The effect of chronic co-administration of fluvoxamine and haloperidol compared to clozapine on the GABA system in the rat frontal cortex

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Abstract

Clinical studies in schizophrenia patients have shown that adding a selective serotonin reuptake inhibitor (SSRI) to antipsychotics can ameliorate negative symptoms that frequently resist standard treatments. It has been proposed that this combined treatment produces a ‘net effect’, different from that of the individual drugs and possibly common to that of the atypical antipsychotic, clozapine, which also ameliorates negative symptoms. The present study was initiated to determine the molecular events in the rat frontal cortex resulting from combined treatment of fluvoxamine and haloperidol compared to clozapine. Rats were allocated to five groups and received a daily intraperitoneal injection with one of the following: haloperidol (1 mg/kg), fluvoxamine (11 mg/kg), clozapine (11 mg/kg), haloperidol (1 mg/kg) plus fluvoxamine (11 mg/kg), or vehicle for 30 d. cDNA arrays were used to screen a broad range of genes in the frontal cortex. Several of the most prominent alterations were taken for analysis in real-time RT–PCR and their related proteins were examined by the Western-blotting technique. The gene expression profile of the combined fluvoxamine plus haloperidol treatment was different from that of the individual drugs. Moreover, clozapine showed some degree of homology with the dual treatment. The protein expression changes, specific to the combined treatment, included glutamic acid decarboxylase (GAD67) and protein kinase Cβ (PKCβ). The latter showed a similar trend following clozapine treatment. The present findings support the existence of a unique mechanism for SSRI–antipsychotic combination, different from that of the individual drugs and suggest that it may involve modification of the gamma aminobutyric acid (GABA) system.

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Introduction

Negative symptoms of schizophrenia such as apathy, loss of emotional response, and social withdrawal respond poorly to antipsychotic treatment. Recent studies reported that adding a selective serotonin reuptake inhibitor (SSRI) such as fluvoxamine (Silver et al., 2000; Silver and Nassar, 1992; Silver and Shmugliakov, 1998; Yasui-Furukori et al., 2004) or fluoxetine (Goff and Evins, 1998) to typical antipsychotics can improve negative symptoms in patients who did not respond to antipsychotics alone. The improvement included key negative symptoms primary to the illness (Silver et al., 2003a) and could be detected as early as 2 wk after treatment onset (Silver et al., 2003c). Since maprotiline, an antidepressant acting by a noradrenergic mechanism was not effective compared to fluvoxamine, amelioration of negative symptoms appears to be related to the serotonergic action rather than to a non-specific antidepressant effect of the drug (Silver and Shmugliakov, 1998). The mechanism underlying the therapeutic action of the combined treatment is unknown. Based on the
clinical observation that the outcome of the combined treatment is different from the individual drugs it is reasonable to expect a parallel effect at the molecular level. Indeed, a recent study has shown that combined administration of olanzapine and fluoxetine results in behaviour, endocrine and immediate-early gene expression changes unpredicted by monotherapy models (Horowitz et al., 2003). In light of the reported abnormalities in dopamine, serotonin, \gamma\text{-}aminobutyric acid (GABA) and glutamate systems in schizophrenia (Carlsson et al., 2001; Ichikawa and Meltzer, 1999; Silver et al., 2000; Wassef et al., 2003; Yan, 2002), we believe that alteration of the dynamic interaction between these systems might distinguish the ‘net effect’ of the combined treatment from the individual drugs.

In a search for molecular alterations that may be related to the clinical outcomes, we studied the effects of chronic co-administration of haloperidol with fluvoxamine in rat brain and compared it to the action of each drug alone and to the action of clozapine, considered as the ‘gold standard’ in respect to the activity against negative symptoms. The frontal cortex was chosen for this study since frontal lobe abnormalities have been linked to negative symptoms in schizophrenia patients and because it is postulated to be a central site for the action of antipsychotic drugs. To enable coverage of a broad range of signalling systems we used microarray as a screening tool and subsequently focused on genes of interest using real-time RT–PCR and Western blotting.

