University of Alberta

The Investigation of Neuroactive Steroids, Kynurenine Metabolites and Glutamate in Schizophrenia

by

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ABSTRACT

Dysfunctional glutamate and γ-aminobutyric acid neurotransmitter systems are considered key players in the pathophysiology of schizophrenia, and recent evidence suggests that neuroactive compounds that modulate these systems, including neuroactive steroids (NASs) and kynurenine metabolites, may also be implicated in the illness. To investigate the role of these neuroactive compounds in schizophrenia, this thesis first examines NAS abnormalities during the chronic stage of illness. This is followed by a series of longitudinal studies examining plasma neuroactive compound and brain glutamate levels in acutely ill patients, where the relationship between plasma and brain markers of glutamatergic neurotransmission is also explored.

In the first experiment, NAS plasma concentrations before and after a pentagastrin-induced panic attack were examined in chronically ill patients with schizophrenia and in patients with panic disorder. Both patient groups had significantly lower levels of the putatively anxiogenic NASs dehydroepiandrosterone (DHEA) and pregnenolone (PREG) prior to panic induction. Also, schizophrenia and panic disorder patients had similar patterns of NAS plasma level fluctuations following panic induction, which may help explain the high comorbidity rates between the two seemingly separate disorders.

In the latter set of experiments, plasma glutamate, NASs and kynurenine metabolites, and brain glutamate were quantified in unmedicated patients with first episode psychosis, and followed throughout the initial course of treatment with atypical antipsychotics. Unmedicated patients with first episode psychosis had elevated plasma 3α,5β-tetrahydroprogesterone concentrations, which normalized with atypical

antipsychotic treatment, and atypical antipsychotics decreased plasma DHEA sulfate, PREG sulfate and kynurenic acid concentrations. Also, there was no relationship between brain glutamate and any of the neuroactive compounds quantified in the plasma. However, this relationship should be re-examined when more sensitive techniques for *in vivo* quantification of brain metabolites are available. These longitudinal studies provide new insight into the role of NASs, kynurenine metabolites and glutamate in first episode psychosis, help ascertain if these systems are implicated in the disease process or in the mechanism of action of antipsychotic drugs, and provide insight into the relationship between peripheral and central measures of glutamatergic neurotransmission.

DEDICATION

This thesis is dedicated to my husband, Scott, for your constant love, understanding encouragement and unfaltering support. Thank you for putting things in their proper perspective.

To my parents, Roman and Irina, thank you for teaching me the value of education, and for your encouragements and support throughout my academic and life endeavors.

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3α,5α-THDOC	3α,5α-tetrahydrodeoxycorticosterone
3α,5α-THP	3α,5α-tetrahydroprogesterone
3α,5β-THP	3α,5β-tetrahydroprogesterone
AC-PC	anterior commissure-posterior commissure
AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid
ANCOVA	analysis of covariance
ANOVA	analysis of variance
BAI	Beck Anxiety Inventory
3β,5α-THP	3β,5α-tetrahyprogrogesterone
BBB	blood-brain barrier
3β,5β-THP	3β,5β-tetrahydroprogesterone
CCK	cholecystokinin
CCK-4	cholecystokinin tetrapeptide
CCK-5	pentagastrin
CCK-B	cholecystokinin type B
CDSS	Calgary Depression Scale for Schizophrenia
Cr	creatine plus phosphocreatine
CSF	cerebrospinal fluid
D_1	type 1 dopamine
D_2	type 2 dopamine
D4	deuterated
DA	dopamine

DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders (4^{th} ed. Text Revised)
EEPIC	Edmonton Early Psychosis Intervention Clinic
EPS	extrapyramidal side effects
FEPs	first episode psychosis patients
GABA	γ-aminobutyric acid
GAD	glutamic acid decarboxylase
GAF	Global Assessment of Function scale
GAT-1	GABA transporter-1
GC	gas chromatography
Gln	glutamine
Glu	glutamate
Glx	a combination of Glu and Gln
GM	gray matter
HAM-A	Hamilton Anxiety Rating Scale
HCs	healthy control subjects
HFBI	heptafluorobutyrylimidazole
3-HKYN	3-hydroxykynurenine
^{1}H MRS	proton magnetic resonance spectroscopy
HPLC	high performance liquid chromatography
HVA	homovanillic acid

IBLC	*N*-isobutyryl-L-cysteine
KYN	kynurenine
KYNA	kynurenic acid
LC	liquid chromatography
LSD	lysergic acid diethylamide
M	metabolite
MANOVA	multivariate analysis of variance
MIS	Magical Ideation Scale
μM	micromolar
mPFC	median prefrontal cortical
mRNA	messenger ribonucleic acid
MS	mass spectroscopy
NAA	*N*-acetyl aspartyl
NAAG	*N*-acetyl-aspartyl glutamate
NAc	nucleus accumbens
NASs	neuroactive steroids
NI-CI	negative ion chemical ionization
nM	nanomolar
NMDA	*N*-methyl-D-aspartate
OPA	o-phthaldialdehyde
PANSS	Positive and Negative Symptom Scale
PCP	phencyclidine
PDs	patients with panic disorder

PFC	prefrontal cortex
PGWB	Psychological General Well-Being Index
PLA	placebo
PREG	pregnenolone
PREGS	pregnenolone sulfate
PROG	progesterone
PSS	Panic Symptom Scale
PVB	parvalbumin
QUIN	quinolinic acid
SCID-1	Structured Clinical Interview for DSM-IV Axis I Disorders
SD	standard deviation
SPE	solid-phase extraction
STAI	State Trait Anxiety Inventory
SZs	patients with schizophrenia
T	time
TDO	tryptophan 2,3-dioxygenase
Trp	tryptophan
VAS-A max	Visual Analogue Scale for maximum change in anxiety
WM	white matter

Chapter 1

Introduction

Versions of parts of this chapter have been published (Shulman Y, Tibbo P (2005), *Can J Psychiatry* 50:695-702; Shulman Y, Tibbo P (2005), *UAHSJ* 2:22-7)

1.1 INTRODUCTION

Twenty percent of Canadians experience a mental illness during their lifetime. Mental illness substantially reduces the quality of life of affected individuals and their families, and costs our economy billions of dollars each year. Schizophrenia is one of the most disabling mental illnesses, and it affects one percent of the Canadian (and world) population. The fourth edition text revised Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) classifies schizophrenia as a psychotic disorder, a category that also includes schizophreniform disorder, schizoaffective disorder, delusional disorder, brief psychotic disorder, shared psychotic disorder, psychotic disorder due to a general medical condition, substance-induced psychotic disorder, and psychotic disorder not otherwise specified (American Psychiatric Association and DSM-IV 2000). Presence of psychotic symptoms (e.g. hallucinations, delusions) is common to all these disorders; however, severity as well as the number of symptoms, duration of illness, mood symptom presentation, and the underlying cause(s) of the disturbance vary between disorders. Schizophrenia has a duration of at least six months, including a period of at least one month (or less if successfully treated) of active-phase symptoms, described as two or more of the following: delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behavior, and negative symptoms (affective flattening, alogia, and avolition). If delusions are bizarre, or hallucinations consist of either a voice keeping up a running commentary on the person's thoughts or actions, or two or more voices conversing, then only one of these symptoms is needed for a diagnosis (American Psychiatric Association and DSM-IV 2000). Due to the duration criteria of schizophrenia,

a person has to be assessed six months after they experience their first psychotic episode before a diagnosis of schizophrenia can be established.

