*Paper I*

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ORGINAL ARTICLE

# Antipsychotic drugs activate SREBP-regulated expression of lipid biosynthetic genes in cultured human glioma cells: a novel mechanism of action?

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**ABSTRACT**
Several studies have reported on structural abnormalities, decreased myelination and oligodendrocyte dysfunction in post-mortem brains from schizophrenic patients. Glia-derived cholesterol is essential for both myelination and synaptogenesis in the CNS. Lipogenesis and myelin synthesis are thus interesting etiological candidate targets in schizophrenia. Using a microarray approach, we here demonstrate that the antipsychotic drugs clozapine and haloperidol upregulate several genes involved in cholesterol and fatty acid biosynthesis in cultured human glioma cells, including HMGCR (3-hydroxy-3-methylglutaryl-coenzyme A reductase), HMGCS1 (3-hydroxy-3-methylglutaryl-coenzyme A synthase-1), FASN (fatty acid synthase) and SCD (stearoyl-CoA desaturase). The changes in gene expression were followed by enhanced HMGCR-enzyme activity and elevated cellular levels of cholesterol and triglycerides. The upregulated genes are all known to be controlled by the sterol regulatory element-binding protein (SREBP) transcription factors. We show that clozapine and haloperidol both activate the SREBP system. The antipsychotic-induced SREBP-mediated increase in glial cell lipogenesis could represent a novel mechanism of action, and may also be relevant for the metabolic side effects of antipsychotics.
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**Keywords: schizophrenia; antipsychotics; lipogenesis; mechanism of action**

## INTRODUCTION

Schizophrenia is a severe psychiatric disorder with a lifetime risk of about 1% in most populations (for a review, see Freedman[1] and Mueser McGurk.[2] Heritability has been demonstrated to play an important role in the etiology, but numerous genetic linkage and association studies have yielded conflicting results, although there are now several promising candidate susceptibility genes.[3] Brain imaging studies of schizophrenic patients have shown various structural and functional abnormalities, including enlargement of the ventricles and volume reductions in specific brain regions,[4,5] even in first episode and drug-naïve subjects.[6–8] On the other hand, degenerative histopathological findings are strikingly absent. These observations hint at a neurodevelopmental origin of the disorder (for a review, see Harrison and Lewis[9] and Liddle and Pantelis[10]).

Interestingly, a number of recent genome-wide expression studies have demonstrated that several genes involved in myelination are downregulated in post-mortem brain tissue from schizophrenic patients.[11–13] These findings are supported by the demonstration that the level of major myelin membrane components, such as sphingomyelin and galactocerebrosides, were reduced in post-mortem schizophrenic brains.[14] In the CNS, myelin is synthesized by oligodendrocytes. The myelin coating of axons is a prerequisite for rapid impulse conduction and maintenance of axonal function. The production of myelin depends upon synthesis of several myelin-specific proteins and lipids. Unesterified cholesterol is a major component of myelin, and although the human brain only accounts for 2% of the body mass, it contains about 23% of the total body pool of cholesterol, produced by *de novo* synthesis (for a review, see Dietschy and Turley[15]). Transcriptional activation of cholesterol biosynthetic genes is thus imperative for the myelination process during development.[16,17] Glial cells serve an important role in the supply of adequate amounts of cholesterol. In addition to its close link to the myelination process, glia-derived cholesterol has recently been demonstrated as essential for synaptogenesis,[18–20] acting as a glial growth factor. These data point at lipogenesis and myelin synthesis as interesting etiological candidate targets in schizophrenia, especially in light of the proposed neurodevelopmental disturbances in schizophrenia.[21]