Methods

Animal protocol

All procedures were in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and were approved by the Technion Animal Ethics Committee, Haifa, Israel. Male Sprague–Dawley rats (Harlan, Jerusalem, Israel), weighing 150–250 g at the beginning of experiments, were housed for an acclimatization period of 1 wk before starting the experiment. Rats were randomly assigned to five treatment groups (six rats per group), which received daily intraperitoneal (i.p.) injections (between 10:00 and 13:00 hours) of haloperidol (Sigma, St. Louis, MO, USA, 1 mg/kg), fluvoxamine (Agis, Tel Aviv, Israel, 11 mg/kg), a solution combining both haloperidol and fluvoxamine (1 mg/kg and 11 mg/kg respectively), clozapine (Novartis Pharmaceuticals Inc., Basle, Switzerland, 11 mg/kg) and vehicle [sterile saline solution containing 2\% dimethylsulphoxide (DMSO) and 70 \text{mm} acetic acid, control group]. The doses of the drugs are in accordance with the literature (Adams and Moghaddam, 2001; Bai et al., 2003; Chong et al., 2002; Daniel et al., 1999; Gao et al., 1997; Silver and Youdim, 2000). The drugs were dissolved in a sterile saline solution containing 2\% DMSO and 70 \text{mm} acetic acid. In order to minimize tissue damage, the site of i.p. injection was varied between right and left side of the rat during the period of treatment. Mild and transient tissue injury was noted in some animals. During the treatment period animals were weighed once a week prior to drug administration. Baseline body weights were not significantly different except for the clozapine group which was significantly smaller [mean weight of 18–21 rats (\pm \text{s.e.m.}): vehicle 216 \pm 6 g, haloperidol 218 \pm 6 g, fluvoxamine 217 \pm 8 g, haloperidol plus fluvoxamine 215 \pm 9 g, clozapine 191 \pm 3 g (clozapine vs. vehicle, \(p < 0.01\)]. The percentage weight gain during the study did not differ significantly between groups (saline 44\%, haloperidol 44\%, fluvoxamine 45\%, haloperidol plus fluvoxamine 45\%, clozapine 52\%). On day 31, 24 h after the last injection, the rats were sacrificed by decapitation. The brains were rapidly removed and dissected for the frontal cortices (olfactory bulbs were removed and a coronal cut was done 2.5 mm from the forehead, for tissue section between Bregma 5.2 and Bregma 2.7). From each brain the right-side organ was inserted immediately into RNAlater\textsuperscript{\textregistered} (Ambion, Austin, TX, USA) solution and was kept at \(-20\) °C. The left side of the organ was frozen immediately for protein analysis.

Drug level determination

Initially we assayed blood samples from study animals (i.e. 24 h after the last dose). This resulted in undetectable levels in the blood. We then conducted an equivalent experiment but sacrificed animals 1.5 h after the last dose. These values are reported here. The levels of fluvoxamine and clozapine were analysed using HPLC with oxaprotiline or fluperlapine respectively as internal standards (Hartert et al., 1992; Weigmann et al., 2001). The level of haloperidol was determined using a liquid chromatography (LC) system coupled with mass spectrometry in the laboratory of Professor Zernig at Innsbruck, Germany.

Total RNA isolation

The frontal cortex tissues were transferred from RNAlater (\(-20\) °C) into cold TriReagent solution (Sigma) and homogenized in a glass Teflon homogenizer. The TriReagent suspensions were mixed thoroughly with chloroform and centrifuged (12 000 g, 20 min, 4 °C). After precipitation with sodium acetate...
radioactive signals were done using AtlasImageTM 2.0 scanner (Fuji Inc.). Quantification and analysis of the radioactive signals were detected with FLA-2000 MP-2040 image plate, Fuji Inc., Tokyo, Japan) and the membranes were exposed to phosphor screen (BAS Two independent experiments were performed. The ated from RNA pools and analysed on cDNA arrays. (Clontech, Palo Alto, CA, USA). Probes were gener- ated from 1176 genes, according to the manufacturer’s protocol (Clontech Rat 1.2 cDNA expression arrays, including 1176 genes, according to the manufacturer’s protocol (Clontech, Palo Alto, CA, USA). Probes were generated from RNA pools and analysed on cDNA arrays. Two independent experiments were performed. The membranes were exposed to phosphor screen (BAS MP-2040 image plate, Fuji Inc., Tokyo, Japan) and the radioactive signals were detected with FLA-2000 scanner (Fuji Inc.). Quantification and analysis of the radioactive signals were done using AtlasImageTM 2.01 software (Clontech). The analysis was done by taking the log, of the ratio for the expressed genes and calculating their mean and standard deviation. Global normalization was done based on the assumption that all of the genes in the array should have an average expression ratio equal to 1 and that the changes in expression of the individual genes balance out so that the total quantity of RNA hybridized from each sample is the same (Quackenbush, 2001). Changes with log2 ratio greater than 2 s.d. of the mean were considered as significant (Kontkanen et al., 2002; Nadon and Shoemaker, 2002).

