Taurine and Glutathione in cerebrospinal fluid and plasma from patients with psychiatric disorders and healthy controls

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2. ABSTRACT

A growing body of results indicate that immunological alterations and oxidative stress are of importance in various mental disorders. The inflammatory changes are possible to detect both in plasma and cerebrospinal fluid (CSF), and different psychiatric disorders exhibit similar changes indicating a common underlying mechanism. The antioxidants are of importance to regulate the redox balance and control the inflammatory processes but the causal relationship between psychiatric disorders and increased oxidative stress is however not fully clarified.

Two important antioxidants; taurine and glutathione (GSH), have been suggested to have central nervous system (CNS)-protective properties. They have been found to fluctuate in several mental disorders including schizophrenia and depression but the clinical relevance need further studies.

The general aim of this thesis was to increase the understanding of taurine and the GSH in depression and schizophrenia, two major mental disorders, in comparison to healthy controls.

Correlations between glutathione and taurine levels in blood and CSF were analyzed in healthy male volunteers and we identified a complex pattern of associations showing that the CSF concentration was influenced by body mass index (BMI), age, intraspinal pressure, plasma concentrations the previous day and possible genetic factors. Electroconvulsive therapy (ECT) is used in the treatment of severely depressed patients. In blood collected before the first and after the third ECT, we found a significant decrease in plasma taurine in patients responding to the treatment, while total glutathione was unaltered. In a group of olanzapine treated patients with schizophrenia or schizoaffective disorders, we analysed taurine and glutathione in plasma and CSF and compared with healthy male and female volunteers. We observed increased plasma taurine levels in patients compared with controls, but no difference in CSF taurine and no alteration in glutathione.

This thesis indicates that taurine might play a role in mental disorders such as depression and schizophrenia. Increased knowledge about the complex regulation of taurine and glutathione might provide new insights into the impact of redox balance in the pathophysiology of psychiatric disorder and contribute to a future personalization of the treatment.
3. POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA


Det övergripande syftet med föreliggande studie var att öka kunskapen rörande taurin och GSH vid depression och schizofreni i jämförelse med friska frivilliga deltagare (kontroller).

Ett komplext samband mellan taurin och GSH nivåer i blod och ryggmärgsvätskan påvisades hos friska manliga kontroller vilket visade att ryggmärgsvätskenivåerna påverkades av body mass index (BMI), ålder, tryck i ryggmärgsvätskekanalen, plasmanivåerna och möjligen även av genetiska faktorer. Från deprim erad patienter som genomgick elektrokonvulsiv behandling (ECT) samlades blod in före den första och efter den tredje ECT-behandlingen. En signifikant sänkning av taurinivåerna påvisades i plasma hos patienter som svarade bra på behandlingen, men inte någon förändring av GSH-nivåerna. Hos en grupp patienter med schizofreni som behandlades med det antipsyotiska läkemedlet olanzapin undersöktes taurin och GSH i ryggmärgsvätska och plasma och jämfördes med värdena från friska manliga och kvinnliga kontroller. En ökad nivå av taurin i plasma påvisades jämfört med kontroller men ingen skillnad påvisades av taurin i ryggmärgsvätska eller GSH i ryggmärgsvätska eller blod.

Studien visar att taurin är involverat i sjukdomsprocessen vid depression och schizofreni. Ökad kunskap om den komplexa regleringen av taurin och GSH kan bidra till en ökad förståelse av oxidativa processers betydelse vid psykiatriska sjukdomar och i framtiden förhoppningsvis bidra till en mer individualiserad behandling.
4. ABBREVIATIONS AND ACRONYMS

AUC  area under the curve  
BBB  blood-brain barrier  
BMI  body mass index  
BPRS  Brief Psychiatric Rating Scale  
CNS  central nervous system  
CSF  cerebrospinal fluid  
CYP2D6  cytochrome P450 2D6 protein  
D2 receptor  dopamine-2 receptor  
DNA  deoxyribonucleic acid  
DSM-IV  Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition  
ECT  electroconvulsive therapy  
GABA  gamma aminobutyric acid  
GAF  Global Assessment of Functioning  
GPX  glutathione peroxidase  
GSH  glutathione  
HPLC  high-performance liquid chromatography  
ICD-10  International Statistical Classification of Diseases and Related Health Problems - Tenth Revision  
MADRS  Montgomery–Åsberg Depression Rating Scale  
MAOI  monoamine oxidase inhibitor  
MINI  Mini-international neuropsychiatric interview  
NAC  N-acetyl cysteine
OLA  olanzapine
ROS  reactive oxygen species
SCID-I  Structured Clinical Interview of DSM-IV Axis I Disorders
SCID-II  Structured Clinical Interview of DSM-IV Axis II Disorders
SOD  superoxide dismutase
SSRI  selective serotonin re-uptake inhibitor
WHO  World Health Organization
5. LIST OF PAPERS

Paper I

Paper II

Paper III

Paper IV
Samuelsson M, Skogh E, Lundberg K, Vrethem M, Öllinger K. Taurine and glutathione in plasma and cerebrospinal fluid in olanzapine treated patients with schizophrenia, Submitted
6. INTRODUCTION

6.1 History

Ancient Greek physicians claimed that mood disorders were diseases of the body and not of the spirit. One of the earliest biochemical hypothesis of any mental disorder reflected the presence of black bile, which affected the brain and led to mood darkening. Hippocrates (460-370 BC) described melancholia (black bile) as a state of aversion to food, despondency, sleeplessness, irritability and restlessness. The understanding of schizophrenia as a brain disease did not develop until the 19th century (Sadock et al., 2009). Today, biological and psychosocial approaches have been largely reconciled with a general recognition that genetic and environmental factors interact and that psychological processes are based in and can influence neurobiological mechanisms (Gelder et al., 2009).

6.2 Nomenclature

In medicine, various terms are used to describe different pathological entities. If there is a clear objective or presumed understanding of the aetiology, the term disease is used. If the aetiology or pathophysiology is unknown, the term disorder is used. Disorder is typically diagnosed as a syndrome based on history, clinical symptoms and occasional laboratory findings. Illness is a term that reflects a subjective awareness of distress and sickness and is used to describe the inability to perform normal social roles. (Gelder et al., 2009)

6.3 Diagnostic systems

There are two major diagnostic systems regarding mental disorders: the ICD-10 (International Statistical Classification of Diseases and Related Health Problems - Tenth Revision; World Health Organization, 1994) that is the World Health Organizations (WHO) classification and used throughout most of the world and the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; American Psychiatric Association, 1994) which mainly is used in the United States. These systems are similar and both use syndrome diagnoses. The major groups of mental disorders are listed in table 1.
Mood disorders

Mood disorders are characterised as a pervasive emotional tone varying along an axis from happiness to sadness, which includes biorhythmic and cognitive disturbances. Major depression is the most common mood disorder and bipolar disorder is the disorder with episodes of depression and mania (or hypomania). Clinically, major depressive episodes often arise from low-grade, intermittent and protracted depressive substrate known as dysthymic disorder. Likewise, is the cyclothymic state the low grade of bipolar disorder. The boundaries between normal and abnormal moods are difficult to define, but the most extreme manifestations of low mood, depression, or elevated mood, mania are clear. (Sadock et al., 2009).
6.4.1 Depressive disorder

Depressive disorder is a syndrome with different symptoms such as low mood, reduced energy and loss of interest (Table 2).

The total annual cost of depression in Europe is estimated at Euro 118 billion in 2004. The WHO estimates that depression causes six per cent of the burden of all diseases in Europe in terms of disability-adjusted life years and is worldwide the number four leading cause of burden of disease. This makes depression a major concern to the personal and economic welfare (Mathers et al., 2007; Maes et al., 2009). Depression is a severe and life-threatening illness, exposing the patient to direct risks, such as suicide and a reduced quality of life, and indirect risks, such as negative interactions with other concurrent diseases. The lifetime prevalence of depression has been estimated as 20 per cent among males and 40 per cent among females; the point prevalence has been estimated as 5 to 10 per cent of the adult population (Sadock et al., 2009).

The aetiology of depression is multifactorial. The monoamine hypothesis, formulated in the 1960s, stipulated that monoamines (serotonin, norepinephrine, and dopamine) are important in depression. Strong links between monoamine disturbances and depression have been established; but it is also clear that other factors are important (Schildkraut, 1965; Hindmarch, 2002; Ruhé et al, 2007, Belmaker, 2008). Monoaminergic systems are now primarily considered as broader neuromodulatory systems, and disturbances are likely secondary or epiphenomenal effects related to aetiology and pathogenesis. Presumably, the effects of monoamines involve the modulation of neurocircuits, which in turn affect mood (Delgado and Moreno, 2000; Sadock et al., 2009).