While there is currently no cure available for schizophrenia, symptom improvement is most commonly achieved with antipsychotic drugs. There are two classes of antipsychotics: first generation ("typical") and second generation ("atypical"). Typical antipsychotics (e.g. chlorpromazine, haloperidol, trifluoperazine) are thought to exert their therapeutic effects by blocking post-synaptic type 2 dopamine (D_2) receptors. However, there is a time lapse between receptor blockade and symptom improvement, where receptors are blocked almost immediately following drug administration, but several weeks pass before symptom improvement is seen (Nestler et al 2001). Typical antipsychotics have many side effects, including sedation, hypotension, dry mouth, blurred vision, constipation, and the motor side effects, tardive dyskinesia (involuntary movement) and extrapyramidal side effects (EPS). EPS are divided into three major categories: 1) parkinsonian syndrome (characterized by rigidity, difficulty in initiating movement, and resting hand and arm tremor); 2) acute dystonia (characterized by sudden spastic contractions of muscles, usually of the face and neck); and 3) akathisia (restlessness and anxiety) (Nestler et al 2001; Sadock et al 2005). Furthermore, while typical antipsychotics can improve positive symptoms (e.g. hallucinations and delusions), they are ineffective at improving the more enduring negative and cognitive symptoms.

The disadvantages of typical antipsychotics have prompted the development of atypical agents (e.g. clozapine, olanzapine, risperidone, quetiapine) which are less likely to cause tardive dyskinesia and EPS, and which may have better efficacy against negative and cognitive symptoms, and can successfully treat some patients who are resistant to

typical agents (Nestler et al 2001). Atypical antipsychotics interact with dopamine (DA), serotonin/5-hydroxytryptamine, muscarinic acetylcholine, adrenergic, and histamine receptors. The mechanism of action of atypical antipsychotics is unclear, but is thought to be predominantly through the serotonin and DA systems. Unfortunately, with the exception of motor symptoms, atypical antipsychotics have similar side effects to those exhibited by typical agents and also cause significant weight gain, and increase the risk of weight-related illnesses, including cardiovascular disease and diabetes (Sadock et al 2005). Furthermore, similar to typical antipsychotics, atypical agents provide symptom improvement, but do not cure schizophrenia. In order to improve treatment for this devastating illness, further research into the etiological and pathophysiological processes of schizophrenia is needed.

1.2 LITERATURE REVIEW

1.2.1 Neurochemical Theories of Schizophrenia

The etiology of schizophrenia is presently unknown; however, insight into the pathophysiological changes associated with the illness is emerging. Many neurotransmitters are implicated in schizophrenia, including DA, serotonin, glutamate (Glu) and γ-aminobutyric acid (GABA).

1.2.1.1 DA and Schizophrenia

The DA hypothesis is presently the best established of the schizophrenia hypotheses. The classical DA hypothesis postulates that hyperdopaminergia in subcortical regions, specifically in the dopaminergic pathway projecting to the limbic

system (mesolimbic tract), is responsible for the positive symptoms of schizophrenia (e.g. delusions and hallucinations) (Sadock et al 2005). This is based on the findings that drugs that enhance DA neurotransmission, such as amphetamine, are psychotogenic in healthy individuals and exacerbate positive symptoms in patients with schizophrenia (SZs), while DA receptor antagonists (e.g. typical antipsychotics) provide effective positive symptom improvement (Sadock et al 2005). Furthermore, increased striatal D_2 receptor occupancy by DA has been found in schizophrenic patients (Abi-Dargham et al 2000), and multiple studies have found elevated D_2 receptor density in the brains of individuals with schizophrenia. However, many of these studies have been conducted on individuals who were taking antipsychotic medication, making it unclear whether D_2 receptor density abnormalities are due to the disease process, or due to the effects of medication (Owen and Simpson 1994). Also, investigations of DA metabolite levels, such as homovanillic acid (HVA) levels, in schizophrenia have produced inconsistent results (Sadock et al 2005).

A major downfall of the classical DA hypothesis is that typical antipsychotics (D_2 receptor blockers) improve positive symptoms, but are ineffective at improving the more enduring cognitive and negative symptoms of schizophrenia. Consequently, the DA hypothesis was expanded to include hypodopaminergia in dopaminergic pathways projecting to the prefrontal cortex (PFC) (i.e. the mesocortical tract), with reduced type 1 DA (D_1) receptor activation in this region. It is now believed that hypodopaminergia in the mesocortical DA system may be implicated in the negative and/or cognitive symptoms of schizophrenia (Abi-Dargham and Moore 2003). The hypodopaminergic arm of the DA hypothesis emerged primarily from indirect evidence from studies that found a

relationship between low cerebrospinal fluid (CSF) levels of HVA, thought to reflect low DA activity in the PFC, and poor performance on working memory tasks in SZs (Kahn et al 1994; Weinberger et al 1988). The most compelling direct evidence for this hypothesis emerged from positron emission tomography studies that showed increased D_1 receptor density in SZs, believed to be a compensatory upregulation of prefrontal D_1 receptors following sustained DA depletion (Abi-Dargham and Moore 2003). While DA does appear to play an important role in schizophrenia, current research does not provide unequivocal evidence for a primary DA lesion in the illness.

1.2.1.2 Serotonin and Schizophrenia

The serotonin hypothesis of schizophrenia emerged prior to the DA hypothesis. It was first believed that a deficiency in serotonin was responsible for the psychotic symptoms of schizophrenia, when it was found that lysergic acid diethylamide (LSD), a potent hallucinogen, had serotonin antagonistic properties (Sadock et al 2005). It was later determined that LSD had both agonistic and antagonistic effects on the serotonin system, thereby possibly implicating either a serotonin deficiency or an excess in schizophrenia (Aghajanian and Marek 2000; Baumeister and Hawkins 2004). With the formulation of the DA hypothesis, the serotonin hypothesis was almost abandoned until the discovery of atypical antipsychotics. As atypical antipsychotics block serotonin receptors, it is currently believed that serotonin receptor over-activity, especially of type 2A serotonin receptors, may be implicated in schizophrenia (Aghajanian and Marek 2000; Baumeister and Hawkins 2004); however, as with DA, serotonin is only a piece of the pathophysiological puzzle in this complex mental illness.