For many years, the dopamine hypothesis has strongly influenced the pathophysiological theories of schizophrenia, based on the dopamine receptor-blocking effects of the first-generation ('typical') antipsychotic drugs. The reintroduction of the atypical drug clozapine, followed by several second-generation antipsychotics (eg risperidone, olanzapine and ziprasidone) and partial dopamine agonists (eg aripiprazole) has been paralleled by many new hypotheses on the mechanisms of action of antipsychotic drugs (for a review, see Miyamoto[22]). All antipsychotics appear to act, at least in part, through inhibition of dopamine D2-like receptors in the mesolimbic frontal brain regions. Interestingly, clozapine seems to have enhanced therapeutic efficacy as compared to typical drugs (eg haloperidol and chlorpromazine) in treatment-refractory schizophrenia,[23] but due to its potentially lethal agranulocytosis-inducing side effect, it is usually not a first-line drug. In addition, clozapine as well as several other antipsychotics are prone to cause other serious adverse effects, of which weight gain and the metabolic syndrome are major clinical challenges (for review, see Allison *et al*,[24] Nasrallah *et al*,[25] Casey *et al*,[26] Melkersson *et al*[27]). The mechanisms behind the superior therapeutic efficacy of clozapine and the drug-related metabolic side-effects remain to be clarified.

In the present study, we have used microarray technology to examine changes in the global gene expression profile in cultured human glioma cells, exposed to haloperidol and clozapine. We identified a cluster of upregulated genes involved in cholesterol and fatty acid biosynthesis, all activated by the sterol regulatory element-binding protein (SREBP) transcription factors. An SREBP-mediated increase in cellular lipogenesis could represent a novel additional mechanism on the therapeutic efficacy and metabolic side effects of antipsychotic drugs.

## RESULTS AND DISCUSSION

To search for potentially novel effects of both typical and atypical antipsychotic drugs, we exposed human cultured glioma cells (GaMg) to haloperidol (10 μM), clozapine (30 μM) or vehicle (lactate, 6 mg/ml) for 24 h, followed by microarray-based global analysis of differential gene expression. The chosen concentrations of the drugs induced comparable responses on gene regulation without any significant cell death (determined with the WST-1 cell proliferation reagent, Roche Diagnostics; data not shown). Among the regulated genes on the microarrays, we identified a cluster of genes ($n = 14$) encoding lipid biosynthetic enzymes that were upregulated by at least one of the drugs, with ratios in the range of 1.7–4.1 (Table 1). The majority of the genes ($n = 10$) were involved in the synthesis of cholesterol, including the rate-limiting enzymes 3-hydroxy-3-methylglutaryl-coenzyme A reductase HMGCR and 3-hydroxy-3-methylglutaryl-coenzyme A synthase-1 (HMGCS1), and also several other steps further downstream in this pathway. In the fatty acid synthesis, the genes encoding the crucial enzymes stearoyl-CoA desaturase (SCD) and fatty acid synthase FASN were among those upregulated. The haloperidol and clozapine-induced upregulation of the cluster of cholesterol and fatty acid biosynthetic genes in the human GaMg cells was verified in replicate biological experiments, using both 15k human NMC cDNA slides (www.mikromatrise.no) and human Applied Biosystems 1700 microarrays version 1.0 for the gene expression analysis (data not shown). In addition, we demonstrated a similar haloperidol and clozapine-induced upregulation of lipogenesis-related genes at 6 and 24 h of drug exposure in a rat glioma cell line (BT4C) (data not shown). These results indicated a broad antipsychotic-induced activation of lipogenesis in glial cells.

To verify and extend these findings, we used real-time PCR to examine the time course of expression of HMGCR and FASN in haloperidol and clozapine-exposed cultured GaMg cells. An enhanced HMGCR expression was clearly evident after 3 h, with a statistically significant increase with time (up to 24 h) for both haloperidol ($P = 0.02$) and clozapine ($P = 0.0003$), as compared to the vehicle-treated control (Figure 1). FASN expression increased significantly with time for clozapine ($P = 0.04$) within the same time frame of incubation, whereas haloperidol failed to reach statistical significance on this aspect ($P = 0.2$) (Figure 1). At 10 μM, clozapine still significantly increased the expression of both HMGCR and FASN at 24 h of drug exposure, whereas both haloperidol and clozapine at 1 μM failed to significantly induce the expression of these two genes (data not shown).

