990). The relationship between treatment-related activation of BDNF gene expression and blood protein concentrations remains to be clarified. The possibility that changes late in the course of treatment (e.g. week 6) may indicate persistence of improvement is also worthy of testing.

The lack of differences in expression of the genes assayed in patients treated with typical and atypical antipsychotics indicates that that the synergistic effect of augmentation is independent of ongoing 5-HT2A antagonistic activity. This may explain the apparently paradoxical clinical findings, observed also here, that adding SSRI to atypical (5-HT2A antagonist) antipsychotics improves rather than antagonizes clinical response (Hiemke et al. 2002; Silver et al. 1996). It is consistent with laboratory findings that fluvoxamine combined with haloperidol have similar effects on GABA-A receptor and its modulating systems to clozapine (Danovich et al. 2010).

Limitations

The primary aim of this study was to identify biochemical accompaniments of augmentation treatment and no control group was included. Lack of placebo control does not allow us to exclude possible contribution of non-pharmacological factors to the clinical improvement. However, the clinical ratings were made blind to the PMC results, and the degree of negative symptom change was similar to that observed in controlled studies performed in a similar patient population (Silver et al. 2000; Silver & Shmugliakov, 1998) so it is unlikely that the clinical improvement in these severely impaired, treatment-resistant patients could be explained by placebo effect although replication with placebo control is desirable. Controlled studies are also needed to determine the extent to which the changes in the genes assayed were due to fluvoxamine alone or to its combination with antipsychotic. Evidence that combined antidepressant+antipsychotic treatment has a greater effect on BDNF levels than each treatment alone (Yoshimura et al. 2010) supports synergistic effects but comparisons with other add-on antidepressants are needed. These should include other SSRIs since some properties of fluvoxamine, e.g. α1 receptor agonist activity (Narita et al. 1996), are not shared by other members of the SSRI class.

As in other peripheral marker studies, the relationship of the PMC changes to those in the brain requires investigation. The question whether the PMC changes may also be informative as to potential side-effects also deserves exploration. The considerable concordance between our findings and reports of abnormalities in similar systems in post-mortem brains of schizophrenia patients (e.g. Arnsten, 2009; Arranz et al. 2003; Benes et al. 1996; Dean et al. 1999; Feng et al. 2001; Jindal et al. 2010; Rasmussen et al. 2010; Song & Messing, 2005) supports an association and our findings make clear predictions which can be tested using brain-imaging techniques (e.g. Yoon et al. 2010). Further research is also needed to investigate the relationships between changes in gene transcription, protein production and functional characteristics and to examine the potential of these PMC changes to serve as biomarkers of therapeutic response.

Conclusion

This study reports, for the first time, changes in expression of GABA-A receptor in PMC of schizophrenia patients in response to SSRI augmentation and in parallel, changes in serotoninergic (5-HT2A, 5-HT7) receptors and intracellular signalling and neurotrophic factor molecules. The changes in gene expression were interrelated, consistent with the known interactions between their parent systems and indicating that treatment produces systematic modulation in dysfunctional systems linked to core illness pathology (Carlsson et al. 2001; Coba et al. 2009; Lieberman et al. 2008). The changes were observed peripherally but the strong associations with clinical symptoms strengthen the possibility that they reflect clinically relevant mechanisms and may predict treatment response.

From a more general perspective, our findings identify potential novel mechanisms for antipsychotic action, highlighting the role of GABA-A receptor modulation and intracellular signalling. The considerable concordance between clinical and preclinical findings, while requiring caution in extrapolation between experimental domains, supports our view that the combined SSRI+antipsychotic paradigm is a useful investigative platform for study of psychotropic drug mechanisms (Silver et al. 2009).
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Statement of Interest

None.

References