1.2.1.3 GABA and Schizophrenia

GABAergic hypofunction in the PFC and the hippocampus have recently been implicated in schizophrenia pathophysiology. Presynaptic changes include decreases in GABAergic neuronal density and GABA synthesis, with compensatory up-regulation of postsynaptic $GABA_A$ receptors and decreased GABA re-uptake. The following section describes GABA dysfunction in schizophrenia in detail.

1.2.1.3.1 *Presynaptic GABAergic Changes*

Multiple postmortem studies have found low GABA levels in the amygdala of SZs, when compared to healthy control subjects (HCs) (Blum and Mann 2002). For example, Perry and coworkers found decreased GABA concentrations in the nucleus accumbens (NAc) and the thalamus of postmortem brain tissue from SZs (Perry et al 1979). Low GABA levels are thought to reflect altered activity or levels of the GABA synthesizing enzyme and GABAergic neuronal marker, glutamic acid decarboxylase (GAD). GAD activity was found to be decreased in the NAc, amygdala, hippocampus, and putamen in SZs (Bird et al 1977), and low levels of GAD messenger RNA (mRNA) have been found in eleven studies, using five different brain banks for the examination of postmortem brain tissue collected from schizophrenic patients (Akbarian and Huang 2006; Benes et al 2007). However, increased GAD levels and activity in the hippocampus (Heckers et al 2002), prefrontal (Gluck et al 2002; Hashimoto et al 2005b) and occipital (Dracheva et al 2004) cortices, have also been reported. High GAD levels/activity may be a mechanism to compensate for GABAergic deficits seen in schizophrenia, or they may

reflect the hypoglutamatergic state hypothesized in the illness (described in the following section).

Findings from several laboratories strongly suggest that reduced GAD mRNA expression and activity are not caused by antipsychotic drug administration. For example, haloperidol had no effect on GAD mRNA expression in the PFC in monkeys (Volk et al 2000), and chronic typical or atypical antipsychotic administration increased GAD levels in the rat brain (Sakai et al 2001). Furthermore, several days of amphetamine treatment, which mimics the hyperdopaminergic state and some symptoms of schizophrenia, decreased extracellular GABA and intracellular GAD levels in the rat NAc (Lindefors et al 1992), and chronic administration of quinpirole (a D_2 receptor agonist) decreased GAD mRNA expression in rat brains (Laprade and Soghomonian 1995). These studies suggest that administration of compounds that mimic some neurochemical abnormalities thought to be present in schizophrenia (i.e. hyperdopaminergia) also causes alterations in GAD consistent with those found in the illness, while antipsychotics have either no effect, or have an opposite effect on these GAD abnormalities.

GABA reuptake also appears to be altered in schizophrenia. In a postmortem investigation, Simpson and coworkers found a decrease in the number of GABA reuptake sites in the amygdala, hippocampus, and the left temporal cortex in SZs (Simpson et al 1989). Similarly, Volk and coworkers found decreased expression of GABA transporter-1 (GAT-1) mRNA (Volk et al 2001), while Pierri and coworkers found decreased GAT-1 immunoreactivity in the PFC in SZs, when compared to HCs (Pierri et al 1999). A decrease in GABA reuptake may serve to compensate for low GABA levels found in SZs. Interestingly, Volk and coworkers also found that GAT-1 mRNA expression was

not altered in monkey PFC following chronic treatment with haloperidol, implying that decreased GAT-1 mRNA expression and GABA uptake sites are not caused by antipsychotics, but rather are part of the disease process, or may be endogenous compensatory mechanisms that serve to correct GABA dysfunction in schizophrenia (Volk et al 2001).

Reports of altered GABAergic neuronal density/number, particularly in the cortex and the limbic system, further implicate GABA dysfunction in schizophrenia. In a postmortem study, Benes and coworkers found decreased nonpyramidal neuronal density in anterior cingulate and prefrontal cortices in SZs when compared to HCs (Benes et al 1991). More recently, Volk and coworkers found a decrease in the number of GAD mRNA-positive interneurons (i.e. GABAergic interneurons) in the PFC (Volk et al 2000), and Benes and coworkers found decreased nonpyramidal neuronal density in the hippocampus (Benes et al 1998). Reduced GABAergic neuronal density/number is likely not caused by antipsychotics, since chronic haloperidol administration increased GABA-immunoreactive terminals in the rat medial PFC (Vincent et al 1994), and patients treated with antipsychotics for the shortest duration of time had the greatest reduction in the number of chandelier axonal terminals and GAD-immunoreactivity in the hippocampus, compared to those treated for a longer duration (Todtenkopf and Benes 1998).

GABAergic chandelier and basket neurons, which express the calcium binding protein parvalbumin (PVB), seem to exhibit the greatest deficits in schizophrenia. Hashimoto and coworkers found reduced PVB mRNA expression in the PFC in patients (Hashimoto et al 2003). Other PVB abnormailities in schizophrenia include decreased number of PVB-immunoreactive neurons in the entorhinal cortex (Reynolds et al 2002),

all regions of the hippocampus (Zhang and Reynolds 2002), and the PFC (Beasley and Reynolds 1997; Beasley et al 2002; Reynolds et al 2002). Interestingly, PVB-containing neuronal cell deficits may be a potential marker for schizophrenia with a genetic, rather than an environmental etiology. PVB-immunoreactive neuronal deficits are most severe in patients with no ventricular enlargement (Reynolds and Beasley 2001), and ventricular enlargement is primarily found in patients with no family history of the illness (Schwarzkopf et al 1991). PVB-containing neuronal loss also supports the neurodevelopmental hypothesis of schizophrenia, which postulates that a disruption of brain development early in life underlies the later emergence of symptoms during early adulthood (McGrath et al 2003). As calcium binding proteins, including PVB, are neuroprotective, and PVB is expressed late during development, a window of vulnerability during early development, prior to the expression of PVB, may result in a subtle neurotoxic challenge that may lead to neuronal loss, and contribute to the emergence of clinical symptoms during adolescence (Reynolds and Beasley 2001).

