Lipogenic effects of antipsychotic drugs in cultured cells and in rat

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Contents

1. Acknowledgements

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2. Abbreviations

AA	Arachidonic acid
5-HT	5-hydroxytryptamine (serotonin)
ApoE	Apolipoprotein E
bHLH	basic-helix-loop-helix
BT4C	Rat glioma cell line
CCF-STTG1	Human astrocytoma cell line
CNS	Central nervous system
DHA	Docosahexanoeic acid
DISC1	Disrupted-in-schizophrenia-1 gene
DTNBP1	Dysbindin-1/ Dystrobrevin-binding protein 1
EFA	Essential fatty acid
EPA	Eicosapentanoeic acid
EPS	Extrapyramidal side effects
EPS	Extrapyramidal side effects
ER	Endoplasmic reticulum
ERP	Event-related potential
FASN	Fatty acid synthase
GaMg	Human glioma cell line
HCN2	Human cortical neuronal cells
HepG2	Human hepatoma cell line
HMGCR	HMG-CoA reductase (3-hydroxy-3-methylglutaryl-CoA reductase)
HMGCS1	HMG-CoA synthase 1 (cytosolic 3-hydroxy-3-methylglutaryl-CoA synthase)

INSIG	Insulin-induced gene
LDLR	Low density lipoprotein receptor
LXR	Liver X Receptor
NHR	Nuclear hormone receptor
NMDA	N-methyl-D-aspartic acid
PLA2	Phospholipase A2
PPAR	Peroxisomal Proliferator Activated Receptor
PUFA	Polyunsaturated fatty acid
SCAP	SREBP Cleavage Activating Protein
SCD	Stearoyl-CoA desaturase (delta-9-desaturase)
SH-SY5Y	Human neuroblastoma cell line
SREBP	Sterol Regulatory Element Binding Protein

3. Summary

The etiology of the serious psychiatric disorder schizophrenia is unknown. Epidemiological studies indicate a high heritability with a complex pattern of transmission. Moreover, structural and genetic findings indicate that both neuronal and glial function is affected, including oligodendrocyte and myelin abnormalities. Antipsychotic drugs are used to treat and ameliorate the symptoms of schizophrenia. Drug-mediated Dopamine D2-receptor blockage is probably necessary for reducing the positive symptoms (hallucinations, delusions) of schizophrenia, but the mechanism(s) involved in the improvement of other schizophrenic symptoms (*negative* and *cognitive*) are less well established. Unfortunately, there are several side effects associated with antipsychotic drug treatment, with increasing focus on weight gain and other metabolic adverse effects.

To gain further insight into the mechanisms of antipsychotic drug action, we applied microarray technology and quantitative real-time PCR to investigate drug-induced changes in global gene expression in various cultured human cell lines, including glial-like cells, neuron-like cells and liver cells. We found that several typical and atypical antipsychotic drugs elevated the expression of genes involved in cellular lipid biosynthesis, including HMG-CoA reductase and fatty acid synthase that are essential enzymes in cholesterol- and fatty acid biosynthesis, respectively. These genes are controlled by the SREBP transcription factors, which we found activated by antipsychotic drugs on the protein level. The increase in gene expression had a functional outcome, as demonstrated by accumulation of intracellular cholesterol and triglycerides. Although with differences in efficacy, most of the tested antipsychotic drugs induced SREBP activation in cell culture. In our studies, the effect was more pronounced in glial cell lines than in neuronal-like cell lines. Antipsychotic-induced

SREBP activation was also seen in hepatocytes, indicating that clinically significant effects might occur in tissues other than the CNS. In a context of therapeutically relevant serum concentrations, clozapine and chlorpromazine appear as the most potent SREBP activators. Interestingly, clozapine has superior efficacy in otherwise treatment-resistant schizophrenia. Clozapine also has the highest liability to induce weight gain, and we further investigated the ability of clozapine to induce lipogenesis *in vivo*. Indeed, in rats exposed to a single intraperitoneal dose of clozapine we observed a marked increase in hepatic lipid levels and altered expression of several genes involved in the control of lipid homeostasis (*e.g. SREBP*, *PPARα* and *LXRα*).

In summary, we have identified transcriptional activation of cellular lipogenesis as a new mechanism of action of antipsychotic drugs. Enhanced cholesterol synthesis in the CNS may have therapeutic implications (glia-produced cholesterol serves as a glial growth factor to promote synaptogenesis and myelination), whereas increased lipid production in the liver and adipose tissues may be linked to the well-known metabolic side effects of these drugs. We propose that our findings provide new insight into the molecular mechanisms of antipsychotic drugs and provide new candidate genes for disease susceptibility and drug response in schizophrenia.

4. Introduction

Antipsychotic drugs often reduce many of the symptoms of schizophrenia. These drugs have been widely used since the early 1950's, but the molecular mechanisms responsible for their therapeutic effects are only partly known. Drug response is individual and optimal treatment is at present often obtained by trial and error. Further insight into the molecular mechanisms involved in drug response could facilitate individualized treatment, with maximum therapeutic outcome and minimum adverse effects, and provide valuable information for the development of new antipsychotic drugs. In this thesis I will describe how we identified a possible new mechanism of antipsychotic drug action.

4.1 Clinical aspects of schizophrenia

4.1.1 Heritability

Schizophrenia is a serious psychiatric disorder with a lifetime risk of approximately 1% for the individual and a prevalence of about 0.4% in the population worldwide. Epidemiological studies indicate that both environmental and genetic factors contribute to the emergence of the disorder. Environmental risk factors associated with schizophrenia include pre-and perinatal complications, winter birth, urban birth and residence, paternal age and the use of CNS-stimulating drugs such as cannabis [1-3]. Evidence that genetic factors are involved in the etiology of schizophrenia comes from family, twin and adoption studies, with the risk of developing schizophrenia increasing with the proportion of genes shared with an affected individual [4] (figure 4.1). Heritability is the calculated proportion of susceptibility to develop a disorder that is attributable to inherited genetic factors, and the heritability of schizophrenia has been estimated to be around 80% [5]. The mode of inheritance for schizophrenia is

complex, probably involving numerous genes of small effect [6, 7]. Several promising schizophrenia susceptibility genes have been identified, and they will be further introduced in section 4.3.5.
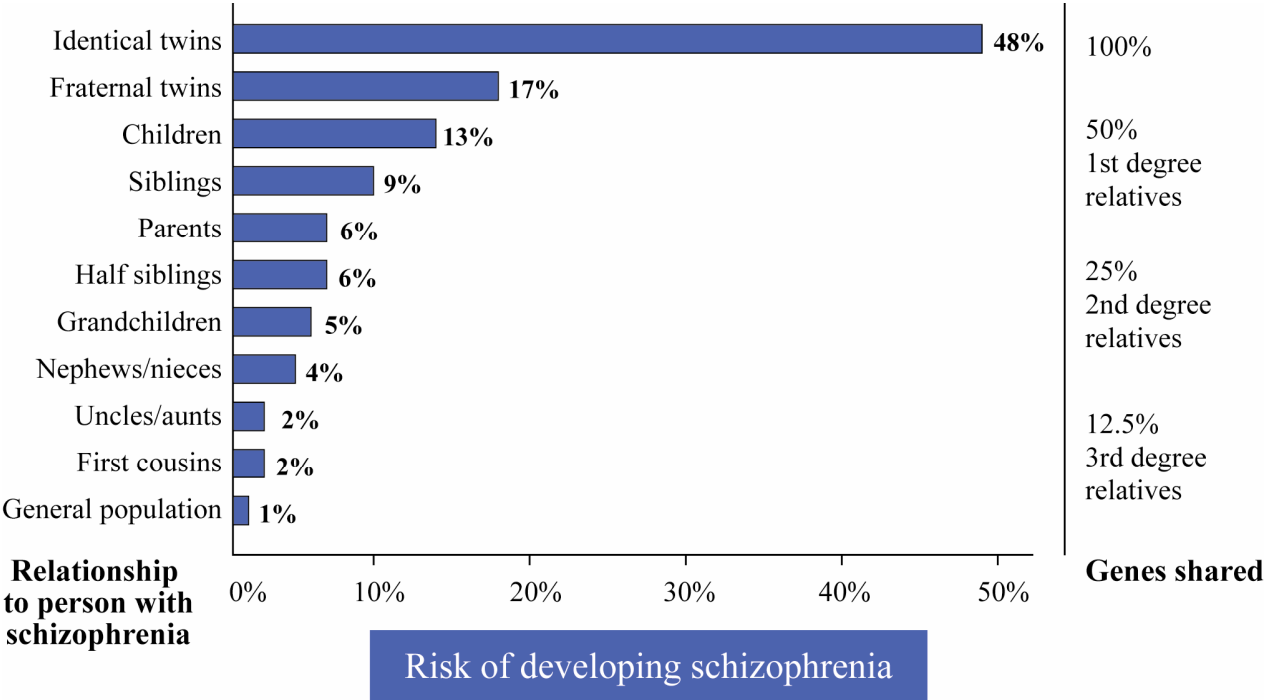
Figure 4.1 Risk of developing schizophrenia according to the genetic closeness to an affected individual
Reproduced from Gottesman et al [4]

4.1.2 Symptoms

Schizophrenia is diagnosed based on the presence and duration of symptoms that fulfil the criteria of the The Diagnostic and Statistical Manual of Mental Disorders IV text revision (DMS-IV-TR) [8] or the International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10) [9]. Schizophrenic symptoms typically emerge during adolescence or early adulthood [10], and are often classified into three categories; *positive*, *negative* and *cognitive* symptoms [11] (table 4.1). *Positive symptoms* include delusions and hallucinations, interpreted as representing reality. These symptoms are often efficiently treated with antipsychotic drugs. *Negative symptoms* include blunted affect, and

social and emotional withdrawal, thereby representing absence of normal behaviour. The negative symptoms are usually the most persistent over time and are difficult to treat and often lead to various add-on medications such as antidepressants [12, 13]. Cognitive impairment is regarded as a prominent characteristic of schizophrenia [14], with *cognitive symptoms* such as poor attention, reduced memory and conceptual disorganization associated with the disorder [15, 16]. The cognitive symptoms are merged into the negative symptoms of schizophrenia [17].

Table 4.1 Schizophrenic symptoms
Modified from Lancon et al [16]

Positive	Negative	Cognitive
Delusions	Blunted affect	Conceptual disorganisation
Hallucinatory behaviour	Emotional withdrawal	Difficulty in abstract thinking
Grandiosity	Poor rapport	Disorientation
Suspiciousness	Social withdrawal	Poor attention
Unusual thought content	Lack of spontaneity	
Lack of judgment and insight	Motor retardation	
	Active social avoidance	

4.1.3 Pathophysiological alterations

Structural abnormalities

There are several macroscopic brain abnormalities associated with schizophrenia, of which reduced cortical brain volume and increased ventricular size are the most consistent [18]. The degree of structural differences varies between patients and these differences become apparent only when comparing large groups of patients and controls. Such changes are also observed in drug-naïve schizophrenic patients and their first-degree unaffected relatives, indicating that they are not a result of the drug treatment or illness itself, but are rather associated with a vulnerability to develop the disorder [19, 20]. Thus, such abnormalities are not suitable as diagnostic criteria. At the microscopic level abnormally clustered neurons and reduced synaptic spine density have been observed in post-mortem brains of schizophrenic patients

13

and this observation has stimulated hypotheses that synaptic connectivity is reduced in the disorder [21-23]. White matter structural abnormalities and reduced levels of myelin are also associated with schizophrenia [24, 25]. Downregulated oligodendrocyte- and myelin specific genes in post-mortem schizphrenic brains supports the theory that abnormal myelination may be involved in the pathophysiology of schizophrenia [26, 27]. Interestingly, regional white matter changes in the prefrontal cortex have been associated with presence of negative symptoms in patients [28], implying that myelin and oligodendrocytes might serve as neuronal targets for future pharmacological treatment with improved effect on negative symptoms.