**Real-time reverse transcriptase–polymerase chain reaction (RT–PCR)**

The RNA prepared for microarray analysis was used for the real-time RT–PCR analysis of genes of interest. Two micrograms of total RNA were denatured and reverse transcribed using random hexanucleotides (0.5 µg/µl). Secondary structures of the template and primer were opened by incubation for 5 min at 70 °C and immediately cooled on ice. A total of 9 µl of mixed reaction containing reaction buffer, dNTP (0.5 mm each), RNasin inhibitor (25 U) and MMLV reverse transcriptase (200 U) were added and samples were incubated at 39 °C for 1 h. For every RNA preparation, a negative control was run in parallel consisting of a direct amplification of the RNA sample, omitting the RT step. Real-time quantitative assessment was performed using LightCycler with FastStart DNA Master SYBR Green I ready-to-use PCR mix kits according to the manufacture’s protocol (Roche Diagnostics, Mannheim, 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 18S-rRNA and comparing to control values assessed the relative expression level of a given mRNA. Amplified products were visualized on 1.5% agarose gel. In addition, the specificity of the PCR product was verified by sequencing using the primers that were used for cDNA amplification.

**Protein determination and Western blotting**

Brain tissue lysates were prepared by tissues homogenization in Tris–sucrose buffer (pH 7.4) (containing a mixture of protease inhibitors; Roche Inc.) and centrifugation at 500 g for 10 min. The supernatants

<table>
<thead>
<tr>
<th>Unigene no.</th>
<th>mRNA</th>
<th>Oligonucleotide sequence (5’–3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X07287</td>
<td>PKCy</td>
<td>F-TTCTTCAAGCAGCCAACCTT T-RGTACGTGGCAFAACGGGAC</td>
</tr>
<tr>
<td>X15468</td>
<td>GABA-Aβ</td>
<td>F-CTTCTCCTCTCCAGAAGTTG</td>
</tr>
<tr>
<td>X04440</td>
<td>PKCβ</td>
<td>F-CTTCTCCTCTCCAGAAGTTG</td>
</tr>
<tr>
<td>M34446</td>
<td>GAD67</td>
<td>F-GTTGATCCACCCAGGAAGGA</td>
</tr>
<tr>
<td>AF003523</td>
<td>BAD</td>
<td>F-GCTTAGGCCCCTTTCGAGGAC</td>
</tr>
<tr>
<td>X01117</td>
<td>18S-rRNA</td>
<td>F-GTAACCGTGAACCCCTT</td>
</tr>
</tbody>
</table>

All templates are initially denatured for 10 min at 95 °C. Amplification is done for 35 cycles. In order to receive melting temperatures of the products (200 bp), melting-curve analysis is done by continues acquisition from 65 °C to 95 °C with temperature transition rate of 0.1 °C/s.
were assessed for the amount of protein using Bradford reagent (Sigma) at 598 nm. Samples containing 30 μg protein were loaded on a NuPAGE 10% gel (Invitrogen, Groningen, The Netherlands). At the end of the electrophoresis, the proteins were transferred to a nitrocellulose membrane using an Xcell SureLock® apparatus (Invitrogen). The membrane was blocked with 5% milk and immunoblotted with primary antibody and subsequently with a second, horseradish peroxidase-conjugated antibody [polyclonal anti-rabbit IgG conjugated with horseradish peroxidase (Cell Signaling Technology, Beverly, MA, USA) or anti-mouse IgG peroxidase conjugate (Sigma)]. Bands were revealed by enhanced chemiluminescence using the detection reagent, ECL (Amerham, Pharmacia, Little Chalfont, Buckinghamshire, UK). Quantification of results was accomplished by measuring the optical density of the labelled bands from the autoradiograms, using the computerized imaging program ‘Total-lab’ (BioSystematica, Tavistock, Devon, UK). The following antibodies were used: affinity-purified rat anti-rat cyclin D3, mouse anti-human Presenilin1, Goat anti-human GABA-A receptor subunit β3 (GABA-A Rβ3) and regulator of G protein signalling (RGS4; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), mouse anti-rat glutamate decarboxylase (GAD67), mouse anti-human protein kinase Cβ and Cγ (PKCβ and PKCγ; Transduction Laboratories, Lexington, KY, USA), rabbit anti-mouse bcl-2-associated death promoter (BAD, Cell Signaling Technology Inc.), monoclonal anti-β-actin (clone AC-15) (Sigma).