1.2.1.3.2 *Postsynaptic GABAergic Changes*

Changes in postsynaptic targets of GABAergic neurons, including pyramidal and nonpyramidal neurons have been found in postmortem studies. Several studies using bicuculline as a selective $GABA_A$ receptor antagonist, revealed higher than normal [^{3}H]-muscimol binding in the hippocampus (Benes et al 1996a), the PFC (Benes et al 1996b), the anterior cingulate cortex (Benes et al 1992) and the superior temporal gyrus (Deng and Huang 2006) in SZs. Increased muscimol binding implies an upregulation of postsynaptic $GABA_A$ binding sites, which is likely a compensatory mechanism for

presynaptic GABAergic deficits. Volk and coworkers also found that SZs have increased α_2 subunit density, which is primarily localized at the axon initial segment of pyramidal neurons, reflecting an increase in $GABA_A$ receptor density at chandelier cell targets (Volk et al 2002). The researchers also found an inverse relationship between α_2 subunit density and GAT-1 density, indicating that presynaptic alterations in chandelier cells are associated with postsynaptic changes in $GABA_A$ receptors at the axon initial segment of pyramidal neurons. This study further implicates chandelier cell deficits in schizophrenia pathophysiology (Volk et al 2002).

1.2.1.3.3 *Possible Clinical Manifestations*

Working memory dysfunction is a cognitive deficit in schizophrenia that is associated with prefrontal cortical abnormalities. Working memory allows an individual to retain an image based on recent sensory information, to integrate the image with cognitive and affective associations and to use the image to plan subsequent behavior (Nestler et al 2001). Information processing, including the input of relevant sensory information and the formation of appropriate behavioral responses, is dependent on long-range connections between the PFC, the thalamus, and posterior cortical areas (Lewis and Gonzalez-Burgos 2000). Pyramidal cells of the PFC receive sensory input from the thalamus, and play a critical role in processing the information prior to sending it to appropriate brain regions. SZs perform poorly on tasks that require proper working memory function, such as the Wisconsin Card Sorting task (Volk and Lewis 2002), and fail to show normal activation of the dorsolateral PFC when performing this task (Lewis and Lieberman 2000). Since prefrontal cortical inhibitory (GABAergic) interneurons play

a role in regulating both the input and output of pyramidal neurons in the PFC, decreased GABAergic tone can disrupt pyramidal neuronal activity, in turn contributing to working memory dysfunction.

Dysfunctional GABAergic neurotransmission in the hippocampus may also be implicated in poor memory and affective symptoms of schizophrenia. Pyramidal neurons of the CA1 sector of the hippocampus receive sensory information from the entorhinal cortex via two inputs: a direct pathway and an indirect pathway through the dentate gyrus and the CA2/3 (Heckers and Konradi 2002). The hippocampus plays a crucial role in regulating both memory and affect by comparing the two inputs from the entorhinal cortex and sending appropriate information to the cortex and limbic structures, respectively (Heckers and Konradi 2002). GABAergic neurons in the CA1 sector of the hippocampus play an important role in feed-forward inhibition of hippocampal pyramidal neurons in the CA1 sector (Benes and Berretta 2001) and GABAergic hypofunction may cause an over-stimulation of the cortical and limbic structures by CA1 pyramidal neurons, possibly contributing to memory dysfunction and affective symptoms, respectively.

1.2.1.4 Glu and Schizophrenia

Glu dysfunction is thought to play a major role in the pathophysiology of schizophrenia. Two opposing hypotheses on how Glu may be altered in schizophrenia have been proposed: 1) Glu hypofunction hypothesis and 2) Glu hyperfunction hypothesis. The Glu hypofunction hypothesis postulates that *N*-methyl-D-aspartate (NMDA) Glu receptors are hypofunctional in individuals with schizophrenia (Abi-

Dargham and Moore 2003; Jentsch and Roth 1999; Krystal et al 1994). It is thought that hypofunctional NMDA receptors located on GABAergic interneurons cause neuronal disinhibition with subsequent increase in Glu release. This in turn causes hyperactivation of non-NMDA Glu receptors and postsynaptic excitotoxic changes, followed by reduced Glu release. The NMDA receptor hypofunction hypothesis is supported primarily by studies examining the clinical effects of phencyclidine (PCP) and other non-competitive NMDA receptor antagonists (e.g. ketamine) which produce a syndrome in normal individuals that closely resembles schizophrenia, and exacerbate symptoms in patients with stable chronic schizophrenia (Itil et al 1967; Javitt and Zukin 1991). Unlike other psychotogenic agents, which produce only positive symptoms, NMDA receptor antagonists are unique because they can also produce negative and cognitive symptoms that are characteristic of schizophrenia (Jentsch and Roth 1999). Behavioral effects of partial deletion of the NMDA-R_1 subunit in mice also implicate NDMA receptor hypofunction in the illness. NMDA-R_1 subunit hypomorphic mice exhibit behavioral abnormalities that may be representative of schizophrenia symptoms, including increased locomotion and stereotypic behavior, which are ameliorated with haloperidol and clozapine treatment, and deficits in social and sexual behavior, which improve with clozapine, but not with haloperidol treatment (Mohn et al 1999). Similar behavioral changes in mice have also been seen following PCP administration (Mohn et al, 1999). Interestingly, Glu hypofunction may also be responsible for the DA dysfunction hypothesized in schizophrenia. While acute PCP exposure increases DA in the NAc and PFC, subchronic (three weeks) exposure to PCP increases DA release in the NAc region and decreases DA in the PFC, thus mimicking the hyper- and hypodopaminergic states

hypothesized in schizophrenia (Carboni et al 1989; Hertel et al 1995; Jentsch and Roth 1999).

The hyperglutamatergic hypothesis of schizophrenia was proposed by Deakin et al (Deakin et al 1989). The basis of this hypothesis is the finding of overabundant glutamatergic synapses in the frontal cortex in SZs (Deakin and Simpson 1997; Simpson et al 1998). In support for the Glu hyperfunction hypothesis Dursun et al found that lamotrigine, a Glu release inhibitor, improved psychopathology when used as an adjunct treatment agent to clozapine in treatment resistant patients (Dursun et al 1999). In a later study conducted by the same group of researchers, where lamotrigine was again used an adjunct to clozapine and four other antipsychotics (risperidone, olanzapine, haloperidol, and fluphenthixol), lamotrigine again proved to be effective in the augmentation of clozapine, but not of the other antipsychotics in treatment resistant patients (Dursun and Deakin 2001). Also, topiramate, a Glu kainate and α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) receptor antagonist, did not improve psychopathology when used as an adjunct to any of the antipsychotics tested in this study (Dursun and Deakin 2001). Since lamotrigine improved symptoms only when added to clozapine, and topiramate, another Glu inhibiting agent, had no efficacy when added to any of the antipsychotics tested, it is likely that the mechanism behind the synergistic effects of lamotrigine and clozapine is independent of the excessive Glu release in patients hypothesized by the researchers. Thus, more research testing the Glu hyperfunction hypothesis of schizophrenia is needed.