Neurophysiological alterations

The cognitive-event related potential (ERP) is an example of a potential risk-indicator in schizophrenia. ERP is an electrophysiological response to external stimuli and includes both automatic (preattentional) and controlled (attention-dependent) processes [29]. Mismatch negativity (MMN) is a preattentional ERP component that is elicited when repetitive auditory stimuli are interrupted by a deviant ("oddball") signal [30]. It has been widely replicated that the MMN response in schizophrenic patients differs from in normal individuals and also seems to be specific to schizophrenia relative to other psychiatric disorders, such as bipolar and major depressive disorder [31, 32]. P50 ERP suppression is another neurophysiological measure altered in schizophrenic patients. In a test situation, subjects are exposed to two clicking sounds within a short time interval, each generating a P50 wave. In normal individuals the P50 wave generated to the second click is much smaller than the first, whereas in schizophrenic subjects the P50 suppression is reduced [33]. Similar to the structural abnormalities mentioned above, altered P50 ERP suppression is not limited to schizophrenic

patients, but may also occur in their relatives [34]. ERP abnormalities have been suggested as a valuable endophenotype for identifying candidate genes involved in cognitive function [35].

4.2 Antipsychotic drugs

Antipsychotic drug therapy is considered a cornerstone in the treatment of schizophrenia. Usually, they reduce or ameliorate the positive symptoms of the disorder. The effect of the presently used drugs on negative and cognitive symptoms is limited, and future drugs should have increased focus on these symptoms. Antipsychotic drugs are categorized based on their clinical efficacy and their side effect profiles. Table 4.2 lists a selection of antipsychotic drugs that belongs to three different classes, as described below.
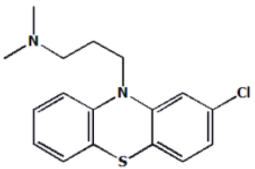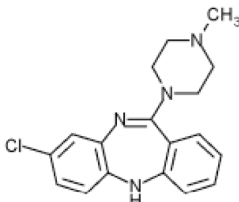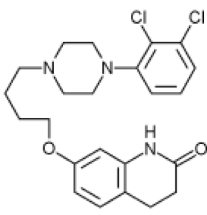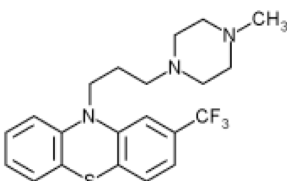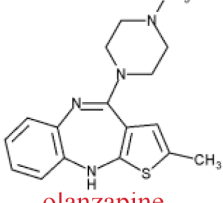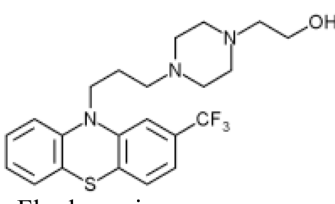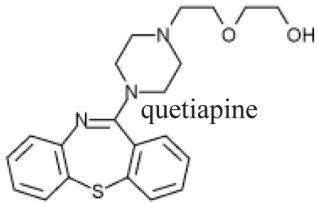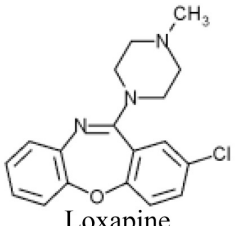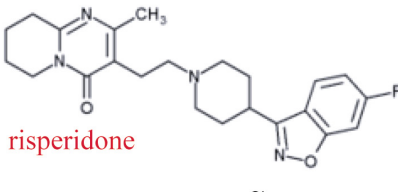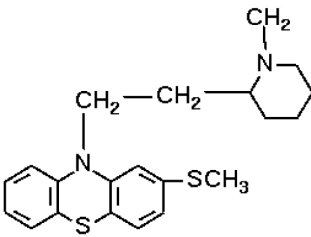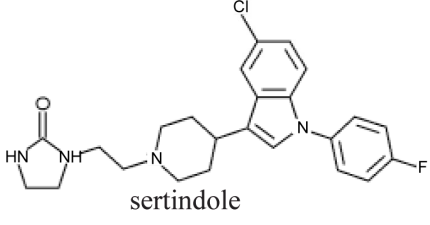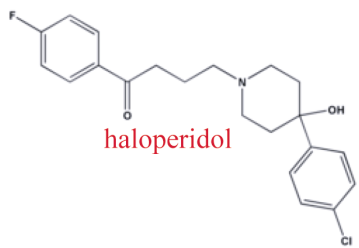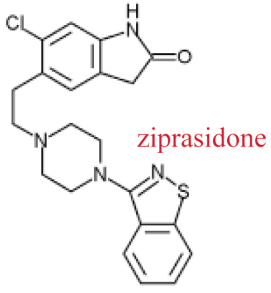
4.2.1 Classification

First generation drugs

The first antipsychotic drug was chlorpromazine. Originally applied to treat preoperative anxiety, its antipsychotic properties were discovered by chance in the early 1950's. The effectiveness of chlorpromazine stimulated the synthesis of other antipsychotic agents, some of which are still in use today. This first generation (typical) antipsychotic drugs are effective against the *positive* symptoms of schizophrenia, but unfortunately they often induce highly unpleasant side effects, such as extrapyramidal side effects (EPS) and hyperprolactinemia [36-39].

Table 4.2 Classification of antipsychotic drugs
Chemical structure of a selection of first-, second-, and third generation antipsychotic drugs as defined by Roth et al [40]. All of these drugs are chemically classified as cationic amphiphiles. Drugs highlighted in red have been investigated in this thesis

1st generation (typical)	2nd generation (atypical)	3rd generation

chlorpromazine

clozapine

aripiprazole

Trifluoperazine

olanzapine

Fluphenazine

quetiapine

Loxapine

risperidone

Thioridazine

sertindole

haloperidol

ziprasidone

Johan Fernø 2006

16

Second generation drugs

Clozapine is the prototype of the second generation (atypical) antipsyhotic drugs and was introduced in the early 1970's. Due to the serious and sometimes fatal side effect of agranulocytosis [41], clozapine was withdrawn from the marked, but was re-introduced and approved by the Food and Drug Administration (FDA) in 1989, since clozapine was found effective in otherwise treatment-resistant patients [42].

In addition to being equally effective as the first generation drugs against the positive symptoms, the second generation drugs seem to have better effect on negative and cognitive symptoms [43, 44]. A meta-analysis demonstrated that some atypicals (clozapine, olanzapine, risperidone) apparently proved better on overall clinical efficacy than other atypicals (ziprasidone, sertindole, quetiapine, remoxipride) and typical drugs (haloperidol), with effect sizes calculated from the Positive and Negative Syndrome Scale (PANSS) [45, 46]. Unfortunately, clozapine and several other atypical drugs are associated with various metabolic disturbances, such as weight gain, hyperglycemia and hypertriglyceridemia [47-50]. These metabolic adverse effects are of great concern since they increase the risk for obesity-related complications and death [51]. They also reduce patient compliance [52, 53]. It is therefore noteworthy that olanzapine, which is associated with considerable weight gain, recently was ranked as the most effective antipsychotic drug in terms of discontinuation rates [54].

Third generation drugs

Aripiprazole and other benzamides function as partial dopamine agonists. Due to their separate mechanism of action, these drugs have been described as the third generation antipsychotic drugs [55]. Aripiprazole is claimed to be effective against positive, negative and

cognitive symptoms of schizophrenia, whereas EPS and metabolic adverse effects appear to be quite infrequent [56].

4.2.2 Mechanisms of action

Although their mechanisms of action are not fully understood, the ability of antipsychotic drugs to block dopamine D2 and other neurotransmitter receptors is considered pivotal for the major clinical effects (figure 4.2).
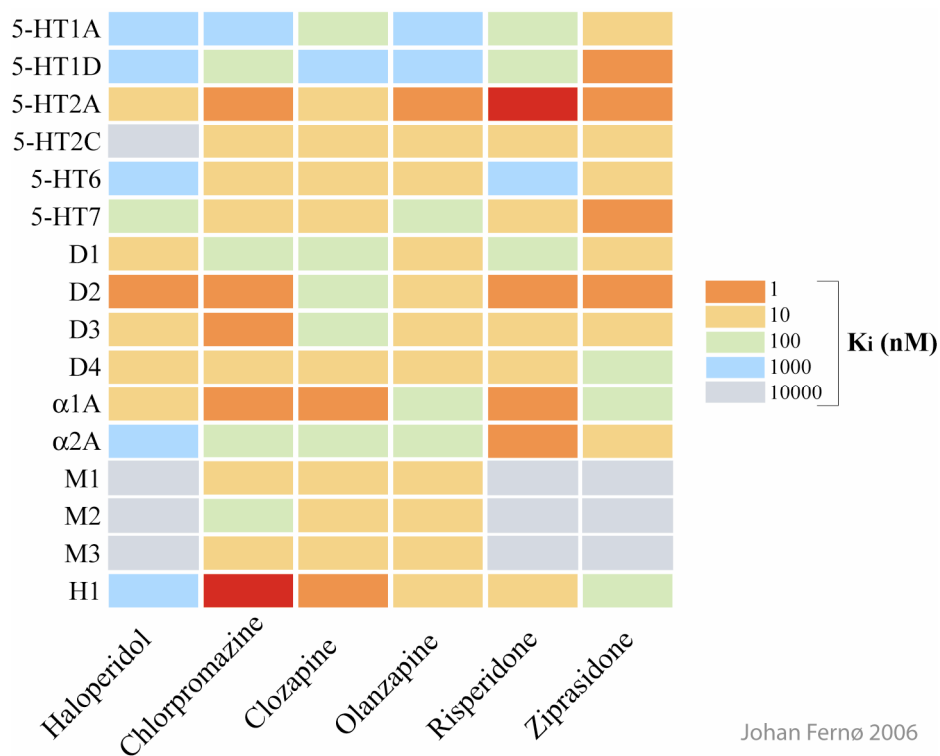
Figure 4.2 Receptor affinities for antipsychotic drugs
Relative neurotransmitter receptor affinities (inverse proportional to K_i-values) for the antipsychotic drugs investigated in this thesis. Modified from Roth et al [57]

D2-receptor antagonism

All established antipsychotic drugs share the property of moderate to high dopamine D2-receptor affinity [58]. The typical antipsychotic drugs exhibit strong dopamine D2-receptor antagonism, with D2-receptor affinity correlated to their ability to reduce psychotic (positive)

symptoms [59-61]. Clinical effects of dopamine receptor blockade can be numerous, and the brain region to which binding occurs seems to be relevant for clinical outcome. Binding to dopamine D2-receptors in mesolimbic circuits probably contributes to the antipsychotic effect, whereas D2-receptor blockade and reduced dopamine firing in nigrostriatal projections may lead to extrapyramidal side effects [38]. In order to balance the therapeutic and adverse effects, it is important to treat patients with optimal drug doses. A D2-receptor blockade of 70-80% is correlated with therapeutic effect with tolerable side effects, whereas occupancy above 80% generally leads to EPS [38, 62]. However, clozapine and quetiapine have therapeutic effect at D2-receptor occupancy as low as in the range 40-60% [38, 63], suggesting that mechanisms other than D2-blockade are important for therapeutic effect.

The role of serotonin (5-hydroxytryptamine; 5-HT)

The diverse receptor binding profiles of atypical drugs suggest that antipsychotic effect can be mediated via receptors other than dopamine [64, 65]. Many antipsychotics demonstrate 5-HT-receptor antagonism, and the combination of strong 5-HT-receptor binding and low dopamine D2-receptor affinity has been suggested as a key mechanism for the improved therapeutic profile observed for several atypical drugs [66]. 5-HT antagonism can lead to increased dopamine signalling in mesocortical projections, which is a proposed mechanism for the more beneficial effects of atypical drugs on the negative symptoms [67, 68]. The 5-HT1-receptor, to which clozapine binds, has been suggested to be involved in reduced anxiety and depression and improvement of cognitive and negative symptoms [44, 69].

N-methyl-D-aspartic acid (NMDA)-receptor antagonism

Ketamine and Phencyclidine (PCP) are NMDA-receptor antagonists that reduce glutamate signalling and that can induce a psychotic state both in patients and in animal models,

including the full range of positive and negative symptoms [70-73]. These findings suggest that glutamatergic neurotransmission should be taken into account when designing new antipsychotic drugs. Specific NMDA-agonists such as the amino acids glycine and D-serine have been demonstrated to promote learning and memory in rats and monkeys [74, 75] and may improve on negative symptoms in schizophrenia [76, 77].

4.2.3 Side effects

Extrapyramidal side effects (EPS)

EPS is the collective term of involuntary movement disturbances such as akathisia, parkinson-like akinesia, dystonia and tardive dyskinesia (TD) [78-80]. The typical antipsychotic drugs are especially prone to induce EPS, which is assumed to be due to their dopamine D2-receptor binding in nigrostriatal circuits [38]. The introduction of atypical antipsychotic drugs was an important improvement in order to reduce these troublesome side effects.