Also other neurotransmitters are implicated in depression. Glutamate and gamma aminobutyric acid (GABA) are the major neurotransmitters for brain signalling, and their receptors have been implicated as possible therapeutic targets in depression (Kendell et al, 2005). In addition, several other amino acids act as neurotransmitters, including aspartic acid, glycine (Cotman and Monaghan, 1986) and taurine (Huxtable, 1992). Also hormones are involved in depression (Sadock et al., 2009).
Table 2. Diagnostic criteria of a depressive episode according to ICD-10

Depression, F32.-

In typical depressive episodes the individual usually suffers from:

1. depressed mood
2. loss of interest and enjoyment
3. reduced energy leading to increased fatiguability and diminished activity. Marked tiredness after only slight effort is common.

Other common symptoms are:

- reduced concentration and attention
- reduced self-esteem and self-confidence
- ideas of guilt and unworthiness (even in a mild type of episode)
- bleak and pessimistic views of the future
- ideas or acts of self-harm or suicide
- disturbed sleep
- diminished appetite.

A duration of at least 2 weeks is usually required for diagnosis. The categories of depressive episodes described in more detail below should be used only for a single (first) depressive episode. Further depressive episodes should be classified as recurrent depressive disorder (F33.-).

F32.0 Mild depressive episode
Diagnostic guidelines: Depressed mood, loss of interest and enjoyment, and increased fatiguability are usually regarded as the most typical symptoms of depression, and at least two of these, plus at least two of the other symptoms described should usually be present for a definite diagnosis. None of the symptoms should be present to an intense degree.

F32.1 Moderate depressive episode
Diagnostic guidelines: At least two of the three most typical symptoms noted for mild depressive episode (F32.0) should be present, plus at least three (and preferably four) of the other symptoms.

F32.2 Severe depressive episode without psychotic symptoms
Diagnostic guidelines: All three of the typical symptoms noted for mild and moderate depressive

Table 2. Diagnostic criteria of a depressive episode according to ICD-10
The cytokine hypothesis states that depression is caused by the stress-related increased production of pro-inflammatory cytokines that in turn increases oxidative and nitrosative damage and causes depression (Maes et al., 2009; Catena-Dell'Ossio et al., 2011). There have also been reports of increased levels of reactive oxygen species (ROS), reduced levels of antioxidants (including glutathione) and alterations of the activity of antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPX), in depression (Sarandol et al., 2007; Kodydková et al., 2009; Stefanescu et al. 2012).

6.5 Schizophrenia

Schizophrenia is characterised by a mixture of positive, negative, disorganisation, cognitive, psychomotor and mood symptoms and affects approximately 0.5 per cent of the world’s population and is worldwide the seventh most costly illness. Positive symptoms of schizophrenia are symptoms that we do not normally experience and negative symptoms are a loss of or diminution of normal functions (Table 3) (Coyle, 2006; Tandon et al., 2009; Sadock et al., 2009).

The disorder can be sub grouped and there is significant heterogeneity in the neurobiology, clinical manifestations, courses as well as treatment responses between patients. Currently, the underlying pathophysiological mechanisms of this disorder are largely unknown. Family, twin and adoption studies have provided evidence of a high heritability of schizophrenia although the development also likely involves environmental factors. Moreover, impaired social outcomes, higher occurrences of other diseases and suicidal rates of 5 per cent have been reported (Tandon et al., 2009; Sadock et al., 2009; Brown, 2011).

No single neurobiological defect is nowadays in focus and studies of schizophrenia interpret studies in broader system contexts. The dopamine hypothesis, which was postulated in 1970, was previously the dominating hypothesis concerning schizophrenia. However, the dopamine hypothesis of schizophrenia is now yielding to a multifactorial view, in which the other monoamines as well as glutamate and GABA are included, with a focus on neurotransmitter interactions in complex neurocircuits (Carlsson et al., 2001; Sadock et al., 2009).
Schizophrenia F20.-

The normal requirement for a diagnosis of schizophrenia is that a minimum of one very clear symptom (and usually two or more if less clear-cut) belonging to any one of the groups listed as (a) to (d), or symptoms from at least two of the groups referred to as (e) to (h), should have been clearly present for most of the time during a period of 1 month or more. Symptom (i) in the above list applies only to the diagnosis of Simple Schizophrenia (F20.6), and a duration of at least one year is required.

(a) thought echo, thought insertion or withdrawal, and thought broadcasting
(b) delusions of control, influence, or passivity, clearly referred to body or limb movements or specific thoughts, actions, or sensations; delusional perception
(c) hallucinatory voices giving a running commentary on the patient's behaviour, or discussing the patient among themselves, or other types of hallucinatory voices coming from some part of the body
(d) persistent delusions of other kinds that are culturally inappropriate and completely impossible, such as religious or political identity, or superhuman powers and abilities (e.g. being able to control the weather, or being in communication with aliens from another world)
(e) persistent hallucinations in any modality, when accompanied either by fleeting or half-formed delusions without clear affective content, or by persistent over-valued ideas, or when occurring every day for weeks or months on end
(f) breaks or interpolations in the train of thought, resulting in incoherence or irrelevant speech, or neologisms
(g) catatonic behaviour, such as excitement, posturing, or waxy flexibility, negativism, mutism, and stupor
(h) "negative" symptoms such as marked apathy, paucity of speech, and blunting or incongruity of emotional responses, usually resulting in social withdrawal and lowering of social performance; it must be clear that these are not due to depression or to neuroleptic medication
(i) a significant and consistent change in the overall quality of some aspects of personal behaviour, manifest as loss of interest, aimlessness, idleness, a self-absorbed attitude, and social withdrawal.

The diagnosis of schizophrenia should not be made in the presence of extensive depressive or manic symptoms unless it is clear that schizophrenic symptoms antedated the affective disturbance. If both schizophrenic and affective symptoms develop together and are evenly balanced, the diagnosis of schizoaffective disorder (F25..) should be made, even if the schizophrenic symptoms by themselves would have justified the diagnosis of schizophrenia. Schizophrenia should not be diagnosed in the presence of overt brain disease or during states of drug intoxication or withdrawal.

Table 3 Diagnosis for schizophrenia according to ICD-10
Studies have also suggested that infections or immunological processes contribute to the aetiology of schizophrenia (Mortensen et al., 2007; Brown, 2011). Studies measuring the peripheral levels of various cytokines in the serum of patients with schizophrenia have provided an inconsistent picture regarding the putative involvement of the immune system in this disease (Potvin et al., 2007). One study of the cytokines in the cerebrospinal fluid (CSF) of first-episode patients with schizophrenia and age-matched healthy volunteers showed that schizophrenia was associated with an activation of the immune system in the brain, at least during the onset of this disease (Söderlund et al., 2009). Moreover, an imbalance in the antioxidant defence system due to persistent oxidative stress has been described, and oxidative damage has been implicated in the pathology of schizophrenia (Dadheech et al., 2008; Bitanihirwe and Woo, 2011).

6.6 Inflammation and oxidative stress

Common for a great variety of diseases including mental disorders is the cytokine induced sickness behaviour (or sickness syndrome). The syndrome is characterised by weakness, malaise, listlessness, anhedonia, hypersomnia, anorexia, social isolation, hyperalgesia, and poor concentration (Dantzer and Kelley, 2007; Saper et al., 2012). Proinflammatory cytokines have also been associated with production of psychiatric symptoms (Konsman et al., 2002; Plata-Salaman, 1998), and the idea that infectious agents contribute to psychiatric disorders has been discussed (Sadock et al., 2009)

Pro-inflammatory cytokines activate neutrophils and macrophages, which produce superoxide radicals and other oxidant substances that induce oxidative stress and reduce cellular antioxidant capacity. Antioxidants defend the body against free radicals through the transformation of these species into less reactive molecules. Free radicals are important in the defence against bacteria and other potentially threatening agents; however, the overproduction of free radicals can cause tissue damage. The immunopharmacology of antioxidants, particularly glutathione (GSH), influences the redox equilibrium in the regulation of cytokines and associated cofactors, thereby controlling oxidative stress. These defensive mechanisms are crucial for survival, and any imbalance that might disturb this intricate oxidant-inflammation association can have detrimental consequences. (Haddad and Harb, 2005; Halliwell, 2006; Halliwell and Guttridge, 2007; Aoyama et al., 2008)
The cells of the human brain utilise 20 per cent of the oxygen consumed by the body but constitute only 2% of the body weight. Consequently, ROS are continuously generated at high rates within the brain during oxidative phosphorylation (Dringen, 2000). The growth and pruning of the neurons in the brain is partially regulated through a redox mechanism that controls the balance between neurodestructive oxidants and neuroprotective antioxidants (Smythies, 1999). Thus, persistent elevated levels of ROS have been implicated in several neurodegenerative disorders (Floyd, 1999).