1.2.1.4.1 *Postmortem Glutamatergic Changes*

Postmortem studies have found abnormal Glu levels, as well as abnormal levels of enzymes and intermediates of Glu metabolism in SZs. For example, Tsai and coworkers found low Glu levels in the hippocampus and PFC in SZs, when compared to HCs (Tsai et al 1995). In the same study levels of *N*-acetyl-aspartyl Glu (NAAG), a neuropeptide that antagonizes the effects of Glu at NMDA receptors, were increased, and the activity of Glu carboxypeptidase II, the enzyme that cleaves NAAG into Glu and *N*-acetyl-aspartyl (NAA), was decreased in the frontal cortex, temporal cortex and hippocampus in SZs (Tsai et al 1995). More recently, Burbaeva et al found decreased enzymatic activity of glutamine (Gln) synthetase, the enzyme that converts Glu into Gln, and increased enzymatic activity of glutamate dehydrogenase, the tricarboxylic acid cycle enzyme that converts Glu into α-ketoglutarate and ammonia, in the PFC of patients (Burbaeva et al 2003). These enzymatic alterations may result in decreased Glu levels in the neurotransmitter pool. Also, as discussed earlier, abnormal prefrontal cortical and hippocampal levels and activity of GAD, the enzyme that converts Glu into GABA, have also been reported (Akbarian and Huang 2006; Gluck et al 2002; Hashimoto et al 2005b; Heckers et al 2002).

Abnormalities in Glu receptor subunit densities and receptor expression found in SZs were reviewed by Goff and Coyle (2001), and are summarized below. Increased kainate receptors in the PFC and decreased AMPA and kainate receptor binding in the hippocampus were found in SZs. However, findings of NMDA receptor density abnormalities have not been consistent. Reports of abnormal Glu receptor expression in schizophrenia patients include low AMPA receptor-encoding mRNA levels in the hippocampus and parahippocampus, as well as low levels of thalamic mRNA encoding

AMPA, kainate, and NMDA receptor subunits. High NMDA receptor subunit NR_{2D} levels in the PFC and low NMDA receptor subunit NR_1 levels were also found in the hippocampus. It is unlikely that NMDA receptor subunit abnormalities are caused by treatment with antipsychotics, as chronic haloperidol administration had no effect on NMDA receptor subunit composition in the rat hippocampus (Goff and Coyle 2001).

1.2.1.4.2 *Brain, CSF and Peripheral Glu Level Abnormalities*

Quantification of brain Glu and Gln *in vivo* recently became possible with the development of high field strength (≥ 3 Tesla) proton magnetic resonance spectroscopy (^{1}H MRS). Increased Gln in the left dorsolateral PFC (Stanley et al 1996), left anterior cingulate cortex and thalamus (Theberge et al 2002; Williamson et al 2003) were found in first episode psychosis patients (FEPs), compared to HCs. Glu and Gln decreased in the thalamus of FEPs following 30 months, but not nine months, of treatment with antipsychotics, a finding that is consistent with reports of low Glu and Gln in the left anterior cingulate cortex of chronically ill medicated patients (Theberge et al 2003; Williamson et al 2003). However, findings of higher than normal Gln in the thalamus of medicated (Theberge et al 2003; Williamson et al 2003) and in the left medial PFC of never-treated (Bartha et al 1997; Williamson et al 1999) chronically ill SZs have also been reported. Also, in a study linking NMDA receptor hypofunction and abnormalities in the Glu-Gln cycle, ketamine administration caused an acute increase in Gln in the anterior cingulate cortex in HCs (Rowland et al 2005).

At lower field strength (< 1.5 Tesla), Glu and Gln are difficult to separate due to the overlap of resonances (generally not an issue at higher field strengths of ≥ 3 Tesla).

As a result, most reliable lower field strength MRS studies report Glu+Gln (Glx). A reduction in Glx in the PFC of chronic patients, compared to drug-naïve FEPs (Ohrmann et al 2006; Ohrmann et al 2005) and HCs (Choe et al 1994; Ohrmann et al 2006; Ohrmann et al 2005), as well as findings of lateralized abnormalities, with left sided deficits of Glx in the hippocampus of SZs, compared to HCs, have been reported (Kegeles et al 2000). Other studies, however, found no differences in Glx in the medial temporal lobe (Bartha et al 1999), dorsolateral PFC (Stanley et al 1995) and the left frontal lobe (Block et al 2000) between SZs and HCs.

Reports of abnormal CSF and peripherally circulating (serum/plasma) Glu level abnormalities further implicate Glu dysfunction in schizophrenia. Kim et al first reported low CSF Glu levels in SZs (Kim et al 1980), but subsequent studies produced inconsistent results, with several reports of no alterations in CSF Glu levels in patients (Do et al 1995; Hashimoto et al 2005a; Perry 1982; Tsai et al 1998). However, the ratio of Gln to Glu was significantly higher than normal in the CSF of drug-naïve FEPs in one of these studies (Hashimoto et al 2005a).

In the few schizophrenia studies examining peripherally circulating (serum/plasma) Glu and Gln levels, abnormalities such as high Glu levels (Tortorella et al 2001; van der Heijden et al 2005) and low Gln levels (Rao et al 1990) in unmedicated chronic patients, and low Glu levels in drug-naïve FEPs (Palomino et al 2007) have been reported, while other studies found no differences in Glu or Gln in acutely ill patients, compared to HCs (Alfredsson and Wiesel 1989). Also, there is evidence to suggest that antipsychotics may exert some of their therapeutic effects by affecting peripheral Glu levels. In two studies, serum Glu concentrations increased after a switch from

conventional antipsychotics to olanzapine in schizophrenic patients (Evins et al 1997; Goff et al 2002), and Tortorella et al also found that plasma Glu levels decreased in neuroleptic-resistant SZs after 12 weeks of clozapine treatment (Tortorella et al 2001), while other atypical antipsychotics had no effects on these levels (van der Heijden et al 2005). In a recent longitudinal investigation, Palomino et al found that plasma Glu levels, which were lower than normal in drug-naïve FEPs, progressively increased, and reached levels that were comparable to normal after six and 12 months of treatment with antipsychotics (Palomino et al 2007). Also, Maeshima et al found that SZs who were taking atypical antipsychotics had higher plasma Glu levels during the remission stage, compared to the active stage of a psychotic episode (Maeshima et al 2007).