Metabolic side effects

Unfortunately, many atypical drugs are associated with metabolic adverse effects, including obesity, hypertension, dyslipidemia and insulin resistance, which are of increasing concern in the treatment of schizophrenia [54, 81]. A meta-analysis of over 80 studies examining weight gain demonstrated that clozapine was the drug associated with the highest average weight gain (Figure 4.3) [48]. The Metabolic syndrome is a collection of risk factors that are associated with cardiovascular disease and increased mortality [82], and a recent study demonstrated that the prevalence of the metabolic syndrome among clozapine-treated patients was significantly higher than in a matched control group [83].
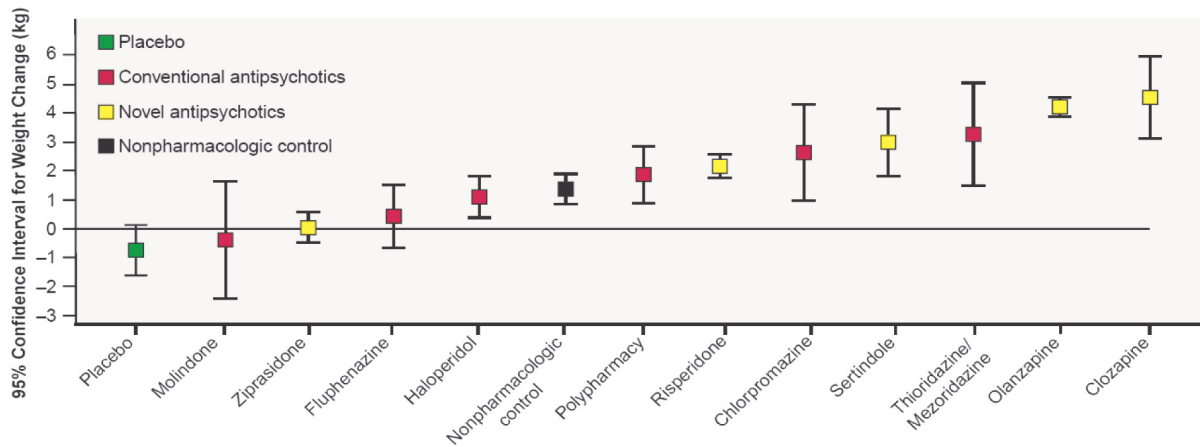
Figure 4.3 Antipsychotic-induced weight gain
Average weight change after 10 weeks on standard doses of different antipsychotic drugs (Random Effects Model). From a Meta-analysis by Allison et al [48]

How antipsychotic drugs induce these metabolic disturbances is not fully established, but several mechanisms have been suggested. First, antipsychotic-induced weight gain appears to induce leptin resistance and elevated levels of circulating leptin [84, 85]. Increased leptin levels correlate with the amount of body fat mass and with human obesity in general [86]. Further studies are required to reveal whether the increased leptin levels are caused directly by the antipsychotic drugs or indirectly via drug-induced obesity. Second, the diverse receptor binding profile of atypical drugs has been suggested to be relevant for the development of metabolic adverse effects, and some receptors are of particular interest [57]. The antagonistic effects of these drugs on 5-HT2C- and histamine H1-receptors seem to be important [87, 88]. The hypothalamic 5-HT2C-receptor has been demonstrated to be involved in metabolic regulation [89, 90] and agonistic stimulation has been demonstrated to reduce food intake [91]. This suggests that the blocking of this receptor with antipsychotic drugs could lead to increased apetite and weight gain. The affinity to the histamine H1-receptor provides the best correlation to antipsychotic-induced weight gain [92, 93]. However, drugs with high 5-HT2C-receptor antagonism can be weight neutral [94] and antipsychotics with low H1-receptor affinity induce weight gain [95], suggesting that other mechanisms for antipsychotic-induced

weight gain exist. Further insight into the mechanism of antipsychotic drug action is imperative for a more comprehensive understanding of drug-induced weight gain.

4.3 Theories regarding the etiology of schizophrenia

4.3.1 Neurotransmitter disturbances

Dopamine

The *dopamine hypothesis* of schizophrenia states that psychotic symptoms are caused by overactive dopamine signalling in subcortical brain areas [96, 97]. This theory emerged based on the dopamine blocking properties of the typical antipsychotic drugs, and the fact that their degree of D2-receptor affinity is correlated with a reduction of the positive (psychotic) symptoms in schizophrenic patients [38, 60, 98]. Furthermore, amphetamine-induced increase of dopamine signalling leads to psychotic symptoms, with higher degree of dopamine release in schizophrenic patients than in age-matched controls [99-101]. In addition, brain-imaging studies have demonstrated increased density and occupancy of dopamine D2-receptors in the striatum of patients with schizophrenia [102, 103].

In contrast to the overactive dopamine innervation in subcortical brain areas, dopamine signalling in the dorsolateral prefrontal cortex (DLPFC) seems to be reduced in schizophrenic patients [104]. The current view on the dopamine hypothesis is that schizophrenic symptoms are caused by an imbalance of dopamine signalling between cortical and subcortical brain areas, with subcortical hyperstimulation of D2-receptor causing the positive symptoms and cortical hypostimulation of D1-receptors causing cognitive and negative symptoms [105]. A recent study demonstrated increased density of dopamine D1-receptor in prefrontal cortex of schizophrenic patients, which was interpreted as a compensatory reaction to decreased cortical dopamine signalling [106].

Glutamate

Glutamatergic hypofunction has been suggested to be involved in the pathophysiology of schizophrenia [107-109]. Integrative approaches suggest that alterations both in dopamine and glutamate signalling, as well as other neurotransmitters such as serotonin and γ-aminobutyric acid (GABA), might be involved in causing schizophrenic symptoms [108, 110]. Most known susceptibility genes for schizophrenia are involved in glutamatergic neurotransmission [111], and glutamate-receptor agonists have been suggested to improve on negative and cognitive symptoms of schizophrenia. However, a recent meta-analysis from the Cochrane Database questions the role of glutamate-receptor agonists as potential antipsychotic drugs and concludes that additional research on glutamatergic mechanisms of schizophrenia is needed [112].

4.3.2 The neurodevelopmental theory of schizophrenia

Insight into the neurotransmitter abnormalities associated with schizophrenia might give hints about the pathophysiology of the disorder, but does not necessarily provide knowledge about the underlying cause. Schizophrenic symptoms have been suggested to be a result of disturbances in normal brain development [113, 114], and the predominant view is that CNS abnormalities in schizophrenia are caused by both environmental and genetic factors [115, 116]. Early environmental disturbances such as viral infections, oxygen deficit and malnutrition of the mother during pregnancy may influence on brain development and have been suggested as risk factors for schizophrenia [117]. The considerable time lag between the presumed brain impairment *in utero* and the manifestation of the clinical symptoms in early adolescence is intriguing. The delay has been explained by late maturation of inter-related neural systems [114, 118]. Such maturation involves trimming of neurons, or pruning, which is the removal of miswired and non-beneficial connections between axons and dendrites that

23

occurs during normal CNS development [117]. Excessive pruning has been suggested to cause the abnormal connectivity of cortical neurons observed in schizophrenia [119, 120]. One possible mechanism for such excessive removal of essential and functional connections is that the pruning process itself is dysfunctional. Another possibility is that the trimming process is normal and that it is the unmasking of already existing deficits that cause schizophrenic symptoms. In either model, the progression towards schizophrenia would be triggered by an erroneous removal of synapses and neurons [121].

4.3.3 Membrane lipid disturbances in schizophrenia

The *membrane hypothesis* of schizophrenia proposes that alterations in membrane phospholipid composition can cause schizophrenic symptoms and be involved in the pathophysiology of the disorder [122, 123]. This hypothesis was originally based on findings of reduced levels of polyunsaturated fatty acids (PUFAs) in red blood cell (RBC) membranes from schizophrenic patients [124, 125]. Further supporting this theory are findings of altered membrane PUFA levels both in fibroblasts [126] and in post mortem brains from schizophrenic patients [127]. The mechanism behind the reduced PUFA levels in cell membranes from schizophrenic patients is unknown, but in accordance with the abovementioned neurodevelopmental theory, both environmental and genetic factors have been suggested [128]. Phospholipase A2 (PLA2)-mediated removal of phospholipid fatty acids is one possible mechanism, which is supported by findings of increased PLA2 activity in serum from schizophrenic patients [129]. Other suggested mechanisms are reduced incorporation of membrane fatty acids [130] and defective conversion of essential fatty acids (EFAs) into polyunsaturated fatty acids [131, 132].

4.3.4 Mitochondria dysfunction

Mitochondria are essential for brain energy production and involvement of mitochondria in schizophrenia was proposed as early as in 1954 [133]. Mitochondrial dysfunction has been suggested to be a part of schizophrenia pathophysiology [134], and independent studies demonstrate decreased brain metabolism and reduced mitochondrial gene expression levels in brains from schizophrenic patients [18, 135, 136]. A role for impaired mitochondrial function in schizophrenia was further supported in a recent study where an integrative approach of genomics, proteomics and metabolomics demonstrated alterations in several metabolic pathways in schizophrenic brains [137]. Processes such as reactive oxygen species (ROS)-induced pathways and β-oxidation were activated, whereas carbohydrate- and lipid biosynthesis decreased. It was proposed that these alterations were directly involved in the myelin degradation associated with schizophrenia [137].

4.3.5 Genetic predisposition and susceptibility genes

Linkage and association studies have identified several genetic loci and genes possibly associated with schizophrenia [111]. Neuregulin-1 (*NRG1*) [138] and dysbindin-1/ dystrobrevin-binding protein 1 (*DTNBP1*) [139] are among the most promising candidate genes identified with linkage studies, with many independent replications [111, 140, 141]. Chromosomal rearrangement studies have identified a balanced translocation t(1;11)(q42;q14) that co-segregate with schizophrenia, and the breakpoint has been mapped to the Disrupted-in-schizophrenia-1 gene (*DISC1*) [142-145]. Table 4.3 shows an overview of schizophrenia susceptibility genes.

Table 4.3 Schizophrenia susceptibility genes and the strength of evidence in four categories
Reproduced from Straub et al [146].

		Strength of evidence (0 to 5+)			
		Association with schizophrenia	Linkage to gene locus	Biological plausibility	Altered expression in schizophrenia
COMT	22q11	+ + +	+ + + +	+ + + +	Yes, +
DTNBP1	6p22	+ + + + +	+ + + +	+ +	Yes, + +
NRG1	8p12-21	+ + + + +	+ + + +	+ + +	Yes, +
RGS4	1q21-22	+ + +	+ + +	+ + +	Yes, + +
GRM3	7q21-22	+ + +	+	+ + + +	No, + +
DISC1	1q42	+ + +	+ +	+ +	Not known
DAOA (G72/G30)	13q32-34	+ + +	+ +	+ +	Not known
DAAO	12q24	+ +	+	+ + + +	Not known
PPP3CC	8p21	+	+ + + +	+ + + +	Yes, +
CHRNA7	15q13-14	+	+ +	+ + +	Yes, + + +
PRODH2	22q11	+	+ + + +	+ +	No, +
AKT1	14q22-32	+	+	+ +	Yes, + +
GAD1	2q31.1	+ +		+ +	Yes, + + +
ERBB4	2q34	+ +			Yes, + +
FEZ1	11q24.2	+ +		+ + +	Yes, + +
MUTED	6p24.3	+ + + +	+ + + +	+ + +	Yes
MRDS1 (OFCC1)	6p24.3	+ +	+ + + +	+	Not known

NRG1 is mainly thought to be involved in synaptic function [111], but of its many isoforms, one is essential for myelination [147]. Due to the many different NRG1 alleles and haplotypes that have been implicated, the functional role of NRG1 in schizophrenia is still unknown [148]. DTNBP1 is located presynaptically in glutamatergic neurons and is reduced at these locations in schizophrenia [149]. A genetic variant of DTNBP1 has been related to cognitive impairment [150]. *DISC1* has a complex pattern of expression, with several isoforms [151]. *DISC1*-expression is observed in several intracellular localizations, especially in mitochondria [152]. This observation is interesting since mitochondria deficits have been reported in schizophrenia and other disorders of the nervous system [134, 153]. It is believed that most of the gene products from the schizophrenia suseptibility genes are involved in neurotransmission and synaptic plasticity [111]. Several genes seem to be related to glutamatergic synapses, supporting the alleged role of glutamate transmission in schizophrenia [111, 154]. There is also genetic support for a role of altered dopamine signalling in schizophrenia, with polymorphisms linked to the Catechol-O-methyl transferase (*COMT*) gene associated with the disorder [111]. A recent study demonstrated and association between schizophrenia and single nucleotide polymorphisms (SNPs) in genes involved in oligodendrocyte function, including the oligodendrocyte lineage transcription factor 2 gene

(*OLIG2*), 2',3' cyclic nucleotide 3' phosphodiesterase (*CNP*) and the tyrosine kinase NRG1 receptor (*ERBB4*). These findings provide genetic evidence for oligodendrocyte abnormalities in schizophrenia etiology [155].