The causal relationship between psychiatric disorders and increased oxidative stress remains unclear. Oxidative stress has been implicated in several mental disorders. In certain disorders, this imbalance persists, even during remission, and in some disorders the imbalance improves with treatment. These findings suggest that oxidants may play a role in the pathology of psychiatric disorders (Herken et al., 2006; Savas et al., 2006; Ng et al., 2008). (Figure 1)

Figure 1. Oxidative stress is induced by several factors including the action of pro-inflammatory cytokines and activation of immune cells during inflammation. Increased oxidative stress has been observed in several mental disorders.
6.7 Treatment

For moderate depression, pharmacotherapy has been equally recommended with psychotherapy, but for severe depression, pharmacotherapy or electroconvulsive therapy (ECT) has been recommended (Socialstyrelsen, 2010). Nearly all patients with schizophrenia will benefit from pharmacological treatment often in combination with specific psychosocial and psychotherapeutic treatments (Sadock, et al., 2009).

Since the monoamine hypothesis of depression was formulated, pharmacological research and treatment have been mainly aimed at monoamine systems. The most commonly used antidepressants are acting on the monoaminergic systems. Also antipsychotic drugs are aiming at monoaminergic systems primarily through blocking dopamine-2 (D2) receptors. New drugs have been developed to specifically regulate D2 and other receptors that are either stimulated or blocked in different proportions (Sadock et al., 2009).

Several psychiatric medications, including mood stabilisers, antipsychotics and antidepressants, are efficacious for a wide spectrum of psychiatric and neuroprogressive disorders. This apparent lack of specificity suggests that the symptoms of a wide range of disorders and their treatments might share aspects of biology and mechanisms of action (Anderson and Maes, 2012).

6.7.1 Electroconvulsive therapy (ECT)

ECT is an effective treatment mainly used for treatment of severe depression (Pandaya et al., 2007). The mechanism by which ECT obtains its antidepressant effect in humans remains inconclusive but some aspects have been elucidated, such as the modulation of monoamines, neuropeptides, hormones, neurotrophic factors and cytokines (Dinan, 2008; Ishihara and Sasa, 1999; Wahlund and von Rosen, 2003).
6.7.2 Pharmacological principles involving antioxidative and inflammatory aspects

N-acetyl cysteine (NAC), an orally administered bioavailable cysteine, increases plasma cysteine levels and restores depleted GSH pools. NAC is proposed as an augmentation strategy for chronic schizophrenic patients and depressive symptoms in bipolar disorders (Berk et al., 2008a and b). Based on preclinical evidence, clinical studies have recently shown that antioxidant precursor treatment might be effective in schizophrenia and bipolar disorders, providing a novel clinical angle to augment suboptimal conventional treatments (Dean et al., 2009).

Many antidepressants, including ECT, exhibit anti-inflammatory activity either through increasing anti-inflammatory cytokines or decreasing pro-inflammatory cytokines (Hestad et al., 2003, Kubera et al., 2004, Maes et al., 2009). Accumulating evidence has shown that mood stabilisers (valproate and lithium) might buffer oxidative defences. (Kim et al., 2002)

The clinical efficacy of antidepressant treatments in depressed patients and the antipsychotic treatments in schizophrenic patients can be enhanced through co-medication with anti-inflammatory agents, such as the cyclooxygenase-2 inhibitor celecoxib, suggesting that antagonising inflammatory pathways might exert a broad spectrum of activity in neuropsychiatric disorders (Müller et al., 2002, 2006).

6.8 Blood-brain barrier (BBB)

BBB efficiently prevents the blood-to-brain passage of a wide variety of substances. CSF is primarily produced in the choroid plexus through plasma ultrafiltration and active filtration. The total volume of CSF is between 120 and 150 ml, and approximately 500 ml is produced daily (Smith and Forman, 1994). The CSF is secreted through venous blood arachnoidal granulations into the cervical lymph nodes (Monks et al., 1999). Compared with plasma, CSF contains lower levels of proteins, amino acids and calcium (Segal, 2001). Moreover, the chemical composition, particularly the protein content, differs depending on the level of the spine from which CSF is collected (Fishman, 1980). In addition, a diurnal rhythm for monoamines has been reported in CSF (Kennedy et al., 2002; Poceta et al., 2009).
6.9 Taurine and Glutathione (GSH)

6.9.1 The relationship between taurine and GSH

Taurine and GSH are two of the most common substances in the body. These substances partly share the same metabolic pathway and use cysteine for their biosynthesis. Both substances have antioxidant properties, and the literature emphasises their protective properties in the central nervous system (CNS) (Albrecht and Wegrzynowicz, 2005; Atmaca, 2004; Dringen, 2000). When broken down GSH can provide cysteine, which is used to generate taurine (Figure 2).

![Figure 2. Metabolic pathways showing the relationship between GSH and taurine biosynthesis.](image-url)
6.9.2 Glutathione (GSH)

GSH represents the major endogenous low-molecular-weight thiol in mammalian cells (Meister, 1988). GSH is a tripeptide composed of glutamate, cysteine and glycine and is produced by de novo synthesis via the γ-glutamyl-cycle (Figure 3) (Njalsson and Norgren 2005; Aoyama et al., 2008).

Figure 3. GSH is composed of glutamate, cysteine and glycine

GSH is present in millimolar concentrations within the cell. In the brain, GSH is considered as a major antioxidant with a concentration of approximately 2 to 3 mM, which is much higher than that in blood or CSF (Monks et al., 1999; Aoyama et al., 2008). In addition, GSH has been implicated as a neurohormone because it has been observed at high concentrations extracellularly in the brain (Dringen, 2000). GSH is also involved in several fundamental biological functions, including transport, free radical scavenging, protection against exposure to xenobiotics, detoxification of carcinogens, redox reactions, biosynthesis of DNA/proteins/leukotrienes, and neurotransmission/neuromodulation (Figure 4) (Ristoff and Larsson 2007; Meister, 1988; Aoyama et al., 2008).

\[ \text{Gly} - \text{Cys} - \text{Glu} \]

\[ \text{SH} \]
Figure 4. Redox cycling of glutathione. Free radicals, R·, are scavenged by GSH, which forms the thiyl radical, GS·. Two GS· radicals can be combined into the oxidized form of glutathione GSSG, which in turn is converted to GSH by glutathione reductase.

Cysteine is the rate limiting substrate, and for GSH synthesis, neurons rely primarily on the extracellular cysteine delivered from astrocytes (Aoyama et al., 2008). GSH is secreted in the liver and released into the plasma, and this pool undergoes a constant state of turnover. Mammalian cells are, however, inefficient at GSH uptake; the cellular uptake is regulated through γ-glutamyltranspeptidase, which degrades GSH and transports the amino acids across the membrane to facilitate resynthesis in the cell (Pastore et al., 2003). The majority of GSH in the cell remains in the cytoplasm, which is also the place of synthesis (Aoyama et al., 2008). The plasma concentration of GSH and cysteine varies with time of day and correlates with the intake of meals. Plasma cysteine levels peak at approximately three hours after a meal, followed by a maximum level of glutathione at 6 hours after the cysteine peak (Blanco et al., 2007). In rodents, a diurnal rhythm of plasma GSH has been observed, which is coupled to the hepatic variation of GSH and depends on food intake (Jaeschke and Wendel, 1985). In humans, a diurnal rhythm for GSH has been observed in bone marrow, with a maximum at 08.30 am (Smaaland et al., 2002), and in platelets, with a maximum at night (Radha et al. 1985). Similarly, rhythmic changes in the oxidative damage of proteins and lipid molecules have been reported. This phenomenon partly reflects the fact that GPX and glutathione reductase activities follow the rhythm of melatonin (Hardeland et al. 2003). Aging is a critical factor for GSH haemostasis, and GSH declines with increasing age in the brain (Aoyama et al., 2008). Cigarette smokers have been reported to have increased blood levels of GSH,
which is likely an induced defence against the toxic products in cigarette smoke (Halliwell and Gutteridge 2007).