To explore the functional consequences of the changes in the gene expression, we determined the HMGCR-enzyme activity and cellular lipid levels (data not displayed). The increased HMGCR gene expression was paralleled by an

**Table 1 Genes encoding enzymes involved in lipid biosynthesis that were upregulated by haloperidol (10 μM) and clozapine (30 μM) exposure of human glioblastoma cells (GaMg)**

| *Gene/enzyme name* | *Gene symbol* | *RefSeq accession number* | *Fold change in gene expression level*[a] | |
|---|---|---|---|---|
| | | | *Clozapine* | *Haloperidol* |
| *Cholesterol biosynthesis* | | | | |
| 3-hydroxy-3-methylglutaryl-coenzyme A reductase | HMGCR | NM_000859 | 1.8 | 2.4 |
| 3-hydroxy-3-methylglutaryl-coenzyme A synthase-1 | HMGCS1 | NM_002130 | 2.2 | 2.2 |
| Diphosphomevalonate decarboxylase | MVD | NM_002461 | ND | 4.1 |
| Mevalonate Kinase | MVK | NM_000431 | 2.2 | 3.4 |
| Farnesyl pyrophosphate synthetase | FDPS | NM_002004 | 1.8 | 3.3 |
| Isopentenyl-diphosphate delta isomerase | IDI1 | NM_004508 | 2.1 | 2.2 |
| Lanosterol synthase | LSS | NM_002340 | 2.8 | 2.5 |
| Lanosterol 14-alpha demethylase | CYP51 | NM_000786 | 2.7 | ND |
| Farnesyl-diphosphate farnesyltransferase-1 | FDFT1 | NM_004462 | ND | 2.0 |
| Squalene epoxidase | SQLE | NM_003129 | ND | 1.8 |
| *Fatty acid biosynthesis* | | | | |
| Stearoyl-CoA desaturase (delta-9-desaturase) | SCD | NM_005063 | 2.5 | ND |
| Fatty acid desaturase 1 | FADS1 | NM_013402 | 1.7 | 3.2 |
| Fatty acid desaturase 2 | FADS2 | NM_004265 | 2.3 | ND |
| FASN | FASN | NM_004104 | 3.1 | 2.2 |

[a]The fold change in gene expression level was determined as the ratio between drug- and vehicle-exposed cells. Positive values correspond to fold increase (upregulation). The data are means of the ratios obtained in two experiments. ND: not determined due to ratios below 1.5 and/or exclusion of data in the filtering process.

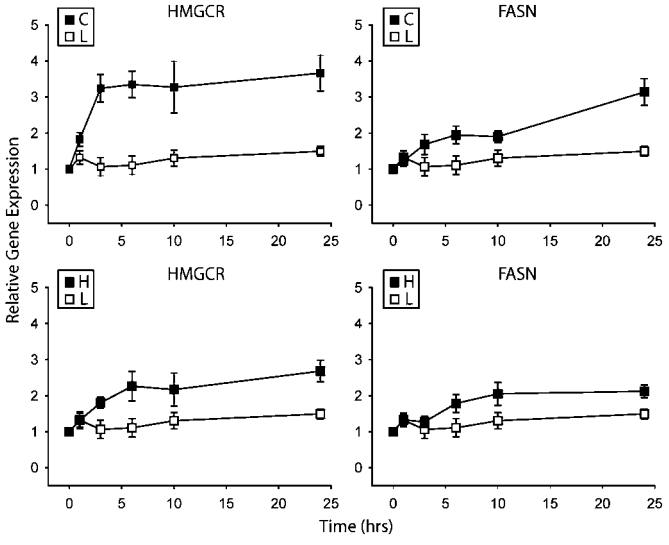


**Figure 1 Clozapine-and haloperidol-induced expression of HMGCR and FAS in GaMg cells. Real-time PCR-based gene-expression analyses were run after exposure with vehicle (L, lactic acid, 6 μg/ml), haloperidol (H; 10 μM) or clozapine (C; 30 μM) for 1, 3, 6, 10 or 24 h. Data are presented relative to the expression of the gene P0. Error bars show ± SEM, and the data are representative of three independent parallel experiments. The levels of statistical significance are given in the text.**