4.4 Control of lipid biosynthesis

Lipids have multifunctional roles in the human body, and can act as energy sources, structural components and signalling molecules. Lipids are supplied from the diet and from endogenous synthesis pathways. This section will introduce key factors involved in several aspects of lipid homeostasis such as lipogenesis, lipid breakdown and lipid transport.

4.4.1 The SREBP system

The Sterol Regulatory Element Binding Protein (SREBP) transcription factors regulate expression of a number of genes involved in biosynthesis and uptake of cholesterol, saturated/monounsaturated fatty acids, triglycerides and phospholipids (figure 4.4) [156-159]. Two different SREBP isoforms, SREBP1 (with two splice variants, SREBP1a and -1c) and SREBP2, are both synthesized as 120 kDa inactive precursors in the endoplasmic reticulum (ER). In the ER they reside in a complex with the SREBP cleavage activating protein (SCAP) and the protein product of the insulin-induced gene (INSIG) (figure 4.5) [160]. At low sterol levels in ER, SCAP undergoes a conformational change and the SREBP/SCAP complex is released from the INSIG protein and transported to the Golgi apparatus where proteolytic cleavage of SREBP occurs by the Site-1 protease (S1P) and Site-2 protease (S2P) [161]. This process releases a 60-70 kDa transcriptionally active basic-helix-loop-helix (bHLH) domain that is translocated to the nucleus where it binds to the sterol regulatory element (SRE). SRE
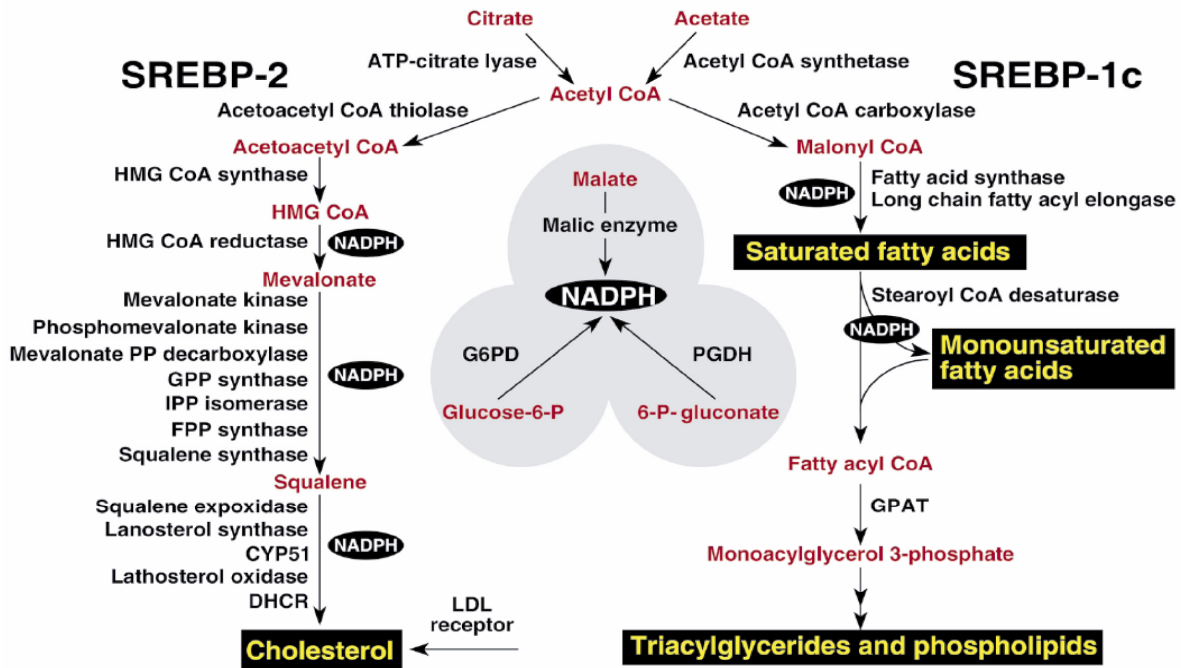
Figure 4.4 Genes regulated by the SREBP transcription factors
SREBP1c preferentially activates genes involved in fatty acid and triglyceride biosynthesis, whereas SREBP2 preferentially activates genes involved in cholesterol biosynthesis and uptake. Reproduced from Horton et al [157]

is present in the promoter of all SREBP target genes and binding of the activated bHLH transcription factor stimulates lipogenic gene expression [157].

Although some degree of overlap occurs, the different SREBP isoforms in principle control separate parts of the lipid biosynthesis pathways. SREBP1c almost exclusively controls the expression of fatty acid biosynthesis genes, such as acetyl CoA carboxylase (*ACC*), Stearoyl-CoA desaturase (delta-9-desaturase) (*SCD*) and fatty acid synthase (*FASN*) [162]. SREBP2 mainly regulates cholesterol biosynthetic and cholesterol uptake genes such as HMG CoA reductase (*HMGCR*), HMG CoA synthase 1 (*HMGCS1*) and Low-density-lipoprotein receptor (*LDLR*) [163, 164]. SREBP1a regulates expression of both cholesterol and fatty acid biosynthesis genes, with most efficient activation of the fatty acid biosynthesis genes [165]. Activation of SREBP transcription factors might also stimulate the before-mentioned

conversion of EFAs to long-chain PUFAs, since the SREBP target genes delta-5- and delta-6-desaturase genes are essential for this conversion [166].

In cultured cells, activation of both SREBP1a and SREBP2 are controlled by cellular sterol levels [167]. Sterol levels also control SREBP2 activity in tissues [168], but the *in vivo* situation is generally more complex. SREBP1c, which is the predominant SREBP1 splice variant in several tissues, is unaffected by sterol levels and is instead regulated by nutritional status [169-171]. Furthermore, SREBP1c has tissue-specific effects with hepatic SREBP1c overexpression in rats leading to accumulation of fat in the liver, whereas adipocyte-specific SREBP1c overexpression leads to a decrease in adipose lipid levels and lowered fat depots [162, 172]. In contrast, adipocyte-specific overexpression of SREBP1a leads to accumulation of fat in mouse adipose tissue [173]. Furthermore, the SREBP system interacts with other metabolically relevant transcription factors, which adds to the complexity of SREBP regulation.

4.4.2 Nuclear hormone receptors

The transcriptional control of lipid homeostasis is complex and involves several transcription factors [174]. The nuclear hormone receptors (NHRs) represents an important class of transcription factors involved in metabolic control [175]. In contrast to the SREBP proteins, for which nuclear abundance is proportional with transcriptional activity, the NHR transcription factors reside in the nucleus and have their activity controlled by ligand binding [176]. Upon activation, the NHRs normally form heterodimers with the retinoic X receptor (RXR) [177, 178]. The liver X receptor (LXR) and peroxisome proliferator activated receptor (PPAR) are metabolically important NHRs, of which the isoforms LXRα and PPARα are abundant in tissues with a high metabolic rate [179, 180]. The NHRs can moderate and be

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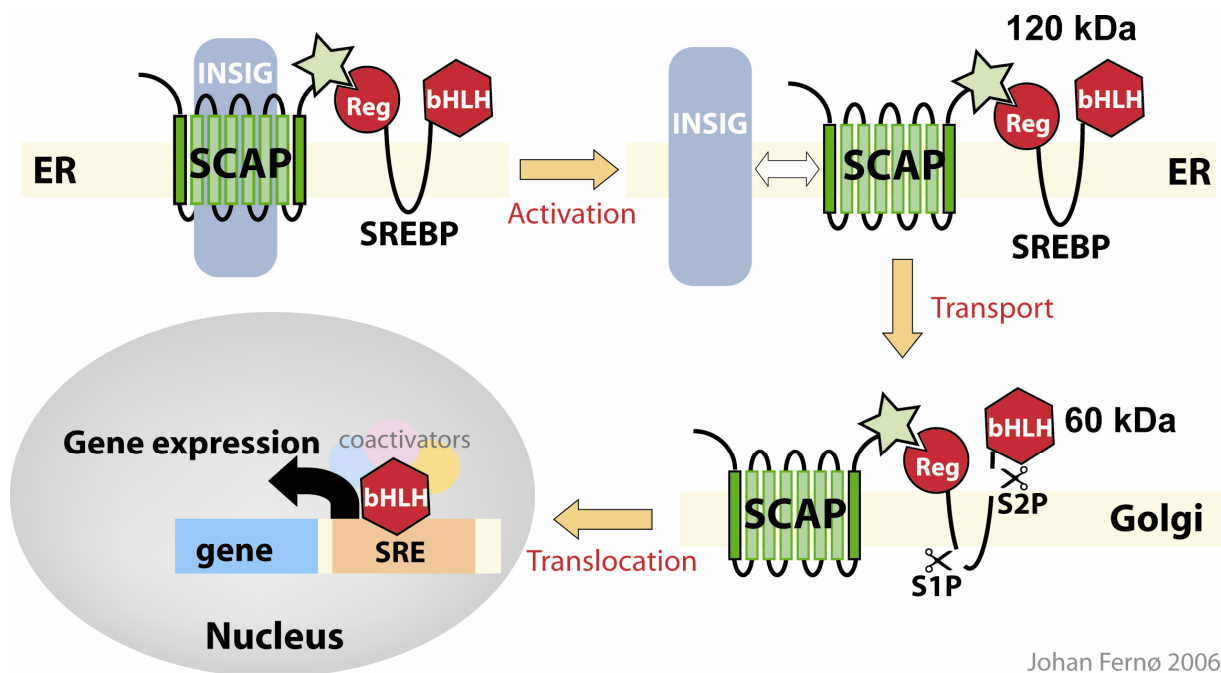
Figure 4.5 Proteolytic activation of the SREBP transcription factors
Cellular sterol levels control the activity of SREBP transcription factors. When sterol levels in ER are low, SCAP (green) undergoes a conformational change and assists the transport of the 120 kDa precursor SREBP (red) to the Golgi apparatus as the initial step in SREBP activation. Production of transcriptionally active SREBP is carried out by proteolytic cleavage by the Golgi-specific S1P and S2P proteases. The mature, transcriptionally active basic-helix-loop-helix (red hexagonal) is subsequently translocated to the nucleus where it activates the expression of genes involved in cholesterol and fatty acid biosynthesis via binding to the sterol regulatory element (SRE) in their promoter region.

influenced by the SREBP system [174, 181, 182], exemplified by LXR-induced activation of

SREBP1c gene expression [183]. LXRα is also involved in cellular lipid export and controls

the expression of genes involved in cholesterol transport, such as Apolipoprotein E (*ApoE*)

and ATP-binding cassette A1 (*ABCA1*) [184]. Drug-induced stimulation of the NHR

transcription factors has been demonstrated to improve several of the symptoms associated

with the metabolic syndrome [175]. LXRα agonists have been suggested to have beneficial

effect on artherosclerosis [183] and stimulation of PPARα-receptors increases fatty acid β-

oxidation, improves lipid blood profiles and reduces the risk of cardiovascular disease [185].

Other PPAR isoforms, such as PPARδ and PPARγ are also involved in metabolic control, and

their stimulation leads to beneficial effects, such as increased energy expenditure and fatty acid oxidation as well as improved insulin sensitivity [186, 187].