### 6.9.2.1 GSH in the CSF

Glutathione-dependent metabolic barrier mechanisms are active in cell culture models resembling the blood-brain barrier (Ghersi-Egea et al., 2006), and in mouse models, GSH assists in the passage of drugs through the BBB (Wijnholds et al., 2000). The passage of GSH itself over the BBB is, however, difficult (Aoyama et al., 2008). The epithelial cells of the choroid plexus, which are the major sites of CSF production, contain high levels of the enzymes involved in the synthesis and degradation of GSH (Anderson et al., 1989). Thus, these cells are considered major producers of GSH in the CSF. It has been reported that lateral ventricle epididymal cells contain the highest levels of GSH and that the meninges contain considerably higher GSH concentrations than neuronal astrocytes, suggesting that these cells play a major role in brain redox haemostasis (Sun et al., 2006). GSH is released from brain cells and might at least in part contribute to the maintenance of the glutathione levels in the CSF (Dringen, 2000).

### 6.9.2.2 GSH in disorders

GSH deficiency or the depletion of GSH in the brain has been implicated in several pathological conditions in which oxidative stress is evident. Decreased levels of GSH have been observed in Parkinson’s disease, Alzheimer’s disease, amyotrophic lateral sclerosis, retinopathy of prematurity, necrotising enterocolitis, bronchopulmonary dysplasia, patent ductus arteriosus and asthma (Benzi and Morett, 1995; Abramov et al., 2003; Njalsson and Norgren, 2005; Halliwell, 2006; Aoyama et al., 2008).

GSH deficits have been observed in the brain and CSF from schizophrenic patients (Mahadik and Mukherjee, 1996; Do et al., 2000). Elevated levels of GSH have been observed in the temporal lobes in first-episode schizophrenic patients reflecting increased levels of oxidative stress (Wood et al., 2009). Compared to control subjects, the levels of GSH are reduced in the postmortem brains of schizophrenic patients in the caudate region (Yao et al., 2006) and in the prefrontal cortex (Gawryluk et al., 2011). GSH-deficient models have revealed...
morphological, electrophysiological, and behavioural anomalies similar to those observed in schizophrenic patients (Grima et al., 2003; Castagne et al., 2004; Steullet et al., 2006), and these observations support the concept that a dysregulation of GSH metabolism is one of the vulnerability factors contributing to the development of schizophrenia (Tosic et al., 2006; Gysin et al., 2007). In stress-induced rats, after restraining for one hour, reduced GSH brain levels have been detected (Chakraborti et al., 2007). The human brain is metabolically active and thus particularly sensitive to an impaired capacity to react against oxidative stress (Aoyama et al., 2008).

Research evidence supports the involvement of GSH in schizophrenia, as postulated by both the dopamine and glutamate hypofunction hypotheses (Do et al., 2000; Grimaa et al., 2003; Steullet et al., 2006; Matsuzawa et al., 2008). Several studies have shown evidence for a genetic link between schizophrenia and GSH (Tosic et al., 2006; Gysin et al., 2007; Saadat et al., 2007; Matsuzawa et al., 2009). Raffa et al. (2009) proposed that a reduction in blood GSH levels is a biological indicator of the degree of severity of the schizophrenia symptoms.

6.9.3 Taurine

Taurine (2-aminoethanesulfonic acid) is a β-amino acid that has a sulfonate group instead of the carboxyl group typically found in proteogenic amino acids (Figure 5). In a strict sense, taurine is therefore not an amino acid, although it is typically referred to as an amino acid in the literature (Huxtable, 1992; Leon et al., 2009; Marcinkiewicz and Kontry, 2012); herein, taurine will be referred to as an amino acid in this thesis.

![Molecular structure of taurine](image)

Figure 5. Molecular structure of taurine

Taurine is not incorporated into proteins and is the most abundant free amino acid in several tissues and cellular components of blood (Aerts and van Assche, 2002; Schuller-Levis and Park, 2003). In the human foetus, taurine is an essential amino acid, as its biosynthetic
capacity is almost negligible. The nutritional requirements for taurine are typically met both through dietary sources and biosynthesis from cysteine and methionine preferentially in the liver and to a smaller extent, in the brain (Aerts and van Assche 2002; Atmaca, 2004). The plasma level of taurine is dependent on diet, which has been illustrated through evidence that vegans only possess 50 per cent compared with expected levels (Hansen, 2001).

Taurine has a wide range of biological functions, including acting as an inhibitory neuromodulator on glutamate and GABA receptors (Huxtable, 1992; Ye et al., 1997; Wu et al., 2005). Taurine also acts as a neurotransmitter and is important for the development and regeneration of the CNS, modulating calcium homeostasis, membrane stabilisation and immune system regulation. The physiological functions of taurine include bile and xenobiotic conjugation, regulation of neuronal excitability, membrane protection, antioxidation, detoxification and osmoregulation (Huxtable, 1992; Schuller-Levis and Park, 2003; Atmaca, 2004; Marcinkiewicz and Kontny, 2012). Taurine also participates in thermoregulation, the alteration of sleep duration and the suppression of eating and drinking (Huxtable, 1992).

6.9.3.1 Taurine in the CSF

Taurine is a highly water-soluble compound and does not readily pass lipid membranes (Huxtable, 1992). Taurine slowly penetrates into the brain, and its uptake systems are not effective (Huxtable, 1992; Oja 1976). Considering the biosynthesis and degradation of taurine in the brain, the regulation of brain taurine has been attributed to transport over the blood-brain and blood-CSF barriers (Keep and Xiang, 1996). The net transportation of taurine occurs in the direction from the blood to the brain due to a high occurrence of active taurine channels in the BBB (Tamai et al., 1995; Lallemand and De Witte, 2004).

6.9.3.2 Taurine in disorders

There is evidence that taurine plays a role in several pathological states. Taurine is involved in the regulation and modulation of anxiety-like behaviour (Chen et al., 2004; Kong et al., 2006); however, Whirley and Einat (2008) showed no evidence of stimulant, antidepressant or anxiolytic benefits of taurine supplementation in mice. Elevated plasma levels of taurine have
been observed in depressed individuals (Altamura et al., 1995; Mitani et al., 2006), and Maes et al. (1998) observed reduced serum levels of taurine after treatment with antidepressant drugs. Interestingly, the severity of depression has been correlated with the taurine concentration in lymphocytes before treatment (Lima, 2003). Aberrant plasma taurine levels have also been observed in patients with acute polymorphic psychosis (Fekkes, 1994). Altered CSF taurine levels have been reported in two studies using pathological male gamblers (Nordin and Eklundh, 1996; Nordin and Sjödin, 2006).

The properties of taurine as an antioxidant compound (Huxtable, 1992; Marcinkiewicz and Kontry, 2012) could counteract to the inflammatory aspect of depression, which is referred to as the cytokine hypothesis of depression (Maes et al., 2009). Taurine counteracts glutamate-induced elevations in intracellular calcium ions to confer protection against neurodegeneration (Wu et al., 2005 and 2009; Leon et al., 2009).

The development of schizophrenia through intrauterine infections in animal models has revealed an associated decrease in hippocampal taurine levels (Winter et al., 2009). The levels of taurine in the brain medial prefrontal cortex were increased in schizophrenic patients, and taurine levels were associated with the illness duration (Shirayama et al., 2010). Bjerkenstedt et al. (1985) observed elevated plasma taurine levels in schizophrenic patients compared with healthy controls. In unmedicated schizophrenic patients, lower CSF taurine levels have been observed compared with healthy controls (Do et al., 1995), whereas in another study, no differences were observed (Korpi et al., 1987).

Based on the hypothesis that taurine might act as an antioxidant, a pharmacological strategy has been developed proposing that taurine treatment would be more effective during the early stages of schizophrenia compared with conventional treatments using anti-psychotics, such as risperidon and olanzapine (OLA) (Paz et al., 2008).
7. AIMS

The primary aim of this thesis was to increase the understanding of taurine and the tripeptide GSH in healthy controls and in individuals with depression and schizophrenia.

Study 1

The aim of this study was to follow the plasma taurine content during one day and by a lumbar puncture the next morning examining if variations in plasma levels of taurine was reflected in different CSF fractions in healthy males.

Study 2

The aim of this study was to follow blood GSH content during one day and by a lumbar puncture the next morning examining if variations in blood and plasma levels of GSH were reflected in different CSF fractions in healthy males.