Statistical analysis

All results were analysed using one-way ANOVA followed by Student’s t test. A change was defined as being significant if the difference between the control and treated groups reached a level of significance of p < 0.05.

Results

Drug level determination

The drug level in the rat brain tissues are presented in Table 2 and are in agreement with the literature (Baldessarini et al., 1993; Bymaster et al., 2002; Igarashi et al., 1995; Zhang et al., 2000). The levels of haloperidol as well as of fluvoxamine are of the same order when given in separated or combined regimens.

Preliminary screening of the mRNA alterations using cDNA arrays

cDNA array was used to generate a list of candidate genes of interest for further study. This method, despite its well-known limitation in quantification of mRNA levels, was employed as a preliminary screening tool to detect plausible related genes without a narrowed a-priori hypothesis. The final list consisted of expressed genes that showed changes greater than 2 S.D. of the average ratio (Table 3). From the genes altered, eight were chosen for further corroboration by real-time PCR (Cyclin D3, PKCγ, PKCβ, BAD, Presenilin 1, GAD67, RGS4, GABA-A Rβ3) and for examination of the corresponding expression at the protein level.

mRNA expression analysis in rat frontal cortex

Quantification by real-time PCR is shown in Figure 1. BAD mRNA concentration was not affected by any of the drugs. Haloperidol and clozapine significantly increased the mRNA levels of GAD67 (~4-fold over control), PKCβ (6- to 14-fold over control), GABA-A Rβ3 (3-fold over control) and PKCγ (11- to 19-fold over control). PKCβ, GABA-A Rβ3 and PKCγ were increased significantly also by fluvoxamine (4.8-, 2.8-, and 8-fold over control, respectively). The combined treatment had no statistically significant effects on

Table 2. Drug concentration in rat brain following chronic treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drug concentration</th>
<th>Haloperidol (Hal)</th>
<th>Fluvoxamine (Flu)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Alone (99)</td>
<td>Combined with Flu (74)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>792 (99)</td>
<td>909 (74)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8155 (329)</td>
<td>9389 (888)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2606 (471)</td>
<td></td>
</tr>
</tbody>
</table>

The levels of fluvoxamine and clozapine were analysed by HPLC (Hartter et al., 1992; Weigmann et al., 2001) and of haloperidol by tandem mass spectrometry (Zernig et al., 2004). The values are given as average of six rats (±S.E.M.) and expressed as drug weight (ng) per wet weight (g) brain tissue. No significant differences were observed in drug concentration between single and combined treatments of haloperidol and fluvoxamine.
any of these genes, although a non-significant increase was noted.

**Western blotting analysis of rat frontal cortices**

The expression changes at the protein level were examined by immunoblot assay. GAD67 protein level was increased by haloperidol treatment (1.6-fold), reduced by the combined treatment (0.5-fold) and unchanged after fluvoxamine or clozapine (Figure 2a). PKCβ protein was increased by haloperidol (2.6-fold) and by fluvoxamine (1.9-fold) treatments, but reduced by the combination (0.75-fold) (Figure 2c). GABA-A Rβ3 protein level was increased by the haloperidol (1.6-fold) and clozapine treatments (1.9-fold) (Figure 2b) and not affected by fluvoxamine alone or in combination with haloperidol. The levels of Cyclin D3, Presenilin 1, RGS4, PKCγ and BAD proteins were not

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**Table 3. Gene expression alterations in the rat frontal cortex**

<table>
<thead>
<tr>
<th>Genbank</th>
<th>Gene Description</th>
<th>Flu*</th>
<th>Hal*</th>
<th>Cloz*</th>
<th>Flu+Hal**</th>
</tr>
</thead>
<tbody>
<tr>
<td>M57276</td>
<td>Leukocyte surface antigen CD53 (Ox-44)</td>
<td>Down</td>
<td>Down</td>
<td>Down</td>
<td>Down</td>
</tr>
<tr>
<td>D83349</td>
<td>Short type PB-cadherin</td>
<td>n.c.</td>
<td>n.c.</td>
<td>Up</td>
<td>Up</td>
</tr>
<tr>
<td>D16309</td>
<td>G1/S-specific cyclin D3 (CCND3)</td>
<td>n.c.</td>
<td>n.c.</td>
<td>Up</td>
<td>Up</td>
</tr>
<tr>
<td>L33413</td>
<td>Receptor for advanced glycosylation end products (RAGE)</td>
<td>Down</td>
<td>n.c.</td>
<td>Up</td>
<td>Up</td>
</tr>
</tbody>
</table>

**Cell adhesion proteins**

- M60525 VGF8A protein precursor
- S49400 Protein tyrosine phosphatase, striatum enriched
- X07287 Protein kinase C gamma type (PKCγ)
- M19007 Protein kinase C beta type (PKCβ); I+II
- X04440 PKCb protein was increased by haloperidol (2.6-fold) and by fluvoxamine (1.9-fold) treatments, but reduced by the combination (0.75-fold) (Figure 2c).