4.4.3 Lipid homeostasis in the CNS

The main cellular components of the brain are neurons and the more abundant glial cells (figure 4.6). Several different types of glial cells exist, all with specialized functions: *Oligodendrocytes* form myelin around axons that provide insulation essential to neurotransmission. *Astrocytes* participate in intercellular communication and provide signals for synapse formation and neurogenesis. The third glial cell type is *microglia* that are involved in immunological reactions [188].
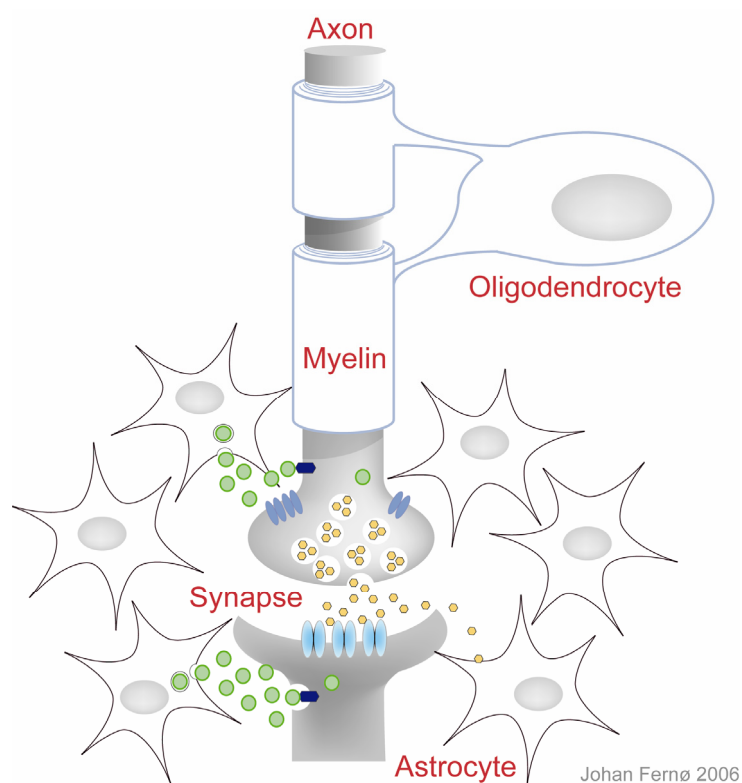
Figure 4.6. Cellular components of the CNS
The main cellular components of the CNS are neurons and the more abundant glial cells. Neurons (shaded grey) are characterized by axons that form connections with other neurons in the form of synapses. Neuronal communication occurs via neurotransmitters (yellow) across the synaptic cleft. Intercellular communication between glial and neuronal cells exists and glia-derived growth factors (green) provide essential signals for intact synapse function.

31

Cholesterol is the major component of myelin and during CNS development increased expression of cholesterol biosynthesis genes parallels the myelination process [159, 189, 190]. In the CNS, cholesterol biosynthesis is synthesized *de novo* by astrocytes and oligodendrocytes, underscoring the importance of glial cells in brain lipid homeostasis [159]. Interestingly, cholesterol was recently demonstrated as a glia-derived growth factor essential for the formation of synapses in culture [191]. The long-chain polyunsaturated fatty acids (PUFAs) such as arachidonic acid (AA; 20:4, n-6), eicosapentaenoic acid (EPA; 20:5, n-3) and docosahexanoic acid (DHA; 22:6, n-3) are enriched in neuronal membranes [192]. These fatty acids are structural components of cellular membranes, but can also be converted into signalling molecules important for normal brain function, such as eicosanoids and docosanoids [193]. In contrast to cholesterol, the majority of the PUFAs are not synthesized in the body itself and must be obtained from the diet [193].

5. Aims of the study

The overall aim of this study was to obtain new insight into the molecular mechanisms of antipsychotic drug action, through identification of drug-induced changes in global gene expression and potential target genes, using clinically relevant model systems.

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Specific aims

1. To use microarray technology and real-time PCR to screen for antipsychotic-induced gene expression changes in cultured human glial cells (paper I).

2. To compare various antipsychotic drugs for their lipogenic effects and ability to affect the expression of SREBP target genes in different CNS-specific cell lines (paper II).

3. To investigate the effect of various antipsychotic drugs on the SREBP transcription factors and their target gene expression in cultured hepatocytes (paper III).

4. To expose rats to an acute dose of the antipsychotic drug clozapine in order to investigate its effects on the SREBP system and lipid homeostasis *in vivo* (paper IV).

6. List of papers

Paper I

Fernø J, Raeder MB, Vik-Mo AO, Skrede S, Glambek M, Tronstad KJ, Breilid H, Lovlie R, Berge RK, Stansberg C, Steen VM. Antipsychotic drugs activate SREBP-regulated expression of lipid biosynthetic genes in cultured human glioma cells: a novel mechanism of action? Pharmacogenomics J 2005; 5:298-304.

Paper II

Fernø J, Skrede S, Vik-Mo AO, Havik B, Steen VM. Drug-induced activation of SREBP-controlled lipogenic gene expression in CNS-related cell lines: Marked differences between various antipsychotic drugs. BMC Neurosci 2006; 7:69.

Paper III

Raeder MB, Ferno J, Vik-Mo AO, Steen VM. SREBP Activation by Antipsychotic- and Antidepressant-Drugs in Cultured Human Liver Cells: Relevance for Metabolic Side-Effects? Mol Cell Biochem 2006; 289: 167-173.

Paper IV

Fernø J, Vik-Mo AO, Havik B, Røst TH, Berge K, Skrede S, Waage JR, Mørk S, Berge RK, Jørgensen HA, Steen VM. Acute clozapine exposure in vivo induces lipid accumulation and marked sequential changes in expression of SREBP, PPAR and LXR target genes in rat liver.

7. Summary of results

Paper I

By the use of microarray technology we demonstrated that in cultured human glioma GaMg cells, the antipsychotic drugs clozapine and haloperidol up-regulate a cluster of genes involved in cholesterol- and fatty acid biosynthesis, including *HMGCR*, 3-hydroxy-3-methylglutaryl-CoA synthase 1 *(HMGCS1)*, fatty acid synthase (*FASN*) and Stearoyl-CoA desaturase (delta-9-desaturase) (*SCD*). The increased gene expression was associated with enhanced HMGCR-enzyme activity and elevated cellular levels of cholesterol and triglycerides. The expression of the lipid biosynthesis genes is controlled by the SREBP1 and SREBP2 transcription factors that are activated via proteolytic protein cleavage. Both clozapine and haloperidol induced proteolytic SREBP cleavage, and antipsychotic-induced activation of SREBP-mediated lipogenesis was suggested as a novel mechanism of antipsychotic drug action, possibly relevant for both therapeutic- and metabolic adverse effects.

Paper II

In this study we aimed at investigating whether the haloperidol- and clozapine-induced SREBP-activation described in paper I is a general feature for antipsychotic drugs. We compared the effect of chlorpromazine, haloperidol, clozapine, olanzapine, risperidone and ziprasidone on SREBP proteolytic cleavage and SREBP-controlled gene expression (*e.g. HMGCR, HMGCS1, LDLR, FASN* and *SCD*) in four CNS-relevant human cell lines. There were marked differences in the ability of the antipsychotic drugs to activate expression of SREBP target genes, with clozapine and chlorpromazine as the most potent stimulators in a

context of therapeutically relevant concentrations, whereas ziprasidone displayed minor activation. Glial-like cells (GaMg glioma and CCF-STTG1 astrocytoma cell lines) displayed more pronounced drug-induced SREBP activation compared to the response in HCN2 human cortical neurons and SH-SY5Y neuroblastoma cells. This indicates that antipsychotic-induced activation of lipogenesis is most prominent in glial cells.

Paper III

Since the observed drug-induced lipogenic effects might be relevant for metabolic adverse effects, we investigated whether the drug-induced activation of lipogenesis observed in CNS-related cell lines also occurs in cultured human hepatoma cells. The effect of antidepressants, antipsychotic- and mood-stabilizing drugs was studied. In general, the drugs that had the highest propensity to induce SREBP activation (clozapine, imipramine, and amitriptyline) are most strongly associated with weight gain. Ziprasidone and buproprion are not associated with weight gain, and did hardly stimulate the SREBP system. The mood-stabilizers did not increase SREBP activation. The results indicate a relationship between drug-induced activation of SREBP in cultured human liver cells and the weight gain associated with antidepressant and antipsychotic drugs.

Paper IV

In order to investigate whether the antipsychotic-induced effects observed in cell cultures also occurred *in vivo*, lipogenic effects were investigated in the liver of rats administered an intraperitoneal dose of clozapine. Clozapine exposure led to hepatic accumulation of lipids and induced changes in the expression levels of several SREBP target genes and of genes controlled by other metabolically important transcription factors, such as PPARα and LXRα. These *in vivo* data show that clozapine affects lipid levels in rat liver and influence on several

transcription factors involved in metabolic control. The present results further support a relationship between drug-induced perturbation of lipid homeostasis in non-CNS tissue and the metabolic adverse effects associated with antipsychotic drugs.

8. Discussion

8.1 Methodological aspects

8.1.1 Cultured cells as a model system for antipsychotic drug response

In order to screen for novel biological processes and candidate genes involved in antipsychotic drug response, we investigated how gene expression was affected by antipsychotic drug exposure in several human cultured cell lines, with emphasis on the human glioma GaMg cell line. Cultured cells provide a simplified model to obtain information of possible relevance to the *in vivo* situation, and they offer the opportunity to selectively investigate cell type-specific responses to experimental conditions. However, cell cultures are often cancer cells, transformed from their original cell population to maintain cell growth. Cultured cells are also detached from their original tissue and thus lack stimuli and feedback from the surrounding environment. When investigating the effect of drugs or other external stimuli, the concentrations used in cultured cells often exceed the "real-life" situation, which adds to the potential bias of the results. Furthermore, cell properties may change in culture over time and it is important to consider the number of cell divisions (passage numbers) when cell culture experiments are carried out.

Antipsychotic-induced stimulation of lipogenic gene expression was found to be a stable phenomenon, occurring at different passage numbers. In our studies we used drug concentrations in the range of 1-50 μM, which in general are much higher than therapeutically relevant serum concentrations. However, due to their lipophilic nature, many psychotrophic drugs have large distribution volumes and are enriched in lipid-rich tissues. Levels of haloperidol and clozapine have been demonstrated to be 10-30 times higher in the CNS than

in serum [194, 195]. This suggests that the high cell culture concentrations still might have therapeutic relevance. Cells were exposed to psychotrophic drugs for 24 hours. Even though therapeutic effect in patients normally has a lag time of several weeks, the gene expression changes identified within the first 24 hours might give important clues about the long-term effects of these drugs. The lower serum concentrations in patients makes it is plausible that the gene expression changes in the clinical situation is smaller, with functional effects developing over time.

In summary, cultured cells represent an inexpensive and easily maintainable model system that can rapidly provide experimental results. Nevertheless, *in vivo* experiments should always be carried out before firm conclusions are drawn.

8.1.2 Gene expression analyses

Microarray technology

In our study we have used microarray (MA) technology as a screening method to identify genes that change their expression in cultured human cells following antipsychotic drug exposure. MA technology is a powerful tool to measure global gene expression in a single experiment. Several different MA technology platforms exist, including single and dual dye methods, cDNA and oligonucleotide probes, as well as commercial and in-house fabricated arrays. We initially used in-house cDNA and oligo arrays with dual dye labelling (Cy3 and Cy5), performed as a dye-swap experiment. A single dye experiment with commercial arrays (Applied Biosystems AB1700 chemiluminescent microarray system) was subsequently carried out. In a recent study by Kuo and colleagues [196], multiple MA technology platforms were compared, and it was demonstrated that, in general, commercial arrays provide more consistent results than in-house arrays. Furthermore, one-dye platforms were more consistent

than two-dye platforms and demonstrated higher correlation with real time PCR measurements [196]. This is in agreement with our experiments, where we experienced problems with dye decomposition, especially with Cy5. Nevertheless, we were able to identify the drug-induced upregulation of lipid biosynthesis-related genes in all the different types of microarray experiments.

Real time PCR

With its high sensitivity, accuracy and wide dynamic range, the real-time PCR technology is the most commonly used method for quantification of gene expression [197]. Detection of PCR products is performed with fluorescent probes or DNA binding dyes, and the term "real-time" derives from the continuous collection of data throughout the PCR amplification process. DNA binding dyes, such as SYBR-green, bind unspecifically to double stranded DNA, which make them very flexible since one dye can be used to detect all genes. On the other hand the risk for unspecific binding and false positive results increases with this less expensive alternative. The more expensive fluorescent probes, that include TaqMan chemistry, provide a more specific detection system.