increase in HMGCR enzyme activity for both haloperidol (10 μM) and clozapine (30 μM), reaching 232 ± 18% ($P = 0.02$) and 307 ± 39% ($P = 0.02$) at 24 h, respectively (mean ± SD, $n = 2$). In addition, the cellular content of cholesterol was increased to 116 ± 17% (nonsignificant; $P = 0.2$) by haloperidol (10 μM) and to 168 ± 29% ($P = 0.02$) by clozapine (30 μM) after 24 h of drug exposure, as compared to the vehicle-treated cells (100%; 118 ± 11 nmol/mg protein) (mean ± SD, $n = 3$). The corresponding triglyceride levels were elevated to 175 ± 30% ($P = 0.01$) and 155 ± 18% ($P = 0.01$) by haloperidol and clozapine, respectively, as compared to the control (100%; 49 ± 3 nmol/mg protein) (mean ± SD, $n = 3$).

The cluster of upregulated lipid biosynthesis-related genes (Table 1) are all known to be under transcriptional control of the sterol regulatory element-binding proteins SREBP-1 and SREBP-2, which are major activators and regulators of the fatty acid and cholesterol biosynthesis (reviewed in Shimano,[30] Horton *et al*[31], and Rawson.[32] The SREBP-1 (with two isoforms, SREBP-1a and SREBP-1c) and SREBP-2 transcription factors are both present as 120 kDa inactive precursors in the endoplasmic reticulum (ER) membrane. Upon activation, the SREBP protein is translocated to the Golgi, escorted by the SREBP-cleavage-activation protein (SCAP), followed by a specific two-step proteolysis to produce a mature transcriptionally active 60–70 kDa fragment.

Since our global gene expression data strongly suggested an SREBP-mediated activation, we decided to study the cellular level of active and inactive SREBP proteins. Western blot analysis demonstrated that antipsychotic drug exposure of the cultured GaMg cells for 3, 6, 16 or 24 h induced a time-dependent proteolytic cleavage of SREBP. A marked increase in the ratio between the 60 and 70 kDa mature

transcriptionally active fragment and the 120 kDa membrane-bound precursor was observed for SREBP-2 and to a lesser extent for SREBP-1 (Figure 2a). The SREBP-1a, which is the most predominant SREBP-1 isoform in cultured cells[33] and SREBP-2 gene expression was only moderately affected by the antipsychotic drugs. SREBP-1a expression had an initial peak in the clozapine-exposed cells, whereas the SREBP-2 transcript level was slightly elevated at 12 and 24 h of drug incubation for both clozapine ($P = 0.01$) and haloperidol ($P = 0.09$) (two-way ANOVA, main effect) (Figure 2b). These results are in line with the initial microarray-based gene expression screening experiments of the human GaMg cells, in which SREBP-2, but not SREBP-1, was upregulated at 24 h of antipsychotic drug-exposure (data not shown). The increased expression of the cluster of lipid biosynthesis-related genes is therefore most likely a result of a direct antipsychotic-mediated proteolytic activation of the SREBP transcription factors, without a preceding elevation of the SREBP gene expression. The reason for the preferential stimulation of SREBP-2 is not known, but it should be noted that in the mouse peripheral nervous system, there seems to be a correlation between myelination and activation of SREBP-2 (but not SREBP-1).[34]