1.2.2 Neuroactive Steroids (NASs) in Schizophrenia

1.2.2.1 What are NASs?

Classical steroid hormones (e.g. cortisol, dihydrotestosterone, and aldosterone) exert their effects via genomic mechanisms. Due to their lipophilic nature, steroid hormones diffuse through the cell membrane into the cytosol, where they bind to their intracellular receptors, which subsequently change conformation and dissociate from associated chaperone molecules (Nestler et al 2001). These bound complexes then translocate to the nucleus, bind as homo- or heterodimers to their respective response elements (DNA sequences in the promoter region of a gene) and regulate gene expression. Thus, when bound to their intracellular receptors, classical steroid hormones act like transcription factors. Steroid hormones require hours to days to exert their effects because they are limited by the rate of protein synthesis (Evans 1988).

In the 1940s, Selye discovered that some progesterone (PROG) metabolites exhibit fast, central effects. In particular, he found that these metabolites had potent sedative and anesthetic effects in rodents, and that these effects were too rapid to be explained by genomic mechanisms of action (Selye 1941; Selye 1942), indicating that some steroids may affect neuronal function via non-genomic mechanisms. The term "neuroactive steroids" was later coined to describe those steroids that exhibit rapid, non-genomic effects on neuronal excitability by interacting with, and modulating the activity of cell surface ligand-gated ion channel receptors, including $GABA_A$ and NMDA receptors (Paul and Purdy 1992; Rupprecht and Holsboer 1999) (Figure 1-1).

NASs can be synthesized in the brain and in peripheral tissue from cholesterol (Figure 1-2), as synthetic enzymes are present in neurons and glia in the brain, and in peripheral endocrine glands. The rate-limiting step of neurosteroidogenesis is the transport of cholesterol though the mitochondrial membrane by the steroidogenic regulatory protein and the peripheral benzodiazepine receptor (Hauet et al 2005). Cholesterol is then converted to pregnenolone (PREG) by the P450 side chain cleavage enzyme in the inner mitochondrial membrane. PREG is then metabolized to other steroids by P450 and nonP450 enzymes in different cellular compartments. All reactions in the neurosteroidogenic pathway, with the exception of those catalyzed by 3α-hydroxysteroid oxidoreductase, are irreversible (Hojo et al 2004; Stoffel-Wagner 2001; Stoffel-Wagner 2003), and steroidogenic enzymatic activity is inhibited when $GABA_A$ receptors are activated (Do-Rego et al 2000).

Brain NASs originate from both peripheral endocrine glands and the brain. Due to their lipophilic nature, NASs are likely able to cross the blood-brain barrier (BBB).

O'Dell et al showed that brain and plasma NASs increase in parallel after acute ethanol administration in rats, and adrenalectomy/gonadectomy prevents this increase (O'Dell et al 2004). These findings implicate peripheral endocrine glands, rather than the brain, as the source of some NASs following ethanol administration, indicating that peripheral NASs can penetrate the BBB.

As NASs cannot bind to intracellular steroid receptors, it was initially thought that they did not exert their effects via genomic mechanisms. However, Rupprecht et al found that the 3α-hydroxy ring A-reduced pregnane steroids 3α,5α-tetrahydroprogesterone (3α,5α-THP) and 3α,5α-tetrahydrodeoxycorticosterone (3α,5α-THDOC) can regulate gene expression by interacting with intracellular PROG receptors when oxidized to 5α-pregnane steroids (Rupprecht et al 1993). Thus, these NASs can regulate neuronal function through both genomic and non-genomic mechanisms. Through their fast modulating effects at ligand-gated ion channels, and possibly through their genomic effects, NASs exhibit neuropsychopharmacological properties. For example, PREG and dehydroepiandrosterone (DHEA) may possess memory-enhancing properties, and 3α-hydroxy ring A-reduced pregnane steroids exhibit sedative, hypnotic, anesthetic and anxiolytic properties (Rupprecht 1997; Rupprecht and Holsboer 2001).

NASs synthesized in the brain and the periphery are among the most selective, potent and efficacious allosteric modulators of the $GABA_A$ receptor complex (Follesa et al 2001). At nanomolar (nM) brain concentrations, which can be found during stressful situations in rats (Purdy et al 1991), 3α-reduced NASs, including the PROG metabolites 3α,5α-THP and 3α,5β-tetrahydroprogesterone (3α,5β-THP), and the deoxycorticosterone metabolite 3α,5α-THDOC, are positive allosteric modulators of $GABA_A$ receptors

(Zinder and Dar 1999). These NASs act by increasing the frequency of chloride channel opening (Macdonald and Olsen 1994; Twyman and Macdonald 1992). At micromolar (μM) concentrations, 3α-reduced NASs also activate $GABA_A$ receptors in the absence of GABA. However, these NASs are not found at such high concentrations physiologically (Follesa et al 2001; LeMelledo and Baker 2002), except during parturtion (Stoffel-Wagner 2003). 3β-reduced PROG metabolites, including 3β,5α-tetrahydroprogesterone (3β,5α-THP) and 3β,5β-tetrahydroprogesterone (3β,5β-THP), act as functional antagonists of $GABA_A$-agonistic steroids, since they inhibit the effects of 3α-reduced NASs, rather than modulate $GABA_A$ receptors directly (Lundgren et al 2003; Prince and Simmonds 1992; Prince and Simmonds 1993).

Other NASs such as DHEA, PREG and their sulfated metabolites, DHEAS and PREGS, are negative $GABA_A$ receptor or positive NMDA receptor modulators (Rupprecht and Holsboer 1999; Twyman and Macdonald 1992). At low μM concentrations PREGS and DHEAS are negative $GABA_A$ receptor modulators (Imamura and Prasad 1998; Park-Chung et al 1999). PREGS acts as a mixed $GABA_A$ receptor agonist/antagonist and DHEAS behaves solely as an antagonist. DHEA is also antagonistic at $GABA_A$ receptors; however it is less potent than DHEAS (Baulieu and Robel 1998; Imamura and Prasad 1998; Park-Chung et al 1999). PREGS is also a potent positive allosteric modulator of NMDA receptors. DHEA and DHEAS also display NMDA agonistic properties (Macdonald and Olsen 1994); however, these effects are not as potent as those seen with PREGS (Baulieu and Robel 1998).