The PCR amplification typically has an initial phase of 10-15 cycles before the fluorescence level from the exponential PCR reaction markedly exceeds the background. The cycle at which this occurs is called the threshold cycle (Ct). Quantification of gene expression levels can be carried out in several ways. In the present study we have used relative quantification, using the standard curve method or the comparative ΔΔCt method. The *standard curve method* accounts for any differences in amplification efficiency between a target gene and a reference ("housekeeping") gene, to which the target gene expression is normalized. In theory, the expression level of the reference gene should remain unchanged across the conditions that

are compared. This requirement represents a potential bias, since in most cases it is not previously known which genes are regulated. The *comparative ΔΔCt method* can be used when the amplification kinetics between target gene and reference gene are similar, *i.e.* that titration curves for the genes have similar slopes. To avoid inefficient amplification it is important to keep the PCR amplification product small (normally <150 bp) [198]. The difference in gene expression (fold change) is calculated between an experimental sample and a calibrator sample, which in our experiments was the untreated control. The comparative ΔΔCt method is useful when assaying many genes in a large number of samples, as the number of reactions is minimized since a standard curve titration for each gene on each run is not necessary.

In summary, microarray- and real time PCR technolgy are powerful tools to investigate gene expression in a high-throughput manner. All the same, it is important to acknowledge the fact that changes in gene expression do not necessarily reflect the protein levels and the activity of the biological processes that the genes are involved in. Inferences about cellular protein levels based on gene expression alone should therefore be careful and verification at protein and functional levels should always be carried out for accurate interpretation.

8.1.3 Rat as an experimental model animal

Rats are frequently used as an animal model to investigate mechanisms involved in antipsychotic drug effects [199, 200]. Gene expression analyses in rat brain have been performed to search for potentially new mechanisms of antipsychotic drug action. The results are ambiguous, but some genes, such as *Apolipoprotein D* (*ApoD)*, have been found affected by antipsychotics in independent studies [201, 202]. A number of rat studies have also been performed to investigate the mechanisms involved in the metabolic adverse effects associated

with several of the antipsychotic drugs. Similar to the brain studies, these results have been conflicting, partly because the phenotype observed for rats does often not match the human clinical situation. Female rats seem more prone to develop antipsychotic-induced weight gain than male rats, which is not the case in humans. In addition, several of the drugs that apparently induce weight gain in rats are not associated with weight gain in humans (table 8.1) [203]. There may be several explanations for the lack of coherence between rats and humans, such as the very different rate of drug metabolism in the two organisms. As an example, the half-life for haloperidol in rats is 1.5 hours compared to 24 hours in man [204]. For olanzapine the half-life is 2.5 hours and 33 hours, respectively [205]. Traditionally, antipsychotic drugs have been administered to rats via intraperitoneal injections [206, 207], subcutaneous injection [208] or gavage [209, 210]. The route of drug administration seems to be important for the phenotype, and recent studies have provided new hope for a more valid rat model for antipsychotic-induced weight gain in humans. When the drugs were mixed in the food *ad libitum,* instead of administered by gavage, the weight of fat tissue in male rats increased [211]. Furthermore, when the food composition was similar to a human diet, with low protein and high fat and carbohydrate contents, the antipsychotic-induced weight gain profile corresponded to what is observed in humans [212]. In this thesis, we exposed rats acutely with an intraperitoneal injection of clozapine and subsequently investigated gene expression changes during a time course of 48 hours. Although the time frame might not be a valid representation of long-term clinical effects in humans, this experimental design may provide valuable insight into the molecular pathways that are initially affected by the drugs directly.

Table 8.1 Rats as an animal model for antipsychotic-induced weight gain
Antipsychotic-induced weight gain seems to occur mainly in female rats. In most studies the propensity of the different drugs to induce weight gain did not match the side-effect profile observed in humans. However, by providing an optimal feeding regime and diet composition, weight gain can occur in male rats, with apparent correlation to drug-induced weight gain in humans [212]

Reference	Gender/type	Drug/route of adm/ dose (mg/kg)/time	Food intake	Phenotype	Reported effects in patients*
Minet-Ringuet et al 2006 [212]	Male/Sprague-Dawley	olanz/*ad lib*/1.0/3w	increased	weight gain	high weight gain
		halo/*ad lib*/1.0/3w	NS	NS	moderate/low weight gain
		zipra/*ad lib*/1.0/3w	NS	NS	no weight gain
Minet-Ringuet et al 2006 [211]	Male/Sprague-Dawley	olanz/*ad lib*/1.0/3w	NS	increased adipocity	high weight gain
		halo/*ad lib*/1.0/3w	NS	increased adipocity	moderate/low weight gain
		zipra/*ad lib*/1.0/3w	NS	moderate increase in adipocity	no weight gain
Arjona et al 2004 [209]	Female/Sprague-Dawley	olanz/gavage/1.2/10d	increased	weight gain	high weight gain
		halo/gavage/0.04/10d	NS	NS	moderate/low weight gain
Goudie et al 2002 [209]	Female/Wistar	olanz/*ad lib*/0.5/12d	ND	weight gain	high weight gain
Baptista et al 2002 [207]	Female/Wistar	risp/b.i.d injection/ 4.5/10d	increased	weight gain	moderate weight gain
	Male/Wistar	risp/b.i.d injection/ 4.5/10d	minor increase	NS	moderate weight gain
Pouzet et al 2003 [203]	Female/Wistar	olanz/gavage/5 and 20/3w	increased	NS	high weight gain
	Male/Wistar	olanz/gavage/5 and 20/3w	NS	NS	high weight gain
Minet-Ringuet et al 2005 [210]	Male/Sprague-Dawley	olanz/*ad lib*/1/2w+6w	NS	NS	high weight gain
		halo/*ad lib*/1/2w+6w	NS	NS	moderate/low weight gain

olanz = olanzapine, halo = haloperidol, risp = risperidone, zipra = ziprasidone, *ad lib* = *ad libitum,* w = weeks, d = days, NS = non-significant difference between drug-exposed and control animal
ND = not measured in the study   * determined from Allison et al. 1999

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8.2 Antipsychotic-induced SREBP activation

8.2.1 Drug-induced SREBP activation and lipogenesis in cultured glioma cells

In order to gain new insight into the molecular mechanisms of antipsychotic drug action, we used microarray technology to investigate global gene expression changes in human cultured GaMg glioma cell lines (paper I). Since no *a priori* biological knowledge or selection of the genes is required before the gene expression analysis, it is possible to identify novel targets and molecular pathways involved in antipsychotic drug response without any specific hypothesis. We demonstrated that antipsychotic drugs upregulate a cluster of genes involved in cholesterol and fatty acid biosynthesis, including *HMGCR*, *HMGCS1*, *FASN* and *SCD,* all controlled by the SREBP transcription factors [157]. The gene expression results were supported by functional data, with a significant increase in cellular cholesterol and triglyceride levels and a doubling of the HMGCR enzyme activity. A time course experiment revealed that antipsychotic-induced proteolytic cleavage of the SREBPs at the protein level occurred within the first 3 hours after exposure, with the most pronounced effect on SREBP2. A corresponding increase in *HMGCR* and *FASN* gene expression was observed, as measured by real-time PCR. In contrast, *SREBP2* gene expression was not increased until 12 hours after exposure, and then only slightly. The antipsychotic-induced increase in lipid biosynthesis-related gene expression is therefore most likely a result of a direct effect on proteolytic activation of the SREBP transcription factors, independent of a preceding elevation of the *SREBP* gene expression.

In order to investigate whether the propensity to induce SREBP activation is a shared feature of antipsychotic drugs, we compared six antipsychotic drugs for their ability to induce SREBP activation and to increase SREBP-controlled gene expression (paper II). All the antipsychotic

drugs activated the SREBP system, but with marked differences in efficacy. The mechanisms by which the antipsychotic drugs activate the SREBP system could, in principle, involve receptor-dependent and -independent processes. Antipsychotic drugs block dopamine D2-like receptors, with binding affinities correlated to antipsychotic effect [60]. Many drugs also bind extensively to other neurotransmitter receptors, *e.g.* 5-HT and histamine H1 receptors [40, 87]. There was no apparent relationship between the receptor binding properties of the drugs and their SREBP-stimulating effect, implying that the SREBP activation is not linked to the receptor binding profiles. This assumption was supported by a recent study from our laboratory, in which several antidepressant drugs, with different receptor-binding properties from antipsychotic drugs, also activated the SREBP system in GaMg cells [213]. Our results suggest that drug-induced SREBP activation is related to some shared chemical property of these psychotropic compounds and is mediated via a receptor-independent mechanism of action. Indeed, all of the antipsychotics and antidepressants that we have investigated are cationic amphiphiles, substances shown to increase the synthesis and accumulation of total cellular cholesterol levels via mechanisms that involve a reduction of cholesterol levels in the endoplasmic reticulum (ER) [214, 215]. The exact mechanism of cationic amphiphile-induced SREBP activation is not fully understood, but a direct interaction with the ER-located SREBP-Cleavage-Activating-Protein (SCAP) has been suggested [216].

We proposed that antipsychotic-induced activation of lipid biosynthesis represents a molecular mechanism of psychotrophic drug action. The possible involvement of lipid-related effects in antipsychotic drug action is supported by a recent study, demonstrating that haloperidol and clozapine treatment alters the expression of lipid-metabolism related genes in the mouse frontal cortex and striatum [201]. However, neither of the genes described in that study was in the cluster of regulated genes identified by us.

8.2.2 Cell type specific effects

Antipsychotic drugs increased SREBP controlled gene expression in various CNS relevant cell lines, demonstrating that antipsychotic-induced SREBP activation is not limited to GaMg cells (paper II). The degree of drug-induced SREBP activation was clearly higher in glial cell lines (GaMg and CCF-STTG1) than in neuronal-like cell lines (SH-SY5Y and HCN2), which is in accordance with the fact that *de novo* lipid biosynthesis in the CNS primarily occurs in glial cells and not in neurons [159, 217, 218]. Any therapeutic effect associated with antipsychotic-induced lipogenesis should thus be mediated mainly via glial cells. The drug-induced SREBP activation evident in the slowly growing HCN2 cells demonstrate that antipsychotic-induced SREBP activation is not a phenomenon specific to cancerous cells. Antipsychotic-induced SREBP activation also occurred in HepG2 hepatocytes (paper III), which was in accordance with our proposal that increased lipid biosynthesis in peripheral tissues might represent an important mechanism of action underlying the metabolic adverse effects associated with several of the psychotropic drugs [81, 219]. Indeed, a recent study suggested that antipsychotic drug effects on glucose transport, lipogenesis and lipolysis in adipocytes could be mechanisms that might explain the weight gain and diabetes associated with atypical drugs [220].

8.2.3 Effects of antipsychotic drugs on the SREBP system in rats

In order to explore how antipsychotic drugs affect lipid metabolism *in vivo*, we examined several aspects of lipid homeostasis in rat liver following an intraperitoneal injection of clozapine. Clozapine was chosen due to its high propensity to induce weight gain in humans [48, 81]. Indeed, hepatic triglyceride levels were elevated to about 2.5-fold, 48 hours after injecting the rats. Our results was concordant with the drug-induced activation of SREBP-controlled lipogenesis observed in the hepatic cell cultures [221], since SREBP1

overexpression in rat liver is known to cause hepatic accumulation of fat [222]. We found increased hepatic expression of some SREBP target genes, such as *LDLR* and *FASN*, as early as one hour after injection. However, in direct contrast to the cell culture results, the expression of all SREBP target-genes was markedly downregulated six hours after clozapine injection, possibly due to negative feedback mechanisms in response to an initial SREBP activation. The reduced expression levels were particularly evident for *SREBP1c* and *FASN* that remained downregulated even after 24 hours. The amount of nuclear SREBP1 protein was strongly reduced and paralleled the gene expression levels. These results demonstrate that acute clozapine treatment affect SREBP activity *in vivo*, but the discrepancy between decreased lipogenic gene expression and increased lipid levels is paradoxical and indicates that hepatic lipid homeostasis is under complex regulation.