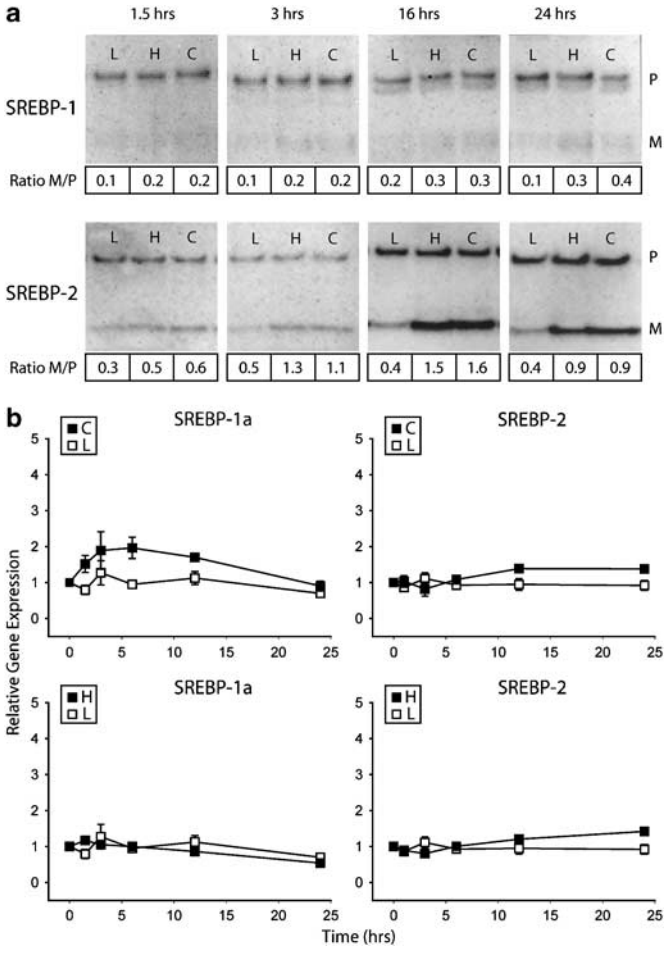


**Figure 2 The effect of clozapine-and haloperidol on SREBP-1 and SREBP-2 activation and gene expression in GaMg cells. The cells were exposed to vehicle (L, lactic acid, 6 μg/ml), haloperidol (H; 10 μM) or clozapine (C; 30 μM) for 1.5, 3, 16 or 24 h (a) SREBP-1 and SREBP-2 precursor (P; 120 kDa) and mature active fragment (M; 60–70 kDa) detected by Western blotting. The semiquantitative M/P ratios are indicated below the columns. The data are representative of two replicate experiments. (b) Clozapine- and haloperidol-induced expression of SREBP-1a and SREBP-2 after exposure for 1, 3, 6, 12 or 24 h in GaMg cells. Real-time PCR-based gene expression analyses were run with SYBR-green as flourescent detector. Error bars show ± SEM, and the data are representative of three independent parallel experiments. The levels of statistical significance are given in the text.**

At present, we are unable to fully explain the mechanism for the antipsychotic-induced SREBP activation. Clozapine and haloperidol are both cationic amphiphiles. Such compounds have previously been shown to promote synthesis and accumulation of total cellular cholesterol by reducing the cholesterol level in the ER, which is the cholesterol-sensing compartment in the cell.[35,36] In a more recent study, Adams *et al*[37] demonstrated that several cationic amphiphiles in high doses (100 μM) apparently mimicked the effect of cholesterol on the conformational change of the SCAP, as assayed *in vitro*. It has also been shown that these compounds were able to inhibit competitively the binding of cholesterol to SCAP.[38] These reports suggest that such amphiphiles could induce SCAP to bind to Insig, thereby inhibiting the SREBP activation. The apparent discrepancy between their data and our present demonstration of an antipsychotic-induced stimulation of SREBP in cultured glioma cells may be due to the differences in experimental conditions.