1.2.2.2 NAS Levels in Schizophrenia

There are several reports of NAS abnormalities in schizophrenia (Table 1-1). Early work by Tourney and Erb revealed low DHEA plasma levels, first thing in the morning, in chronically ill, unmedicated SZs, compared to HCs (Tourney and Erb 1979). In a preliminary investigation the same group also found that abnormal diurnal rhythm of plasma DHEA, but not of DHEAS, distinguished SZs from controls with 100 percent accuracy (Erb et al 1981). More recent studies found significantly elevated DHEA and DHEAS, with a trend toward lower PREG and 3α,5α-THP plasma levels in unmedicated FEPs (Marx et al 2004; Strous et al 2004). Interestingly, DHEA and PREG levels were inversely correlated with negative symptom severity (Marx et al 2004), and DHEAS levels were inversely correlated with illness severity and aggressive behavior (Strous et al 2004). Elevated DHEA levels were also found in chronically ill medicated patients (di Michele et al 2005; Gallagher et al 2007), and Oades and Schepker reported elevated plasma DHEAS levels in medicated young men with psychosis, but no difference in DHEAS plasma levels in medicated young women with psychosis, when compared to HCs (Oades and Schepker 1994). Similarly, Shirayama et al found no difference in DHEAS concentration between patients and control subjects (Shirayama et al 2002), and Ritsner et al found no difference in DHEA or DHEAS levels between SZs and HCs (Ritsner et al 2004). However, in this study the ratios of cortisol to DHEA and cortisol to DHEAS in the plasma, which were 8.9 and 0.05, respectively, were significantly higher in patients than in HCs. Although the ratios did not correlate with schizophrenia symptom severity assessed by the Positive and Negative Symptom Scale (PANSS), the cortisol to DHEA ratio correlated with depression, anxiety, anger and hostility levels in SZs. In a recent study conducted by the same group, significantly higher than normal serum DHEA

and lower serum DHEAS levels were found in chronic male schizophrenic patients, and these steroids were positively associated with dysphoric mood and positive symptom severity (Ritsner et al 2006a). Correlation analysis in a different study also found significant associations between DHEAS levels and the ratio of DHEAS to cortisol in the plasma and cognitive function, including verbal memory, executive function and memory for faces, in chronic, medicated schizophrenia patients (Silver et al 2005). Also, Spalletta et al found that in a small sample size (two postmenopausal women and six men) of chronically ill schizophrenic patients, 3α,5α-THP levels were directly correlated with aggressiveness, measured by the Modified Overt Aggression Scale, and with hostility, measured by summing the scores obtained from the PANSS items suspiciousness/persecution, hostility, and uncooperativeness (Spalletta et al 2005).

In a recent postmortem investigation, altered brain DHEA, PREG and 3α,5α-THP levels were found in brain tissue collected from chronically ill, medicated SZs. Significantly higher PREG and DHEA levels in the posterior cingulated cortex and significantly higher PREG, lower 3α,5α-THP, and a trend toward higher DHEA levels ($p = 0.06$) in the parietal cortex, were found in patients, compared to levels found in normal subjects (Marx et al 2006b). The authors also found that brain PREG and 3α,5α-THP concentrations exceeded, while brain DHEA levels were comparable to those found in serum or plasma. Furthermore, NAS brain level concentrations were at the physiologically relevant nM range in this study. In another recent study conducted by the same research group, PREG was implicated in the neurobiology of suicide in SZs. In their study, Bradford et al found that a small sample size of SZs who committed suicide

had significantly lower PREG levels in the parietal cortex, compared to SZs who died of other causes (Bradford et al 2007).

Findings from studies examining NAS levels in schizophrenia are inconclusive and at times contradictory. However, they do suggest that NASs may be implicated in schizophrenia pathophysiology. It is unclear if altered plasma levels are due to the disease process, or if they are secondary to the effects of medication, as there are currently no longitudinal investigations of NAS levels in drug-naive SZs and of the effects antipsychotics and symptom improvement on these levels. This type of study would provide insight into NAS abnormalities present in schizophrenia, and help establish the relationship between abnormal levels and various symptom and treatment domains.

1.2.2.3 Antipsychotics Alter Neuroactive Steroid Levels

Atypical antipsychotics may exert some of their therapeutic effects by altering NAS levels. The atypical agents olanzapine (Marx et al 2000; Marx et al 2003) and clozapine (Barbaccia et al 2001; Marx et al 2003) dose-dependently increased cortical, striatal, and/or plasma 3α,5α-THP concentrations, and clozapine increased cortical, striatal, and plasma 3α,5α-THDOC concentrations (Barbaccia et al 2001) in rats, following acute administration. Clozapine also retained the ability to increase these NASs after chronic administration (Barbaccia et al 2001). In a recent animal study, acute clozapine administration to rats significantly increased PREG in the cortex, hippocampus and serum, while acute olanzapine administration significantly increased PREG in the cortex and hippocampus (Marx et al 2006a). Interestingly, a positive association between serum and hippocampal PREG levels was also found in this study, indicating that blood

PREG levels may reflect levels in the brain. Risperidone (Marx et al 2000) and haloperidol (Barbaccia et al 2001; Marx et al 2000) had no effect on 3α-reduced NAS levels, and quetiapine, ziprasidone and aripiprazole had no effect on PREG levels (Marx et al 2006a) in these studies. In another study, clozapine, but not haloperidol, decreased DHEA and DHEAS concentrations in the rat cerebral cortex (Nechmad et al 2003).

It is possible that antipsychotic-induced NAS level alterations contribute to the antipsychotic effects of these drugs. Reduced GABAergic neurotransmission may be implicated in schizophrenia (Benes and Berretta 2001). Thus, antipsychotic-induced increases of 3α-reduced NASs, which are positive allosteric $GABA_A$ receptor modulators, and decreases of DHEA and DHEAS, which are negative allosteric $GABA_A$ receptor modulators, may augment GABAergic tone and contribute to symptom improvement. Consistent with this hypothesis, the PROG metabolite 3α,5α-THP has been reported to suppress DA neurotransmission by increasing GABAergic tone in rodents (Khisti et al 1998; Motzo et al 1996), and to cause the same behavioral changes as haloperidol (Khisti et al 2002). Ugale et al used the conditioned avoidance response and apomorphine-induced climbing paradigms in rats to provide behavioral evidence implicating 3α,5α-THP-mediated GABAergic modulation in the antipsychotic-like action of olanzapine (Ugale et al 2004). The investigators found that drugs that interfere with 3α,5α-THP synthesis block olanzapine's inhibitory effect on conditioned avoidance response and apomorphine-induced climbing behavior. Furthermore, DHEAS, a negative $GABA_A$ receptor modulator, blocked the effects of olanzapine in this study, indicating that antipsychotic-induced DHEA and DHEAS level decreases (Nechmad et al 2003) may also be important for the actions of atypical antipsychotic agents.

PREG level elevations may also be an important mechanism of action of some antipsychotics. PREG has been found to enhance memory and learning in rodent models (Flood et al 1992), and therefore may improve cognitive dysfunction in SZs. Furthermore, PREG can be converted to PREGS, a positive NMDA receptor modulator. Since NMDA receptor hypofunction may be implicated in schizophrenia, enhancing NMDA receptor function may be therapeutically beneficial.