8.2.4 Interplay between metabolically important transcription factors

How could downregulation of lipid biosynthesis genes be paralleled by a marked increase in hepatic triglycerides? When addressing this disagreement it should be remembered that it is mainly the balance between lipid biosynthesis (in principle stimulated by the SREBP-controlled lipogenesis) and lipid breakdown (in principle stimulated by PPARα-controlled fatty acid β-oxidation) that determines hepatic lipid levels. Some of the genes involved in these processes are given in table 8.2.

In our rat experiments PPARα-, LXRα- and SREBP-controlled genes demonstrated similar expression profiles. This was surprising, given the opposite biologic functions of these transcription factors (table 8.2). A marked reduction in PPARα-controlled gene expression may explain the observed accumulation of hepatic triglyceride levels, since this indicates that

Table 8.2 Function of transcription factors involved in metabolic control
The table summarizes the genes investigated in paper IV and the transcription factors that control their expression levels. The main functions of the proteins encoded by the genes are indicated

Transcription factor	Target gene	Function
SREBP1c	*FASN*	Fatty acid biosynthesis
SREBP2	*HMGCR*	Cholesterol biosynthesis
	HMGCS1	Cholesterol biosynthesis
	LDLR	Cholesterol uptake
LXRα	*ABCA1*	Cholesterol efflux
PPARα	*ACOX1*	Fatty acid oxidation
	HMGCS2	Ketogenesis
-	*SOAT1*	Cholesterol esterification

the hepatic fatty acid β-oxidation and the degree of lipid breakdown is reduced. A recent study provides a hypothesis that might explain our paradoxical observations.Hepatic triglycerides accumulated in mice with a liver-specific knockout of the *FASN* gene [223]. It was proposed that since *de novo* synthesized fatty acids act as endogenous PPARα-agonists, the absence of "new fat" synthesis reduced the hepatic PPARα-mediated β-oxidation, leading to the hepatic lipid accumulation. According to the authors, lipid import from adipose tissue to the liver was increased, and since this "old fat" could not stimulate hepatic PPARα-mediated β-oxidation, the lipid accumulation in the liver increased [223]. The reduced PPARα-controlled gene expression and subsequent accumulation of hepatic triglycerides observed in our experiment may thus be an indirect effect of a transient clozapine-induced "knock-down" of *FASN* gene expression. The mechanism responsible for the SREBP-controlled downregulation is unclear, but might be related to a negative feedback, responding to an initial SREBP activation. The sequential activation of SREBP and PPARα target genes, with subsequent parallel expression profiles, supports that an interplay between these transcription factors has taken place in the clozapine-treated rats (figure 8.1). The nuclear hormone receptor LXRα also seem to promote a similar expression profile, which is in

agreement with the extensive "cross-talk" between PPARα, LXRα and SREBP1c that has been reported [181, 182].

In summary, acute injection of clozapine in rat induces hepatic accumulation of lipids, probably as a consequence of reduced fatty acid β-oxidation, which in turn is caused by a drug-induced decrease in *de novo* fatty acid biosynthesis. Increased import of fat from peripheral adipose depots might also contribute to the phenotype. The mechanism behind the severe decrease in SREBP activity is unclear, but might be caused by a negative feedback, in response to an initial SREBP activation.
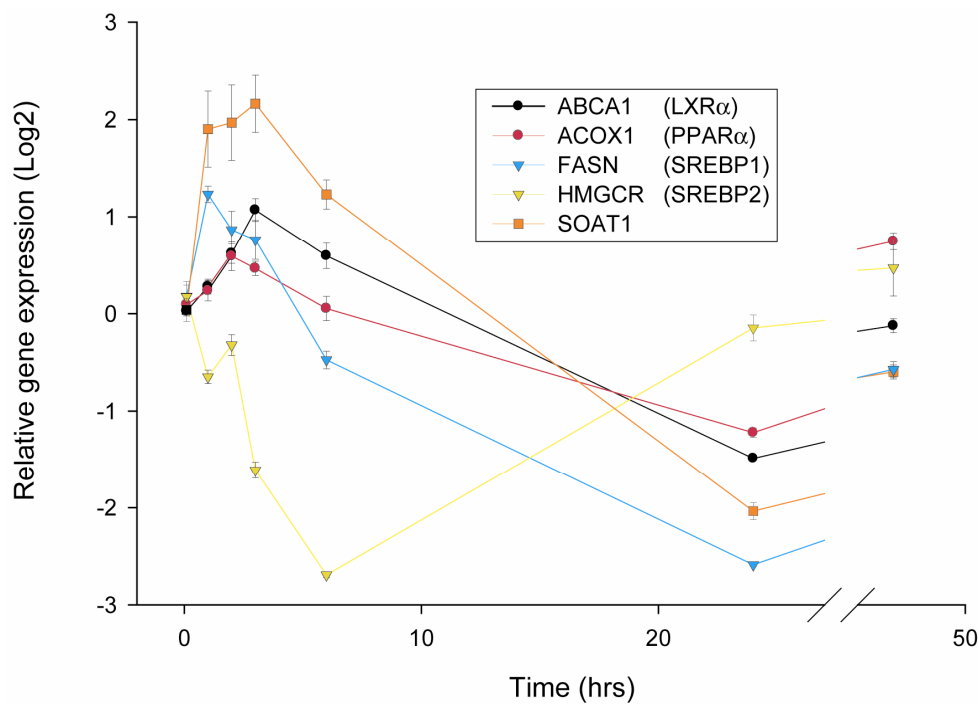
Figure 8.1 The effect of clozapine exposure on hepatic gene-expression in rat
Expression profiles of genes controlled by the various metabolic transcription factors given in table 8.2 (in brackets) following a single intraperitoneal injection of clozapine (50 mg/kg)

8.3 Potential therapeutic implications of antipsychotic-induced SREBP activation

Although the human brain accounts for only 2% of the total body weight, it contains about 25% of total body sterol content [159]. Cholesterol is important for various processes in the CNS, and drug-induced increase in cholesterol biosynthesis might have clinical impact via different cholesterol-dependent processes. This part of the discussion will focus on processes with potential relevance to some pathophysiological alterations associated with schizophrenia.

8.3.1 Cholesterol as an important component in myelination

Oligodendrocyte abnormalities and reduced myelin levels have been postulated to be involved in schizophrenia pathophysiology [46, 224]. Gene expression studies have demonstrated downregulation of myelin-related genes in post mortem brains from both schizophrenic and bipolar patients [26, 27], and it was recently reported that polymorphisms associated with the myelin-related transcription factor OLIG2 confer susceptibility to the disorder [155]. Furthermore, white matter changes in the prefrontal cortex seem to be associated with the presence of negative symptoms in the patients [28, 225]. 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 [226, 227]. Taken together, these findings indicate that reduced myelination might lead to schizophrenic symptoms.

Given that cholesterol is an essential component in myelin, our observation of the antipsychotic-induced increase in cholesterol biosynthesis is interesting. During CNS myelination, cholesterol biosynthesis is increased [159] and cholesterol availability in the

myelin-forming oligodendrocytes has been proposed as the rate-limiting factor for brain maturation [228].

Cholesterol biosynthesis in the CNS is primarily confined to glial cells, with neurons generally having a low capacity to synthesize lipids [193, 229]. Our cell culture results indicate that antipsychotic-induced stimulation of cholesterol biosynthesis is a glial-specific phenomenon, and this effect would be expected to also occur in oligodendrocytes, since they are specialized glial cells. It is thus possible that antipsychotic-induced cholesterol biosynthesis could have a therapeutic effect by providing essential building blocks for myelin to counteract the decreased myelination in brains of schizophrenic patients.

8.3.2 Cholesterol and ApoE in synaptogenesis

Reduced number of synapses and decreased synaptic connectivity has been implicated in the pathophysiology of schizophrenia. The presence of astrocytes is essential for synapse formation to occur in cultured CNS neurons, suggesting that glial-derived growth factors are required [230]. Cholesterol and ApoE particles were shoen to act in concert as a glial-derived synaptogenetic factor [191], which is in accordance with the inability of neurons to synthesize endogenous cholesterol, as observed in cultured neurons [231, 232]. ApoE mediates cholesterol transport from glial to neuronal cells and cholesterol uptake is via low-density-lipoprotein receptors (LDLRs) (Figure 8.2) [218].
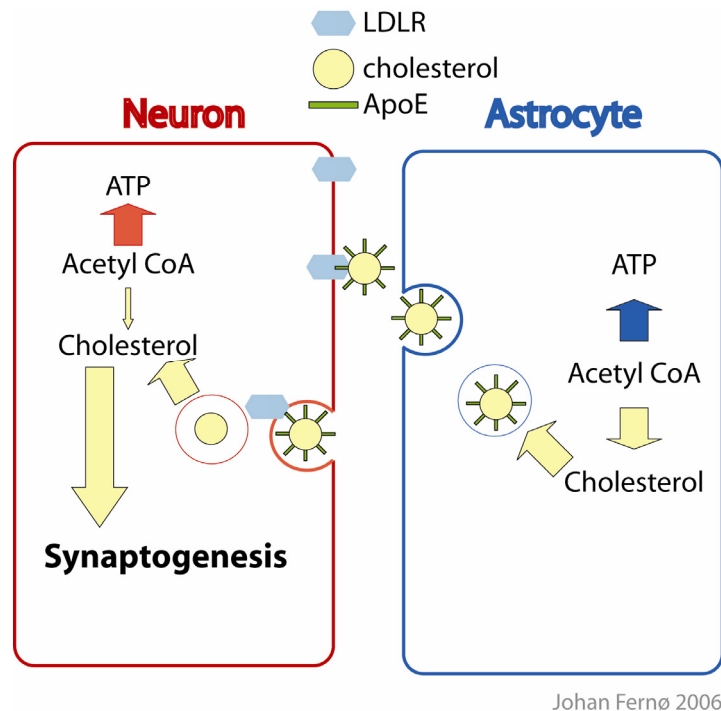
Figure 8.2 Cholesterol production and transport in neurons and glial cells
Illustration of a model for cholesterol production and transport from astrocytes to neurons. Glial-derived cholesterol (yellow) is transported from astrocytes to neuronal synapses by ApoE particles (green). Cholesterol uptake is mediated through LDL-receptors (light blue), located in the neuronal membrane. Glial-derived cholesterol can subsequently be used in processes essential for the neurons, such as synaptogenesis [191]. Modified from Pfrieger et al [218].

We have demonstrated that in the glial GaMg cells, antipsychotic drugs increase cholesterol biosynthesis, *LDLR* gene expression and ApoE gene expression and protein levels (paper II and Vik-Mo, Fernø et al, unpublished results), which indicates that these drugs induce the synthesis of all the essential components of the glial-derived synaptogenic factor. Given the description of schizophrenia as a disease of the synapse [233], the antipsychotic-induced elevation of a synaptogenetic factor represents a potential new therapeutic mechanism of action. Whether antipsychotics stimulate synaptogenesis in the brain *in vivo* and whether synaptogenesis is involved in therapeutic effect remains elusive and should be the scope of future studies. If clinically relevant, this effect cannot be related to a specific antipsychotic effect, since antidepressant drugs also increase cholesterol biosynthesis in glial cells [213]. Instead, the effect might be linked to some common symptoms or deficits that are present in

both schizophrenia and major affective disorders (*e.g.* cognitive dysfunctions). This possibility is underscored by the therapeutic breadth of these classes of drugs, with both antidepressant and antipsychotic drugs used in psychotic, bipolar and depressed patients. Interestingly, synaptic pathology has been observed both in schizophrenia and in mood disorders [234].

8.3.3 Lipid rafts in receptor-mediated neurotransmission

Cholesterol is essential to maintain cell membrane fluidity and in most cells cholesterol accounts for 20-25% of the plasma membrane [159]. Cholesterol is not homogenously distributed throughout the membrane, but enriched in microdomains called lipid rafts [235]. Embedded in lipid rafts are various signalling proteins and receptors, including the glutamate receptors [236] that represent potential pharmacological targets in the treatment of schizophrenia [109, 237]. Cholesterol depletion in lipid rafts can cause a gradual loss of synapses, and it was suggested that the described synaptogenic effect of glia-derived cholesterol could be mediated by cholesterol feeding to neuronal lipid rafts [238]. The same study demonstrated that depletion of cholesterol affects the stability of certain neurotransmitter receptors. Although speculative, it is thus possible that in addition to positive influence on synaptogenesis, antipsychotic-induced increase of glial cholesterol biosynthesis could mediate therapeutic effect by influencing neurotransmitter receptor stability.