It has recently been shown that haloperidol and clozapine treatment alters the expression of some lipid-metabolism-related genes in the mouse frontal cortex and striatum, although neither of these genes were among the cluster of regulated genes identified by us.[39] The SREBP-mediated stimulation of the cholesterol and fatty acid biosynthesis in glial cells may represent a novel and clinically relevant action of antipsychotic drugs, due to the important role of cholesterol in myelination and synaptogenesis.[15,18,19] There is accumulating evidence for white matter changes in the brain of schizophrenic patients (reviewed in Davis *et al*[21]), and a meta-analysis revealed a small decrease in the whole brain white matter volume.[5] Regional white matter changes in the prefrontal cortex seem to be associated with the presence of negative symptoms in the patients.[40,41] It is also noteworthy that a large subset of patients with the rare late-onset form of metachromatic leukodystrophy, a severe demyelinating CNS disorder, display psychotic symptoms.[42,43] Interestingly, the atypical antipsychotic clozapine seems to have enhanced therapeutic efficacy as compared to typical drugs (eg haloperidol and chlorpromazine) in treatment-refractory schizophrenia.[23] The concentrations of haloperidol (10 μM) and clozapine (30 μM) that gave comparable SREBP activation in the glioma cell cultures were approximately 400 and 15-fold higher than the upper therapeutically relevant serum concentrations of these drugs. The upregulation of the lipid biosynthesis-related

genes was also significant at 10 μM of clozapine, which is five-fold above the therapeutically relevant level. Although great care should be undertaken when extrapolating results from cell culture to serum and CNS conditions, it seems possible that clozapine, but not haloperidol, may induce these effects *in vivo*, as a far more potent activator of SREBP and lipogenesis in the clinical situation. Whether such effects could be relevant for the superior therapeutic efficacy of clozapine need further clarification.

Although speculative, our results may also shed new light on the mechanisms of antipsychotic-induced weight gain, dyslipidemia and diabetes type II in schizophrenic patients. These serious metabolic side effects are particularly prevalent during treatment with several atypical drugs, including clozapine (reviewed in Allison *et al*,[24] Casey *et al*[26] and Melkensson and Dahl[27]). Further studies should therefore explore the effects of antipsychotic drugs on SREBP-stimulated lipogenesis in liver- and fat cells, with the aim of further understanding the biological processes that underlie drug-induced weight gain and the metabolic syndrome.

## MATERIALS AND METHODS

### Cell Culturing and Drug Exposure

A human glioma cell line (GaMg)[28] was cultured in monolayer in EasYFlasks or six-well plates (NUNC), using Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and PenStrep (100 U/ml final concentration). The cells were exposed to clozapine (30 μM), haloperidol (10 μM) or vehicle (lactic acid, 6 μg/ml) for up to 24 h, followed by trypsination and subsequent RNA extraction (see below). Haloperidol and clozapine were from Janssen-Cilag (Beerse, Belgium) and Sigma-Aldrich (St Louis, MO, USA), respectively.

### RNA Extraction and Microarray Analysis

Total RNA from vehicle- and drug-exposed GaMg cells was extracted by the GenElute™ RNA extraction kit (Sigma-Aldrich), quality-controlled and quantitated on a Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA, USA), and stored at −80°C until use. RNA was converted to fluorescense-labeled cDNA with the FairPlay™ aminoallyl kit, using 10 μg of total RNA per sample and Cy5 or Cy3 as flourescent dyes (Amersham International, UK), according to the manufacturer's recommendations.