**Cell cycle, growth and apoptosis**

- M60525 VGF8A protein precursor
- AF00352 cbl-2-associated death promoter (BAD)
- U97143 RET ligand 2 (RET 2)

**G-protein and their effectors**

- U34958 Transducin β1 subunit
- S49400 Protein tyrosine phosphatase, striatum enriched
- M16309 G1/S-specific cyclin D3 (CCND3)

**Neurotransmitter systems**

- X15468 GABA receptor β3
- M34445 67-kDa glutamic acid decarboxylase (GAD67)
- J05231 Neuronal acetylcholine receptor protein α5 subunit precursor (ACRA5)

**Growth factor**

- D49846 Growth factor receptor-bound protein 2 (GRB2)

**Transcription factors**

- L29259 Elongation factor SIII p15 subunit

**Voltage-gated channels**

- L39018 Sodium channel protein 6 (SCP6)
- X62841 Voltage-gated potassium channel protein 3.4
- U37026 Sodium channel SCNB2, β2 subunit, brain

**Others**

- U39207 Cytochrome P-450
- J05107 Corticosteroid 11-β-dehydrogenase isozyme 1
- D82363 Presenilin 1 (PSNL1); S182 protein

**Effect of SSRIs on the GABA system**

Male Sprague–Dawley rats were treated (i.p. injection, 30 d) with haloperidol (Hal, 1.1 mg/kg), fluvoxamine (Flu, 11 mg/kg), a combination of drugs (1 mg/kg and 11 mg/kg respectively), clozapine (Cloz, 11 mg/kg) or vehicle. Hybridization array analysis was performed using Atlas Rat 1.2 cDNA expression arrays, according to the manufacturer’s protocol (Clontech). Genes that were affected by the combined treatment in the same direction (up- or down-regulation), in two independent experiments were chosen as a reference point and compared to the results of the other treatments.

* 6 rats; ** 12 rats, n.c., not changed; Up, ratio > 1.5; Down, ratio < 0.6.
changed with any of the drug treatments (data not shown).

Discussion

The most notable results of the current study are the distinctive protein alterations related to GABA system regulation (GABA-A Rβ3, GAD67 and PKCβ) following the combined treatment of haloperidol and fluvoxamine. This outcome is not a result of a pharmacokinetic effect on drug metabolism since their concentrations in the brain were similar in the individual and combined treatments.

The changes observed at the protein level had limited concordance with those at the mRNA level (haloperidol induction of GAD67, PKCβ, GABA-A Rβ3; no effect of fluvoxamine on GAD67 and increase in PKCβ; increase of GABA-A Rβ3 by clozapine and no effect on BAD by any of the treatments). Such disparity has been reported previously (Freeman et al., 2001) and might reflect post-translational processing of the protein. In addition, mRNA changes present in the brain region assayed may be manifested at the protein level only in its projections.

Our findings can be linked to current models of GABA regulation: PKCβII regulates GABA-A receptor function by direct phosphorylation of its β subunits β1, β3 (Brandon et al., 2000; Poisbeau et al., 1999; Sands et al., 1998; Wang et al., 2002). Treatment with haloperidol modulates PKC via D2 dopaminergic receptors (Silva et al., 1995; Yurko-Mauro and Friedman, 1995) while treatment with fluvoxamine augmentation or clozapine, may regulate PKC via serotonergic receptors (Ago et al., 2005; Feng et al., 2001; Yan, 2002), thereby suggesting a potential common pathway for the effect to these drugs. GAD67, another element discussed here, controls the levels of GABA and has been proposed as the key control point linking dopaminergic, glutaminergic (Kalkman and Loetscher, 2003) and serotonergic (Di Cara et al., 2003) systems.

The results of the present study indicate that in the rodent frontal cortex haloperidol may alter GABAergic tone by increasing GAD67 and PKC protein levels. Adding fluvoxamine may oppose the effect of haloperidol.