8.3.4 PUFAs and SREBP

The nervous system is enriched in essential polyunsaturated fatty acids (PUFAs). PUFAs, such as arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) are located in cellular membranes and small changes in their abundance can lead to membrane dysfunction with a broad range of implications, including alteration in receptor binding,

neurotransmission and signal transduction [127, 239]. Reduced AA-levels have been found in post mortem brains from schizophrenic patients, suggesting that altered membrane lipid composition can be involved in schizophrenia pathophysiology [127]. Interestingly, long-chain PUFAs can be converted from dietary precursors in astrocytes [217], via a process that involves the SREBP controlled enzymes Δ5- and Δ6-desaturases [240] (figure 8.3). This opens for the possibility that antipsychotic-induced SREBP activation may confer therapeutic effect by inducing the conversion of essential fatty acids to long-chain PUFAs. On the other hand do PUFAs reduce the transcriptional activity of SREBP1c, indicating a complex interplay between PUFA levels and SREBP activity [241]. Whether antipsychotic-induced activation of the SREBP system has an effect on membrane PUFA levels remains elusive and should be a scope of future studies.

Figure 8.3 *De novo* PUFA synthesis
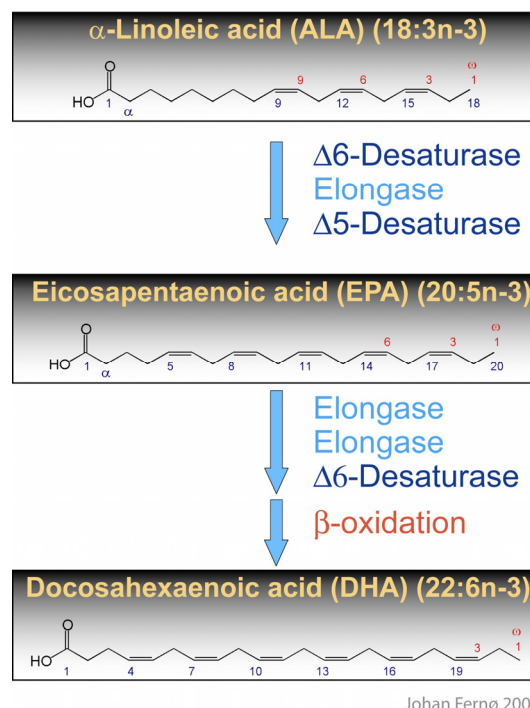Essential fatty acids can be converted to long-chain PUFAs by a process of elongation, desaturation and β-oxidation. The figure displays only omega-3 (n-3) fatty acids, but a similar process occurs for omega-6 fatty acids with AA as an end product. The enzymes that are normally involved are the Elovl2 and -5 elongases and the SREBP-controlled Δ5- and Δ6 desaturases (dark blue text) [174]

8.4 Possible metabolic implication of antipsychotic-induced changes in lipid homeostasis

8.4.1 The role of SREBP in metabolic control

Non-alcoholic fatty liver has been described as the hepatic manifestation of the metabolic syndrome in humans [242]. Transgenic overexpression of SREBP1 in rat leads to hepatic accumulation of fat in liver, and has been suggested as an animal model for hepatic steatosis in humans [162, 222]. Hepatic steatosis has been proposed as a metabolic side effect associated with antipsychotic drugs [243, 244]. Thus, our findings of psychotropic drug-induced elevation in SREBP-controlled gene expression in liver cells (paper III) provide a plausible mechanism for some of the metabolic disturbances associated with these drugs.

A marked increase in triglyceride levels was observed in rats injected intraperitoneally with clozapine, 48 hours after injection. This is interesting, since clozapine is one of the antipsychotic drugs with the highest propensity to induce weight gain [81]. Based on our cell culture results, we assumed that this hepatic fat accumulation was a direct result of clozapine-induced SREBP activation. However, after a possible initial activation, both the SREBP-controlled gene expression and the nuclear levels of SREBP1 were dramatically reduced in the rat liver (paper IV). The elevated levels of liver lipids might be explained by an observed reduction in fatty acid β-oxidation, which was probably indirectly caused by the reduced SREBP activity.

Do our results imply that drug-induced SREBP activation is confined to cultured cells and that SREBP activation cannot cause hepatic steatosis and other metabolic adverse effects caused by these drugs *in vivo*? The fact that some SREBP target genes were upregulated one

hour after clozapine injection suggests that an initial SREBP activation did take place, and that the reduced SREBP activity observed in rat liver might have been a result of a negative feedback response. In chronically treated humans the serum concentrations of antipsychotic drugs are increased over time and the low steady state concentrations makes it unlikely that a dramatic feedback inhibition would occur. In fact, in blood from patients treated for at least three weeks with the atypical drug olanzapine as monotherapy, the expression of the SREBP-controlled genes *FASN* and *SCD1* was moderately increased (Vik-Mo AO, Birkenaes A, Fernø J et al, unpublished results). Taken together, our results demonstrate that the SREBP system is affected by antipsychotic drugs, both *in vitro* and *in vivo*. Although the response in man seems to differ from rat, our *in vivo* findings indicate that rats provide a useful model system to identify factors affected in humans.

8.4.2 Antipsychotic-induced effects on nuclear hormone receptor-controlled gene expression

Drug-induced stimulation of the PPAR and LXR nuclear hormone receptors (NHRs) has been demonstrated to have beneficial effects on several aspects of the metabolic syndrome [175]. Activation of the PPARα/γ/δ and LXRα transcription factors is associated with improved insulin sensitivity [245], better serum lipid profile [246, 247], increased energy expenditure [248] and prevention of artherosclerosis [249]. In the clozapine-treated rats, the hepatic expression levels of several NHR target genes were decreased. Given their role in stimulation of lipid breakdown and lipid efflux, the reduced expression of NHR target genes might explain the observed accumulation of hepatic lipids. We proposed that the downregulation of NHR-controlled genes was a consequence of reduced SREBP expression, and not an effect of clozapine itself. However, since the SREBP system was not downregulated in patient blood samples, a reduction in PPARα-mediated β-oxidation would not be expected to have any

relevance for metabolic disturbances associated with these drugs in patients. However, an *in vitro* lucferase reporter gene assay demonstrated that clozapine and haloperidol act directly as antagonists on PPARα, PPARγ, PPARδ and LXRα and reduce their transcriptional activity (paper IV). Hence, the reduced NHR-controlled gene expression observed in clozapine treated rats could have been caused both by decreased SREBP activity and by a direct antagonistic effect of clozapine. These complex mechanisms of action are speculative and further studies are needed.

In summary, the SREBP activation induced by psychotropic drugs may provide a novel molecular mechanism of action, involved in one or several of the metabolic adverse effects associated with these drugs. In our experiments, the SREBP system is affected both by drugs that induce metabolic disturbances in humans, and drugs that do not. This apparently reduces the likelyhood that SREBP activation is associated with the drug-induced metabolic adverse effects. However, it is important to consider the experimental design before drawing conclusions. In the cell culture experiments, the effects of the various drugs were primarily compared at molar concentrations. In one study (paper II), we systematically transformed the molar concentrations of the drugs into therapeutic relevant units, yielding clozapine and chlorpromazine as the most potent activator of the SREBP system in the context of clinically relevant concentrations. Indeed, these two drugs both have high propensity to induce weight gain [48]. It is also important to consider the lipophilic character of these drugs, with their large distribution volumes leading to drug accumulation in lipid rich tissues. Indeed, concentrations in brain, liver and adipose tissue are 10-30 times higher than their serum concentrations [195, 250, 251]. Thus, the therapeutic relevance of SREBP activation probably varies among the drugs, with the concentrations used in the cell culture experiments highly clinically relevant for some, but not for others.

Studies with the highly active antiretroviral therapy (HAART), which is used in the treatment of HIV infected patients, have suggested that drug-induced effects on the SREBP and PPAR transcription factors might be relevant to metabolic adverse effects. HAART induces metabolic adverse effects, and the occurrence of lipodystrophy and hyperlipidemia has been linked to overexpression of SREBP1 and decreased PPARγ expression in liver biopsies from patients [252]. Further supporting SREBP as a potential target gene for the interindividual variation in drug-induced weight gain is the finding that SREBP polymorphisms are associated with obesity and type-2 diabetes in a non-psychiatric French cohort [253].

9. Concluding remarks

In this study we have reported that antipsychotic drugs activate SREBP controlled lipogenic gene expression in cell cultures and in rats, and preliminary results indicate that such effects also occur in humans. In the cell culture experiments, SREBP activation was associated with elevated levels of cholesterol and triglycerides. Acute clozapine treatment *in vivo* also led to a marked accumulation of lipids in rat liver, but the effects on SREBP gene expression were ambiguous. We proposed that the activation of cellular lipogenesis is relevant for both the therapeutic effect and the adverse metabolic effects of these drugs. This thesis establishes the first steps in a translational research approach, where *in vitro* experiments provide molecular targets for further investigation in clinical studies. The dose-dependent SREBP activation in cell cultures suggests that activation of SREBP in patients are most pronounced in the tissues where antipsychotic drugs have accumulated to high concentrations. Due to the lipophilic character of these drugs, this would be in brain, liver and adipose tissue [195, 250, 251].

Fibrates and other lipid-lowering drugs that stimulate the nuclear hormone receptor transcription factors are used in the treatment of the metabolic syndrome [175]. Our results indicate that antipsychotic drugs act as antagonists on these transcription factors. In parallel with SREBP activation, these results provide another mechanism that might be relevant for the metabolic adverse effects associated with several antipsychotic drugs. Whether effects on nuclear hormone receptors in the CNS are relevant for therapeutic effect remains elusive. Based on our results, we here launch a potentially new mechanism of antipsychotic drug action and provide several novel candidate genes that may be involved in treatment outcome. Further investigations should reveal whether specific variants of these genes are associated

with the therapeutic outcome or occurrence of metabolic adverse effects in patients receiving antipsychotic drugs.

It is interesting that some of the drugs with best therapeutic effect in terms of therapeutic efficacy and continuous treatment [54, 254] are the ones that seem to be most related to the adverse metabolic effects [48, 81]. Indeed, weight gain has been suggested as a prognostic indicator of therapeutic outcome [255]. This was supported by a recent study, demonstrating an association between initial beneficial therapeutic response to clozapine and long-term weight gain [256]. The correlation suggests that the molecular mechanisms behind therapeutic and metabolic adverse effects could to some degree be shared. The antipsychotic-induced activation of the SREBP transcription factors might represent such a shared mechanism of action, which should indeed be the scope of future studies. If adverse effects are inevitable side effects in order to gain optimal therapeutic effect, the design of novel drugs without metabolic adverse effects might be difficult. An alternative approach would then be to develop drugs that suppress the effects of antipsychotic drugs in metabolically relevant tissues without affecting the therapeutic CNS processes.

10. Future perspectives

In order to investigate the role of the metabolically active transcription factors SREBP, PPAR and LXR and their target genes in antipsychotic drug response, further studies should be performed both in animal models and in patients. In order to resemble the clinical situation, long-term antipsychotic drug treatment in animal models should be performed. Due to their lipophilic character, psychotropic drugs accumulate in CNS tissue over time, and it is possible that the drug concentrations required for inducing the gene expression changes in the CNS will occur after weeks rather than days. For the purpose of the metabolic adverse effects, a rat model responding to psychotropic drugs in a manner resembling the clinical situation was recently developed [212] and should be applied to investigate the effect on the metabolic transcription factors in various organs. The effect of co-treatment of antipsychotic drugs and hypolipidemic drugs that mediated their therapeutic effect via activation of LXR and PPAR transcription factors should also be explored.

Gene expression changes in blood samples can mirror expression in the brain [257] and investigation of expression changes of candidate genes in blood and in biopsies from liver and adipose tissue from drug-treated patients might provide valuable information. If SREBP activation is related to therapeutic efficacy and/or metabolic side effects, it is possible that differential gene expression patterns with respect to SREBP-stimulated lipogenesis in blood samples from patients can function as a predictor of therapeutic outcome. Furthermore, studying polymorphisms in SREBP- and related candidate genes could provide gene variants that determine therapeutic outcome, which will facilitate a more individualized treatment with antipsychotic drugs.

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