To screen for drug-induced differentially expressed genes, two parallel competative hybridizations of the drug-exposed samples ('test') against the corresponding vehicle-treated samples ('control') were performed in a 'forward' (Cy5 in 'test' sample and Cy3 in 'control') and 'reverse' (Cy3 in 'test' sample and Cy5 in 'control') dye-swap procedure. All hybridizations were carried out on 35k human oligonucleotide-based microarrays provided by the Norwegian Microarray Consortium (NMC, www.mikromatrise.no). The microarrays are spotted on Corning UltraGAPS glass slides, using the Operon Human Genome Oligo Set Version 3.0, with 34.580 70-mer probes representing 24.650 genes and 37.123 gene transcripts (Operon Biotechnologies, Huntsville, AL, USA). Automated hybridization was performed for 12 h at 45°C with the ChipMap80 kit (including the ChipHybe80 buffer) on the Ventana Discovery system (Ventana Medical Systems, Tucson, AZ, USA). Scanning of the slides was performed on a GenePix 4000B fluorescence scanner (Axon Instruments, Foster City, CA, USA) with pixel size of 10 μm, followed by image and data analysis, using GenePix Pro (Axon Instruments) and J-Express (MolMine, Bergen, Norway; www.molmine.com) software. The image raw data were filtered based on signal intensity, excluding spots in which more than 60% of the pixels were below a threshold value given as the median + 2SD of the local background intensity. The remaining spots were subjected to global lowess normalization. Differentially expressed genes were defined as those with ratios above 1.5 (ie upregulated) or below 0.67 (ie downregulated).

### Real-Time PCR Analysis

Total RNA was extracted, controlled and stored as described above. From each of the samples, 100 ng RNA was reverse transcribed to cDNA using TaqMan RT reagents (Applied Biosystems, Foster City, CA, USA). The final concentrations of the reagents were as follows: 1 × TaqMan RT buffer, 5.5 mM $MgCl_2$, 2 mM dNTP mixture, 2.5 μM random hexamers, 0.4 U/μl RNase inhibitor and 1.25 U/μl Multiscribe reverse transcriptase in RNase-free water to a total volume of 50 μl. The reaction mix was incubated at 25°C for 10 min (primer annealing), 48°C for 30 min (synthesis) and 95°C for 5 min (enzyme inactivation). The resulting cDNA samples were stored at −20°C.

All subsequent real-time PCR experiments were performed on an ABI Prism 7900$^{HT}$ sequence detector system using 96-well plates. The PCR reaction solution for 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) and fatty acid synthase (FASN) contained 1.25 μl 20 × TaqMan® Gene Expression Assays (Applied Biosystems) and 12.5 μl 2 × TaqMan Universal PCR Master Mix. The SREBP-1a and SREBP-2 PCR reaction solution contained 12.5 μl 2 × SYBR green (Medprobe), together with forward and reverse primers (Sigma-Aldrich) in a final concentration of 300 nM. All PCR reactions contained 5 μl of cDNA reaction mix and RNase-free water to a total volume of 25 μl. The real-time PCR was run as follows: 50°C for 2 min (UNG incubation) and 95°C for 10 min (AmpliTaq Gold activation), followed by 40 cycles of 95°C for 10 s and 60°C for 1 min. For each sample, the gene expression was quantified by the standard curve method and normalized against the expression of the ribosomal protein P0 gene. The standard curve consisted of five points that were obtained by a two-fold serial dilution of control RNA, starting out at 250 ng, together with a 'nontemplate' control; all performed in triplicate. P0 was preferred as the endogenous control over GAPDH and β-actin, which both gave qualitatively similar results in pilot experiments (data not shown).

### Western Blotting

GaMg cells were seeded into six-well plates as described above, and left to adhere overnight. To reduce the level of

SREBP activation, the medium was then supplemented to contain 20% FBS 3 h prior to the addition of the drugs or vehicle. The cells were exposed to the drugs or vehicle in the medium containing 20% FBS for 1.5, 3, 16 and 24 h. Collection of total cell protein was performed by washing the adherent cells twice with cold PBS before adding RIPA lysis buffer (containing 15 mM NaCl, 50 mM Tris, 0.5% sodium deoxycholate, 1% NP-40 and 0.1% SDS) with protease inhibitor (Roche Diagnostics, IN, USA). The cell lysates were standardized according to their protein content as determined by the Bio-Rad technique (Bio-Rad Laboratories, Richmond, CA, USA) and subjected to SDS-PAGE using NuPage gels and nitrocellulose membranes on the Western Breeze system (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's recommendations. The membranes were incubated overnight at 4°C with purified monoclonal anti-human mouse antibodies against SREBP-1 (IgG-2A4, Cat. n. 557036) or SREBP-2 (IgG-1C6, Cat. n. 557037) (BD Biosciences Pharmingen, San Diego, CA, USA). The blots were probed with secondary antibody solution, followed by detection using Chemiluminescence reagent (Invitrogen) according to the manufacturer's instructions. Quantification of Western blots was performed by computer-assisted densitometry and image scanning with Fuji Las-1000 luminescent image analyzer (Fuji, Japan) and Image Gauge v4.0 software (Fuji).