Study 3

The aim of this study was to examine if plasma taurine and GSH levels were affected in depressed patients after treatment with three ECT.

Study 4

The aim of this study was to compare the blood and CSF levels of taurine and GSH in well-treated and fairly well-functioning outpatients with schizophrenia medicated with oral olanzapine with control subjects. In the patient group, we also investigated whether the taurine and GSH levels were correlated with symptoms or the level of functioning.
8. MATERIALS AND METHODS

8.1 Population

8.1.1 Healthy male controls

Thirty-six healthy male volunteers were recruited among medical students, hospital staff members and their relatives. These individuals were subjected to a medical check-up, including laboratory tests (electrolytes, blood, kidney, liver and thyroid) and a physical examination. The volunteers were required to have been medication-free for at least one month and free from any form of substance abuse; smoking was allowed. The volunteers were subjected to semi-structured interviews using Structured Clinical Interview of DSM-IV Axis I Disorders (SCID-I) and Structured Clinical Interview of DSM-IV Axis II Disorders (SCID-II). The exclusion criteria included somatic or psychiatric disorders. Thirty-six medication-free and physically healthy volunteers were considered eligible. Five volunteers were excluded due to lumbar puncture failure, and one volunteer did not appear for the study examination and was therefore excluded. CSF and plasma were collected from all 30 of the eligible males, but due to laboratory failure, only 26 of these samples were used in the analysis of reduced GSH (Table 4).

<table>
<thead>
<tr>
<th>Inclusion phase</th>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least 1 week before day 1</td>
<td>8 a.m.</td>
<td>12 noon</td>
</tr>
<tr>
<td>Inclusion/exclusion criteria</td>
<td>Blood sample</td>
<td>Blood sample</td>
</tr>
<tr>
<td>SCID-1 interview</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCID-2 interview</td>
<td>Blood for genotyping</td>
<td></td>
</tr>
<tr>
<td>Laboratory screening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical examination</td>
<td></td>
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<tr>
<td>Informed consent</td>
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</tbody>
</table>

Table 4. Healthy male volunteers, time schedule for examinations
8.1.2 Healthy female controls

Fifty-eight healthy female volunteers were recruited through advertisements at Linköping University. The exclusion criteria included pregnancy, ongoing severe somatic disease, history of mental illness, ongoing medication, including hormonal contraceptives taken during the preceding three months, or taking part in any other study. The volunteers were subjected to a medical check-up, including laboratory tests (electrolytes, blood, kidney, liver and thyroid) and a physical examination. The volunteers were subjected to a semi-structured interview using Mini-International Neuropsychiatric Interview (MINI). Eleven individuals were excluded because of a personal history of mental illness or a history of mental illness in first-degree relatives. Three individuals were excluded because of routine laboratory test results, and one individual was excluded because of technical difficulties in the venous blood tests. Five volunteers left the study because of problems returning to the follow-up visits. Two volunteers started treatment. Ten individuals left the study because of menstrual cycle problems. One individual left the study because of infection during the second visit, and ten individuals rejected lumbar puncture. Thus, 15 physically and mentally healthy female volunteers were considered eligible for the study (Table 5).

<table>
<thead>
<tr>
<th>Inclusion phase</th>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least 1 week before day 1</td>
<td>8 a.m.</td>
<td>8 a.m.</td>
</tr>
<tr>
<td></td>
<td>12 noon</td>
<td>Fasting state</td>
</tr>
<tr>
<td>Inclusion/exclusion criterias</td>
<td>Blood sample</td>
<td>Blood sample</td>
</tr>
<tr>
<td></td>
<td>Blood sample</td>
<td>Blood sample</td>
</tr>
<tr>
<td>MINI 5.0 interview</td>
<td>Blood for</td>
<td>Lumbar puncture</td>
</tr>
<tr>
<td></td>
<td>genotyping</td>
<td></td>
</tr>
<tr>
<td>Laboratory screening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical examination</td>
<td></td>
<td></td>
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<tr>
<td>Informed consent</td>
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<td></td>
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</tbody>
</table>

Table 5. Healthy female volunteers, time schedule for examinations
8.1.3 Depressed patients receiving ECT

Patients treated with ECT at the University Hospital in Linköping were asked to participate in this study. The inclusion criterion included a diagnosis of depression, while the exclusion criteria included younger than 18 years in age, involuntarily committed, subjected to ECT within three months prior to this study, or the inability to understand pertinent information. A senior psychiatrist established the diagnoses using the complete clinical presentation and a MINI interview, or two senior psychiatrists conducted a clinical diagnosis. To estimate the severity of the depression, the psychiatrist performed Montgomery–Åsberg Depression Rating Scale (MADRS) scoring before the first ECT treatment. Initially, 30 patients were included in the study; however, 3 patients refused to participate after inclusion. Venipuncture failed in 3 patients, and 1 patient received acute ECT before venipuncture. Thus, 23 patients were eligible for the study (Table 6). A total of 19 patients were adequately treated with oral antidepressants but did not respond, and 4 patients were treatment naïve.

<table>
<thead>
<tr>
<th>Inclusion phase After decision of ECT but before ECT number 1</th>
<th>ECT number 1</th>
<th>ECT number 3</th>
<th>1-4 days after ECT number 3</th>
<th>After completed ECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inclusion/exclusion criteria</td>
<td>Blood sample before ECT</td>
<td>Blood sample</td>
<td>Renewing of the information to patient about the study</td>
<td></td>
</tr>
<tr>
<td>MINI interview or a clinical diagnosis by two senior psychiatrists</td>
<td></td>
<td>MADRS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MADRS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Informed consent</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Table 6. Depressed patients receiving ECT
8.1.4 Olanzapine-treated patients with schizophrenia or schizoaffective disorder

Fifty-four Caucasian outpatients diagnosed with schizophrenia or schizoaffective disorder according to DSM-IV criteria were identified and screened for inclusion. All patients were prescribed OLA as the only antipsychotic drug treatment. No individuals were first-episode patients, and all but three patients had received prior treatment with antipsychotic drugs other than OLA. The patients had been treated with medication containing OLA for between 0.2 and 11 years (median 2 years) and had been administered the same dose of OLA (2.5-25 mg/day) for at least 14 days. Concomitant drugs were benzodiazepines and/or zopiclone in 10 patients and lithium in three patients. Only somatically healthy patients, as judged by routine laboratory analyses (electrolytes, blood, kidney, liver and thyroid measurements) and a physical examination, were eligible. Of the 54 patients, 37 patients (schizophrenia n=31 and schizoaffective disorder n=6) were eligible and agreed to participate in the study. The Brief Psychiatric Rating Scale (BPRS) and Global Assessment of Functioning (GAF) were used to evaluate the symptoms and level of function, respectively. Plasma was collected from all patients (n=37), and CSF was successfully collected from 24 patients (Table 7).

<table>
<thead>
<tr>
<th>Inclusion phase</th>
<th>Day of testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inclusion/exclusion criteria</td>
<td>8 a.m.</td>
</tr>
<tr>
<td></td>
<td>Fasting state</td>
</tr>
<tr>
<td>Psychiatric interview</td>
<td>Blood sample</td>
</tr>
<tr>
<td>BPRS</td>
<td>Lumbar puncture for those who agreed to this testing</td>
</tr>
<tr>
<td>GAF</td>
<td></td>
</tr>
<tr>
<td>Laboratory screening</td>
<td></td>
</tr>
<tr>
<td>Physical examination</td>
<td></td>
</tr>
<tr>
<td>Informed consent</td>
<td></td>
</tr>
</tbody>
</table>

Table 7. Olanzapine treated patients
8.2 Ethical considerations

All volunteers and patients received verbal and written information, and written informed consent was obtained. Approval for this study was obtained from the Ethics Committee of the Linköping University Hospital (ethical permits numbers: male volunteers M143-04, female volunteers M125-07, patients receiving ECT M187-08, and patients with schizophrenia M75-09). The Swedish Medical Products Agency also approved the study of patients with schizophrenia. The principles embodied in the Declaration of Helsinki were adhered to.

8.3 Collection of blood and CSF

For the male controls, the study comprised two days (Table 4). On day 1, blood samples were drawn at 8.00 a.m., noon, 4.00 p.m. and 8.00 p.m. Subsequently, the volunteers were requested not to eat anything after midnight until the next day (day 2), when a blood sample was drawn, and a lumbar puncture was performed at 8.00 a.m. There were no restrictions concerning posture or rest before the puncture.