There are obvious limitations in extrapolating laboratory findings; however, there is heuristic value in considering possible clinical implications of our study. The alteration in GABAergic elements as seen from fluvoxamine plus haloperidol and its similarity to that following clozapine are of interest in light of evidence for cortical abnormalities of the GABA system in schizophrenia (Blum and Mann, 2002; Guidotti et al., 2000; Woo et al., 1998).

The increase in PKCβ mRNA and protein levels following the individual fluvoxamine or haloperidol treatments was a novel finding and is complementary to earlier report of elevated PKCβ levels in post-mortem brains of schizophrenia patients on antipsychotic drugs (Hakak et al., 2001), indicating that PKCβ changes are related to the treatment. PKCβ protein

Figure 1. Real-time PCR analysis of gene expression for selected mRNAs. Male Sprague–Dawley rats were treated (i.p. injection, 30 d) with haloperidol (1.1 mg/kg), fluvoxamine (11 mg/kg), a combination of both drugs (1 mg/kg and 11 mg/kg respectively), Clozapine (11 mg/kg) or vehicle. Total RNA isolated from rat frontal cortices was reverse transcribed. cDNA was amplified in real-time PCR using suitable primers for PKCβ, PKCγ, GABA-A Rβ3 GAD67 and BAD (as listed in Table 1). The relative expression level of a given mRNA was assessed by normalizing to the housekeeping gene 18S-rRNA. Each point represents an average of two pools, three rats in each pool. Control values were arbitrarily set as 100%. Data (mean ± S.E.M.) is expressed as percent of control. Student’s t test * p < 0.05; ** p < 0.01; drug group compared with control group.
levels were significantly reduced following the combined medications of haloperidol and fluvoxamine and a similar trend was noted with clozapine. This similarity is consistent with the hypothesis suggesting these treatments share a common final pathway. PKC signalling pathway abnormalities have been related to...
impaired learning and working memory in various animal models (Nogues, 1997). Cognitive impairments and working memory deficit, in particular, are core symptoms of schizophrenia, and are associated with negative symptoms (Silver et al., 2003b; Strauss, 1993). This suggests that PKC alterations may be involved in the psychopathology of cognitive and negative symptoms. Indeed, reduced PKC density and transduction have been reported in schizophrenia (Battaini, 2001; Dean et al., 1997; Pongrac et al., 2002; Wang et al., 1999).

The GAD67 protein differences found between haloperidol and clozapine may be a discriminate biomarker between ‘typical’ and ‘atypical’ antipsychotics. The finding that adding fluvoxamine to haloperidol reduced levels of GAD67 suggests a possible relevance of GAD67 protein alteration to negative symptoms improvement. Since fluvoxamine alone did not affect GAD67, the effect on the GABA system may be indirect. Reduced GAD67 levels have been reported in post-mortem brains of schizophrenia patients (Blum and Mann, 2002; Guidotti et al., 2000; Kalkman and Loetscher, 2003; Vawter et al., 2001; Volk et al., 2000) and our findings suggest this may be a result of drug effects.

The increase in the level of the β3 subunit of GABA-A receptor by haloperidol and by clozapine is in agreement with results of Sands et al. (1998) and Zink et al. (2004) respectively. Increased GABA-A receptor-binding sites have been reported in chronic schizophrenia patients (Guidotti et al., 2000; Hakak et al., 2001) preferentially in the cortex (Benes et al., 1996; Blum and Mann, 2002), a finding usually interpreted as a compensatory up-regulation of post-synaptic receptor sites due to loss of GABAergic neurons (Deutsch et al., 2001). Taken together with the results of a previous study (Zink et al., 2004), our findings indicate that these changes may be secondary to drug treatment.

The significance of the finding that the GABA-A subunit was up-regulated following clozapine, but not by the combined haloperidol plus fluvoxamine treatment, is not clear. One possibility is that receptors other than GABA-A (Qin et al., 1994; Sands et al., 1998) may be involved in negative symptoms production. In this light, it is of interest that in clinical studies, adding d-cycloserine to clozapine reportedly worsened negative symptoms (Goff et al., 1999).

It should be noted that while our discussion focused on potential mechanisms underlying treatment response, the possibility that some of the observed changes relate to treatment side-effects (reviewed in Silver, 2003) should also be considered.

In summary, our study indicates that the effects of the combined fluvoxamine plus haloperidol treatment given chronically differ from those of the individual drugs, particularly on the expression of GABA-A receptor modulators. This raises the possibility that alterations in GABAergic components may be involved in the mechanisms underlying the therapeutic effect on negative symptoms in schizophrenia and indicates the need for further study.

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Statement of Interest

None.

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