### Enzyme Analysis

GaMg cell cultures were washed and detached by scraping in PBS. The cells were collected by centrifugation and resuspended in a buffer composed of 100 mM sucrose, 40 mM $K_2HPO_4$ (pH 7.2), 30 mM EDTA, 50 mM KCl, 0.25% (v/v) Triton X-100. The protein content was determined with Bio-Rad protein assay (Bio-Rad Laboratories). HMG-CoA reductase activity was determined by an assay based on the method described by Brown *et al*[29] The samples were preincubated for 20 min at 37°C in a mixture of 0.1 M potassium phosphate (pH 7.4), 5 mM dithiothreitol, 10 mM $Na_4EDTA$, 3 mM NADP, 12.5 mM glucose 6-phosphate and 25 μg/ml glucose-6-phosphate dehydrogenase, in a total volume of 180 μl. The reaction was initiated by adding 20 μl 0.4 mM [$^{14}$C]-HMG-CoA (2.2 μCi/ml) (NEC642; Perkin–Elmer, Boston, MA, USA). After 30 min at 37°C, the activity was terminated by the addition of 25 μl 6 M HCl. Measure of 5 μl of 4 μM DL-[$^3$H]-mevalonic acid (5 μCi/ml) (TRA155; Amersham) was added as internal standard to correct for incomplete recovery. After incubation for 30 min at 37°C, the product mixture was isolated by thin-layer chromatography on Silica gel 60 (Merck, Darmstadt, Germany) and the radioactivity of [$^3$H] and [$^{14}$C] were determined by scintillation counting.

### Lipid Analysis

Cells were exposed to the drugs for 24 h as previously described and subsequently washed twice with ice cold PBS and detached by scraping in PBS. The cells were collected by centrifugation and re-suspended in a buffer composed of 100 mM sucrose, 40 mM $K_2HPO_4$ (pH 7.2), 30 mM EDTA, 50 mM KCl, 0.25% (v/v) Triton X-100. Lipids were measured on a Technicon axon system (Miles, Tarrytown, NY, USA), diluting 4 μl of the cell lysate to a total volume of 250 μl. Cholesterol was measured using the Technicon RA kit (product number: T01-1684-02; Miles), with the following reagents given in final concentrations: 44.7 mM sodium hydroxybenzoate, 8.1 mM sodium cholate, 0.7 mM aminoantipyrine, 1285 U peroxidase (horseradish), 160 U cholesterol oxidase and 125 U cholesterol esterase (pancreatic). Triglycerides were measured with the Technicon RA kit (product number: T01-1868-02; Miles), with the following reagents given in final concentrations: 250 kU/l lipase (microbial), 6250 U/l L-α-glycerophosphate oxidase (microbial), 5000 U/l peroxidase (horseradish), 1125 U/l glycerol kinase (microbial), 0.625 mM ATP, 0.625 mM 4-aminoantipyrine, 12.5 mM $MgCl_2$ and 3.5 mM 4-chlorophenol.

### Statistical Analysis

The gene expression results were statistically evaluated by an analysis of variance (ANOVA), based on a factorial design with two groups (antipsychotic drug *vs* control) × six test occasions (time points). Enzyme and lipid data were analyzed using a two-sided Student's *t*-test. All tests were conducted with Statistica® software. A significance level of 0.05 was used.

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## DUALITY OF INTEREST

None declared.

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