The female controls and patients with schizophrenia were requested not to eat anything after midnight until the next day, when a blood sample was drawn, and a lumbar puncture was performed at 8.00 a.m. There were no restrictions concerning posture or rest before the puncture (Tables 5 and 7).

In the patients receiving ECT, fasting blood samples were collected after one night of sleep before the first ECT session and at least 24 hours after the third ECT when fasting morning samples were drawn (Table 6).

8.3.1 Venepuncture

8.3.1.1 Plasma

For the collection of blood plasma, blood samples were obtained in heparinised vacutainers and centrifuged in a Sigma 203 centrifuge at 3500 rpm (1438 g) for 10 minutes. The plasma was separated immediately and frozen at −70°C until further analysis.
8.3.1.2 Whole blood

Blood samples for cytochrome P450 2D6 protein (CYP2D6) genotyping were collected in EDTA vacutainers and stored at -20°C until further analysis. Blood for the analysis of reduced glutathione was collected in vacutainers and immediately deproteinised after the addition of 1 volume of blood to 4 volumes of ice-cold 5% (wt/vol) metaphosphoric acid (Lang et al., 2002). After centrifugation, the clear supernatants were removed and frozen at -20°C until further analysis.

8.3.2 Cerebrospinal fluid (CSF)

Because previous studies have shown that the technique used for CSF sampling is important (Nordin et al., 1982; Nordin, 1989; Eklundh, 2000, Teunissen, 2009), a standardised procedure was used. At approximately 8.00 a.m., a disposable needle (BD Whitacre Needle 0.7 x 90 mm) was inserted at the L 4-5 level with the subject in the right decubitus position. For convenience, a pillow was placed under the subject’s head. Intraspinal pressure was measured using a disposable spinal fluid manometer (Optidynamic®, Mediplast) before and after the collection of CSF. In healthy controls, three 6-ml CSF fractions were collected, and in patients with schizophrenia, two 6-ml fractions were collected. The CSF was allowed to drip into a plastic test tube, and the CSF collection time was recorded using a stopwatch. The 6-ml fractions of CSF were protected from light and centrifuged in a Sigma 203 centrifuge at 3500 rpm (1438 g) for 10 minutes within 30 minutes after the puncture. Each 6-ml sample was divided into three 2-ml aliquots, which were stored in a freezer (-70°C) until further analysis. The neuroaxis distance (the length of the spine from the external occipital protuberance to the puncture site) was measured in the lying position.

8.4 Analysis of the samples

8.4.1 Analysis of reduced glutathione (GSH)

GSH was determined from whole blood using high-performance liquid chromatography (HPLC) on a Kromasil 100-5C18 reverse-phase column (Hichrom, Reading, UK) as previously described (Honegger et al., 1989). The GSH was detected using a BAS LC-4B Amperometric Detector (Bioanalytical Systems Inc., West Lafayette, IN, USA) equipped with
a gold electrode at 0.50 V. The mobile phase was delivered at 0.5 ml/min and contained 0.1 M sodium phosphate buffer, pH 2.5, supplemented with 0.1 mM EDTA and 1 mM n-octyl sodium sulphate and 5% methanol. The GSH concentration in each sample was calculated from a standard curve using standard solutions of GSH.

8.4.2 Analysis of the total amount of GSH (reduced and oxidised)

In the plasma and CSF, the total amount of GSH (reduced and oxidised) was analysed spectrophotometrically from the continuous reduction of 5,5’-dithiobis-(2-nitrobenzoic acid) according to Akerboom and Sies (1981) using a standard curve. Two hundred microliters of CSF were mixed with 0.1 M potassium phosphate buffer supplemented with 1 mM EDTA, pH 7.0, containing 5,5’-dithiobis-(2-nitrobenzoic acid) and 48 µM NADPH. The reaction was initiated after the addition of 0.003 U/ml glutathione reductase (Roche). The reaction was measured spectrophotometrically at 412 nm, and the absorbance change due to the reduction of 5,5’-dithiobis-(2-nitrobenzoic acid) per minute was used as an indirect measurement of the total glutathione content (Figure 6).

Two reactions are coupled:

Enzyme catalyzed reduction of GSSG to GSH by the enzyme glutathione reductase:

\[
GSSG + NADPH \rightarrow 2 \text{GSH} + NADP^+
\]

Then DTNB is reduced to TNB by glutathione:

Figure 6. Summary of the reactions used to analyse the total GSH in the blood and CSF.
8.4.3 Analysis of taurine

Using HPLC on plasma and CSF samples, taurine was separated, along with other amino acids, using a Biochrome 30 amino acid analyser. The amino acids were detected using spectrophotometry. The EZ Chrom Elite programme was used for the final determination of concentrations (Jeppsson and Karlsson, 1972; Ekberg et al., 1974; Brattström et al., 1988). The procedure was performed at Labmedicin Skåne (Klinisk kemi Malmö, Sweden), which has an accredited laboratory for the analysis of amino acids in plasma and CSF.

8.4.4 Genotyping

Genomic DNA was isolated from whole blood using the QIAamp® DNA Blood Mini Kit (QIAGEN Ltd). The CYP2D6 alleles *3, *4, *6, *7 and *8 were analysed using TaqMan® Pre-Developed Assay Reagents for allelic discrimination (primers, probes, positive controls: part numbers 4312554, 4312555, 4312556, 4312557 and 4312558) and the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The CYP2D6*5 allele (total deletion of the gene) was detected using long PCR, followed by 1% agarose gel electrophoresis (Hersberger et al., 2000). The CYP2D6 gene duplication, which usually confers ultra-rapid metabolism, was analysed using long PCR as described previously (Steijns and van der Weide, 1998). When neither the CYP2D6 variants *3, *4, *5, *6, *7, *8 nor gene duplications were detected, the allele was classified as functional CYP2D6*1.

8.5 Electroconvulsive Therapy (ECT)

ECT was performed in the morning after at least six hours of fasting. The patients were administered atropine before anaesthesia, which was induced with thiopental, and muscle relaxation was induced with succinylcholine. The patients were ventilated with 100 per cent oxygen. Right unilateral ECT treatments with ultrabrief or brief pulse waves were performed using a Mecta spECTrum 5000Q device (MECTA Corp, Oregon), and the dosage was adjusted according to age, sex and outcome. The seizure was monitored using electroencephalography and a stopwatch.

Because several ECTs are required to induce an anti-depressive effect the effect of a series of three ECT was examined. Fluctuations in several amino acids (but not in taurine) have been
detected up to 24 hours after ECT in a previous report (Palmio et al., 2005). To avoid such changes, fasting blood samples were collected at least 24 hours after the third ECT. Additional factors that might affect the levels of taurine and glutathione include muscle relaxation or anaesthetic agents which might act neuroprotective, possibly due to their reduction of extracellular glutamate concentrations (Miao et al., 1998). A study design using a sham group would have eliminated these confounding factors but could not be used due to ethical considerations. Each patient served as his or her own control.

8.6 Psychiatric rating scales and diagnostic instruments

8.6.1 Mini-International Neuropsychiatric Interview (MINI)
MINI (Sheehan et al., 1998) is a short semi-structured clinical interview aiming to diagnose psychiatric disorders according to the diagnostic systems of the DSM-IV (American Psychiatric Association, 1994) and ICD-10 (WHO, 1994). The interviewer uses a decision tree structure that makes it possible to skip the associated questions, and the duration of the interview is approximately 15 minutes. The Swedish version 5.0.0 was used. MINI was used in studies three and four.

8.6.2 Structured Clinical Interview of DSM-IV Axis I Disorders (SCID-I)
SCID-I (First et al., 1997a) is a semi-structured diagnostic interview divided into separate modules aiming to diagnose psychiatric disorders according to the diagnostic system of the DSM-IV (American Psychiatric Association, 1994). The modules begin with an entry question that allows the interviewer to skip the associated questions. The duration of the interview is approximately one hour for a non-psychiatric patient, but the interview can last for up to two hours for psychiatric patients. SCID-I was used in studies one, two and four.
8.6.3 Structured Clinical Interview of DSM-IV Axis II Disorders (SCID-II)

SCID-II (First et al. 1997b) is a semi-structured interview to assess personality disorders according to the diagnostic systems of the DSM-IV (American Psychiatric Association, 1994). The SCID-II Personality Questionnaire is used as a screening tool before the interview, and the assumption is that a subject who responds with a "no" on the questionnaire would also have answered "no" to the same question in the interview. SCID-II was used in studies one, two and four.

8.6.4 Montgomery–Åsberg Depression Rating Scale (MADRS)

MADRS is a ten-item depression rating scale designed to be particularly sensitive to treatment (Montgomery and Åsberg, 1979; Keller, 2003). The ratings are based on a clinical interview that starts with broadly phrased questions concerning symptoms to more detailed questions that allow a precise rating of severity. The duration of the interview is approximately 15 minutes. MADRS was used in study three.

8.6.5 Brief Psychiatric Rating Scale (BPRS)

BPRS measures the severity of the signs and symptoms of psychopathology. After a mental status interview, the interviewer rates the patient symptoms. The rating takes 2-3 minutes after the interview (Overall and Gorham, 1962; Sadock et al., 2009). BPRS was used in study four.

8.6.6 Global Assessment of Functioning (GAF)

GAF (American Psychiatric Association, 1994) is a numeric scale (0 through 100) used to subjectively rate the social, occupational, and psychological functioning of adults. The scale reports the judgment of the clinician concerning the overall level of functioning in the individual. The maximum level (a score of 100) indicates efficient functioning over a wide range of activities; the minimum level (a score of one) indicates a persistent danger that the individual will hurt him/herself or others. GAF was used in study four.
8.7 Statistical methods

The statistical analysis was performed using the Statistica 8 software programme (StatSoft, Tulsa, OK, USA, 2007). A p-value < 0.05 was considered significant.

Study 1

The plasma concentration of taurine versus time was plotted, and the area under the curve (AUC) was measured using the linear trapezoidal method (Roland and Tozer, 1980). An overall difference was analysed using a within-subject analysis of variance (ANOVA). Repeated post-hoc comparisons using t-test for dependent samples were performed. To evaluate associations, multiple regression analyses employing the best subset regression technique were used (Hill and Lewicki, 2006).

Study 2

The Shapiro-Wilk W test was used to assure the normal distribution of the material. A t-test for dependent samples was used when comparing the laboratory data. Multiple regression analyses employing the best subset regression technique were used to determine associations (Hill and Lewicki, 2006). Pearson’s correlation analysis was used to determine correlations.

Study 3

A t-test for dependent samples was used to compare the laboratory data before and after ECT, and a t-test for independent variables was used to compare the groups before the first ECT. Pearson’s correlation analysis was used for the correlation analysis. For the MADRS values, a Wilcoxon matched-pair signed-rank sum test was used to compare the data before and after three ECT, and a Mann-Whitney U test was used to compare the groups before the first ECT. Each patient served as his or her own control.

Study 4

Kolmogorov-Smirnov and Lilliefors tests were used to verify normal distribution. A t-test for independent variables and Pearson’s correlation analysis were used for the data analysis.
9. RESULTS AND DISCUSSION

9.1 Paper I

In this paper, the relationship between plasma and CSF taurine was studied in healthy males. The results showed a difference between the plasma concentrations of taurine at 4.00 p.m. and 8.00 p.m. on the day before the CSF sample was obtained, indicating a diurnal rhythm. A previous study showed the rhythmicity for all amino acids in plasma, except taurine, threonine, glutamate, alanine, tyrosine and total tryptophan (Riggio et al., 1989). However, a diurnal rhythm for taurine was observed in the rat striatum, which peaks at 8.00 p.m. (Fernández-Pérez et al., 2010). If circadian metabolic control is directly clock-regulated or controlled through circadian rest activity and food intake remains unknown (Dallmann et al., 2012). Whether there is an interaction between plasma taurine and melatonin function is an intriguing question, as both compounds play a role in the regulation of biorhythms (Durlach et al., 2002).

Brain taurine is to a small extent dependent on biosynthesis and degradation in the brain (Huxtable, 1989), while the primary regulation of brain taurine is attributed to the flux in and out of the brain at the blood-brain and blood-CSF barriers through a Na⁺-dependent transporter. The main flux occurs from the blood to the brain (Keep and Xiang, 1996; Lee and Kang, 2004; Ohtsuki, 2004). A possible correlation between taurine in plasma and CSF has been questioned (McGale et al., 1977; Hagenfeldt et al., 1984). In this study no correlation was observed, but when using multiple regression analyses, plasma taurine levels at 4.00 p.m. on day 1, intraspinal pressure and body mass index (BMI) impacted the CSF/plasma ratio. These results are consistent with our observation that the level of taurine at 4.00 p.m. influences the CSF/plasma ratio the following morning. Based on the results of this study, it is reasonable to conclude that there is a complex association between taurine in plasma and CSF, at least in healthy males. (Table 8)
It has been previously reported (Nordin et al., 2003) that CSF taurine is influenced by the cytochrome P450, CYP2D6 genotype. Among the volunteers of our study, we observed three poor metabolisers with a higher AUC (area under the plasma concentration versus time curve) for taurine calculated between 8.00 a.m. to 8.00 p.m. (0-12 h) on day 1 compared with the 27 extensive metabolisers. Although the number of observations was too small to make a statistical evaluation, this result confirms the hypothesis that the CYP2D6 genotype could influence taurine transport from the blood to the CSF or in the opposite direction.

In summary, our findings indicate that taurine has a complex association between blood and CSF, which is potentially genetically predisposed and influenced by plasma taurine on the day before CSF sampling and BMI and intraspinal pressure measurements.
9.2 Paper II

In this study, the relationship between GSH in plasma and CSF was studied in healthy males. When analysing 3 consecutive fractions (0-6, 7-12 and 13-18 ml) of CSF, GSH peaked in fraction 2 (Figure. 7). The CSF is produced in the choroid plexus, and it has been suggested that the content of consecutive fractions of CSF might reflect a timeline of its production; i.e., the first fraction represents CSF produced the previous day while the following fractions mirror the content of CSF produced during the night and early morning, respectively. Therefore, disrupted gradients of monoamines in the CSF have been suggested in previous studies to represent a diurnal rhythm (Nordin et al., 1995; Kennedy et al., 2002; Poceta et al., 2009), and our results indicate that the GSH levels might also vary in such a way.

Figure 7. Glutathione concentrations in CSF. Three fractions (0-6, 7-12 and 13-18 ml) of CSF were collected. Concentration of total glutathione (reduced and oxidized) is presented as mean ± SEM. Fraction 2 is separated from fractions 1 and 3 (p<0.002).

In plasma, a minor reduction in concentration was observed when comparing the GSH level at 4 p.m. on day 1 and the sample collected at the time of the lumbar puncture. The difference is small, and its physiological impact is difficult to evaluate. In blood collected at different
times, no overall differences in the GSH concentrations were observed. Previous studies in both mice and humans have suggested that plasma GSH levels are coupled with food intake and peak several hours after a meal (Jaeschke and Wendel, 1985; Blanco et al., 2007). However, we observed no differences in blood or plasma GSH when comparing the fasting and non-fasting samples (i.e., the samples taken at 8 a.m. on days 1 and 2). The time points for meals and sampling were, however, not adjusted to reveal such a correlation in our experimental setup. The differences between the three CSF fractions might mirror a diurnal rhythm of GSH that corresponds with daytime food intake, but because no corresponding fluctuation was observed in the blood or plasma, this correlation is unlikely.

The second fraction of CSF correlated negatively with blood GSH drawn at noon the previous day and at the time of the lumbar puncture. The negative correlation between blood GSH at the time of the lumbar puncture and CSF total glutathione content in the second fraction indicates that the interrelationship is more complex and does not solely reflect a diurnal rhythm. Because confounding factors are of importance when analysing CSF (Eklundh, 2000), a regression analysis was performed and revealed an association between GSH in plasma at 8.00 p.m. the day before the lumbar puncture and the second and third fraction of the CSF. Age was also a confounding factor in the second fraction. The hypothesis of multifactorial influence on the diurnal rhythm was strengthened by the fact that confounders varied depending on the fraction analysed. Considering the previously possible identified relationship between taurine levels and polymorphisms of the CYP2D6 protein (Nordin et al., 2003; Paper I), we determined if there were any differences between levels of GSH in the blood and CSF in poor and extensive metabolisers. No such differences were observed in this study, but the low number of poor metabolisers makes this evaluation uncertain (n=2).

In conclusion, we observed associations between glutathione in plasma and CSF fractions two and three. Because of the disrupted gradient in the CSF with higher levels of glutathione in the second fraction, these results must be taken into consideration when planning future studies of GSH in the CSF.
9.3 Paper III

This study was undertaken to investigate whether GSH and taurine levels were altered in depressed patients treated with ECT. Previous studies have shown that glutathione levels are increased after treatment with mood stabilisers in depression (Wang et al., 2004). However, the serum levels of taurine are reduced after treatment with antidepressant drugs (Maes et al., 1998). In this study, the analysis of the blood samples before and during ECT treatment showed that plasma taurine levels were lowered during treatment, which is consistent with the results of previous research regarding plasma taurine and depression. However, GSH was not affected.

To further analyse the overall decrease in plasma taurine levels following a series of three administrations of ECT, the data were sub grouped according to responders and non-responders after three treatments. The subsequent analysis revealed that the plasma taurine levels were significantly reduced in patients who responded to ECT but not in non-responders, even if they also showed a reduction in taurine levels (Table 9). Several studies have shown elevations of plasma taurine levels in depressed patients (Altamura et al., 1995; Mitani et al., 2006), and our findings are consistent with previous studies that have shown a reduction in serum taurine levels during treatment with antidepressants (Maes et al., 1998).

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Taurine (μmol/L)</th>
<th>before ECT1</th>
<th>after ECT3</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (23)</td>
<td></td>
<td>59.5±16.0</td>
<td>53.3±11.8</td>
<td>0.01</td>
</tr>
<tr>
<td>Responders (7)</td>
<td></td>
<td>57.4±8.3</td>
<td>49.6±13.5</td>
<td>0.03</td>
</tr>
<tr>
<td>Non-responders (16)</td>
<td></td>
<td>60.5±18.6</td>
<td>54.9±11.0</td>
<td>0.09</td>
</tr>
</tbody>
</table>

* Taurine was compared using student’s t test, significant results are marked in bold letter

Table 9. Plasma taurine in patients before the first and after the third ECT therapy.

Notably, with the exception of four patients, all of our patients received previous antidepressant medication in adequate doses and for an adequate time without responding before ECT was given. Nevertheless, these patients responded significantly to ECT, and seven patients were considered responders after three ECT administrations. The decreases in MADRS scores were significant in both groups (Figure 8). For both the responders and non-responders, the decrease in the MADRS score was consistent with or even larger than
previous results obtained following pharmacological treatment for 1-2 weeks (Januel et al., 2003). This raises the question if patients might benefit from a more early treatment with ECT when on unsatisfactory pharmacological treatment.

Figure 8. MADRS scores determined before the first and after the third ECT administration. The patients were grouped depending on the treatment outcome into responders (MADRS score ≤15; n=7) and non-responders (MADRS score >15; n=16). The values are presented as the means ± SD.

Our results indicate that taurine might be involved in depression but the pathophysiological mechanism is not yet clarified.
9.4 Paper IV

Similar to the alterations of taurine and glutathione observed in depressed patients, schizophrenic patients showed elevated plasma taurine levels (Bjerkenstedt et al., 1985) and reduced GSH levels (Raffa et al., 2009) compared with healthy controls. In this study, taurine and GSH levels were analysed in 37 schizophrenic patients treated with OLA and 45 healthy controls. Plasma taurine was increased in patients compared with controls, but there were no other differences between the patients and controls regarding taurine in CSF (Figure 9) and GSH levels in plasma or CSF. After subgrouping the male and female patients, the plasma taurine levels were increased in both groups compared with controls. In the control group, females had lower plasma taurine levels compared with male controls, but in the patient group, no statistically significant differences between females and males were detected. In contrast, Do et al. (1995) showed lower taurine levels in the CSF in unmedicated patients with schizophrenia compared with controls. An unexplored possibility is that the taurine level in CSF was normalized due to properties of OLA.

![Figure 9](image_url)

Figure 9. Taurine concentrations in the plasma and CSF of patients with schizophrenia compared with controls. The top of the histogram shows the mean value, and the whiskers show the standard deviation.

Raffa et al. (2009) hypothesised that a decrease in blood GSH reflects a decreased CSF GSH level and that blood GSH is a biological indicator of the degree of the severity of schizophrenia symptoms and not related to the daily dosage of typical antipsychotics. However, the pool of plasma GSH is considered to be the pool that is most sensitive to
oxidation and is also easily available for exchange with other compartments, such as the CSF. In this study, no correlations between taurine and GSH levels and the symptoms or function of the disorder (GAF and BPRS with subscales) were observed.

In the comparisons between controls and patients with schizophrenia, the groups were not fully identical. The controls were somewhat younger and had lower BMIs. Regarding the female controls, these individuals were in the same phase of the menstrual cycle and had to be free from any anticontraceptives, which might theoretically have impacted the results of this study. We attempted to standardise the procedure of lumbar puncture, but some confounders were difficult to address. In contrast to other studies, which often used patients with other disorders as controls, this study employed healthy controls.

In summary, higher levels of taurine in plasma were observed in patients with OLA-treated schizophrenia compared with controls, implicating the involvement of taurine in the pathophysiology of the disease. The absence of differences between patients and controls regarding GSH in plasma or CSF is an interesting finding per se in the perspective of earlier reports of a dysregulation of GSH metabolism in schizophrenia. We propose that the relatively high level of function and treatment with OLA might have influenced our results.
10. CONCLUDING REMARKS

In this thesis we have studied taurine and GSH in depression and schizophrenia and in healthy controls. Changes in taurine and GSH have previously been described in both the plasma and CSF of individuals with mental disorders; and orally given augmentation therapy with taurine and GSH precursors have shown positive results on depression as well as schizophrenia treatment. Due to this and their antioxidant and CNS-protective properties, studies on taurine and GSH as markers of disease state are of importance and may have potential clinical relevance.

In studies one and two, we observed a complex relationship between the plasma and CSF concentrations of taurine and GSH, respectively. This makes it possible to estimate CSF concentrations of taurine and GSH from blood samples, at least in healthy males.

In the third study the plasma taurine levels were lowered during improvement of depression when treated with ECT. GSH was, however, not affected. Taurine may thus be involved in depression but it is still unknown in what way.

In the fourth study plasma taurine was found to be higher in OLA treated schizophrenic patients than in controls, otherwise no differences emerged.

Plasma taurine was thus affected in both the depression and schizophrenia study. Glutathione levels in whole blood and plasma was not changed in depressed and schizophrenic patients when compared to healthy controls. In addition no differences in glutathione CSF was found in schizophrenic patients.

Thus, this thesis indicate that taurine might play a role in mental disorders such as depression and schizophrenia, and that the level of taurine may be altered during different treatment regimes, and possible to follow by a blood test. The correlation between blood and CSF levels of taurine and glutathione has a complex pattern, which is influenced by, for example: BMI, age, intraspinal pressure, plasma concentrations previous day and possibly genetic factors, as shown in this thesis
11. FUTURE PERSPECTIVES

Oxidative stress has been implicated in several mental disorders although the causal relationship remains unclear. In certain disorders, the redox imbalance persists, even during remission, and in some disorders, the imbalance improves with treatment. These findings suggest that oxidants may play a part in the pathology of psychiatric disorders. Considering the deviant GSH and taurine levels in several neurological and psychiatric disorders, further studies are needed to evaluate the possibility to follow the course of the illness by sampling of taurine and GSH.

Many of the mental disorders have a cyclicity and it is a challenge to help the patients to remain in recovery and not relapse. There is a great need for new laboratory parameters that might indicate an elevated risk for relapse. Changes in inflammatory parameters have been found in recovered patients but the clinical relevancies of these findings are unsure. Future studies should not solely examine the levels of taurine and GSH in the acute phases of the disorder but also during recovery. Such knowledge might improve the personalisation of the treatment.

GSH has shown deviant values in several studies but not in ours. Whether the reduced, oxidized or the balance between reduced/oxidised forms is the most important parameter to analyse requires further examination.

The efficacy of ECT is well known and well documented. Our results are thus in line with earlier studies. Even so, the result has to be high lightened. Even if 19 of 23 patients were medicated in adequate dosage and time did the entire group reduce their MADRS-score with nearly 50 per cent after three ECT sessions. This raises many questions. The most important question is if patients might benefit from a more early treatment with ECT when on unsatisfactory pharmacological treatment?

When looking at the past century, it is rational to suggest that the aetiological considerations regarding mental disorders have made great progress. Yes, it is undoubtedly so. However, what about the ancient Greeks who over 2500 years ago proposed that the presence of black bile leads to mood darkening through its influence on the brain? Indeed, this theory might be more accurate than most clinicians have considered. Even if the theories of today do not involve the influence of the planet Saturn, as proposed by ancient Greeks, taurine was first discovered in the bile of the Greek animal Taurus (hence the name).
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