While animal studies implicate NAS level alterations in the therapeutic efficacy of some atypical antipsychotics, this has not been confirmed in clinical investigations. Only one study has examined the effects of antipsychotics on NAS levels in humans. This study examined the effects of six weeks of clozapine treatment on 3α,5α-THP and 3α,5α-THDOC plasma levels in nine (five men and four women) drug-resistant SZs. While symptom improvement was seen in patients, no significant variations in circulating NAS levels during the course of clozapine administration were found (Monteleone et al 2004). It is difficult to draw conclusions from this study due to the small sample size examined. Furthermore, although the patients discontinued all medication (except for typical antipsychotics) for at least two weeks prior to commencing treatment with clozapine, the effects of long-term treatment with antipsychotics prior to the study may have affected the experimental results. Therefore, the effects of antipsychotics on NAS levels in clinical samples remain elusive, and warrant further investigation.

1.2.2.4 Antipsychotic Potential of NASs

NASs such as DHEA and DHEAS may possess intrinsic antipsychotic properties, possibly via positive NMDA receptor modulation (Rupprecht 2003). DHEAS has also

been reported to increase the number of NMDA receptors in the rat brain (Wen et al 2001). Since NMDA receptor hypofunction is implicated in the illness, DHEA and/or DHEAS therapy may be beneficial for some patients. In addition to enhancing NMDA receptor function, DHEA can also be converted into androsterone, a positive allosteric $GABA_A$ receptor modulator (Miyamoto et al 2003), and may therefore augment GABAergic tone, thus possibly improving symptoms. DHEA and DHEAS can also enhance DA release in the frontal cortex. As hypodopaminergia in the mesocortical DA system is implicated in schizophrenia (Majewska 1999), DHEA- and DHEAS-induced enhancement of DA release in the frontal cortex may be a mechanism by which these NASs contribute to symptom improvement.

The hypothesis that DHEA is therapeutic in schizophrenia is supported by clinical studies examining DHEA and DHEAS in SZs. Harris et al found an inverse correlation between DHEA plasma levels and symptom ratings on the Brief Psychiatric Rating Scale and performance in some measures of memory in medicated, chronic patients with schizoaffective disorder and schizophrenia (Harris et al 2001), and Strauss et al found that DHEA administration to SZs improves negative symptoms (Strauss et al 1952). In two recent double-blind, placebo (PLA)-controlled studies, Strous et al found that DHEA, when given as an adjunct to antipsychotics, improves negative, depressive and anxiety symptoms after six weeks (Strous et al 2003) and improves negative symptoms after 12 weeks (Strous et al 2007) of administration to chronic SZs. However, these results were not replicated by a different group (Ritsner et al 2006b).

As mentioned earlier, clozapine decreases cortical DHEA and DHEAS in rats (Marx et al 2003). While a reduction in DHEA or DHEAS may result in increased

GABAergic tone, it may also result in decreased NMDA receptor function. Thus, DHEA therapy may be beneficial for those with predominant glutamatergic pathology, but detrimental to those with predominant GABAergic pathology.

More research is needed examining the effects of DHEA and DHEAS in schizophrenia. While some studies show that DHEA or DHEAS therapy improves some symptoms in schizophrenia (Harris et al 2001; Strauss et al 1952; Strous 2005), other studies reveal that low levels of these NASs may be beneficial for patients (Ugale et al 2004). Thus determining which symptoms improve and which get worse with DHEA/DHEAS therapy, and assessing each patient's symptom profile and primary pathological changes in the brain will determine which patients would benefit from DHEA therapy. Furthermore, DHEA can be metabolized *in vivo* to other pharmacologically active steroids with differential effects, making it difficult to assess whether the effects of DHEA therapy are caused by DHEA or its metabolites. Therefore the pharmacokinetic profile of DHEA and variability of DHEA metabolism between individuals need to be considered when examining the effects of DHEA and determining if DHEA therapy is appropriate.

PREGS is another positive allosteric NMDA receptor modulator (Rupprecht 2003). Although its therapeutic potential has not been investigated in schizophrenia, it modulates NMDA receptors in a similar way as DHEA and DHEAS, and may therefore prove to be beneficial for some symptoms of schizophrenia. Indeed, in a preliminary PLA-controlled study investigating the therapeutic potential of PREG, the immediate precursor of PREGS, it was shown that following a two week PLA lead-in phase, stable, chronic, medicated SZs receiving PREG (100-500 mg/day) as an adjunct to antipsychotic

medication for four to eight weeks had greater negative symptom improvement, compared to patients who received PLA as an adjunct to antipsychotic medication, and increased serum PREG levels were positively correlated with cognitive improvement in those patients who received PREG (Marx et al 2007). The authors also found that patients who were given PREG had higher PREGS and 3α,5α-THP, with no changes in DHEA or DHEAS plasma levels, compared to patients who were given PLA (Marx et al 2007), indicating that the addition of a sulfate group to PREG, and/or PREG metabolism along the PROG arm (see Figure 1-2) may be important mechanisms that contribute to PREG's antipsychotic effects.

PROG and its metabolites may also possess antipsychotic properties. Schizophrenia symptom severity varies across the menstrual cycle (Hallonquist et al 1993), and women are more vulnerable to psychosis and the onset of schizophrenia both postpartum and after menopause (Hafner et al 1993), which may be attributed to a drop in PROG levels. Rupprecht et al investigated the neuroleptic properties of PROG in rats by examining the effect of PROG on apomorphine-induced disruption of prepulse inhibition of the acoustic startle response (Rupprecht et al 1999). Prepulse inhibition is an animal model for measuring sensorimotor gating, which is disrupted in some individuals with schizophrenia. Apomorphine-induced disruption of prepulse inhibition in rats is antagonized by both typical and atypical antipsychotics, and is therefore considered to be an animal model for antipsychotic drug action. Rupprecht et al found that PROG antagonizes apomorphine-induced disruption of prepulse inhibition; this is an effect caused by PROG itself, rather than the PROG metabolite 3α,5α-THP, since 3α,5α-THP did not restore prepulse inhibition in this study (Rupprecht et al 1999). They also found

that PROG dose-dependently decreases locomotion, an effect similar to that produced by both typical and atypical antipsychotics. In contrast to haloperidol, PROG did not induce catalepsy and did not antagonize amphetamine-induced stereotypy, indicating that PROG and its metabolites do not cause EPS, and as such have a similar side effect profile to atypical antipsychotics (Rupprecht et al 1999).

These data are difficult to explain at this time and may appear contradictory to the finding that PROG plasma levels are elevated in men newly diagnosed with schizophrenia (Prior et al 2003). A possible explanation may be that PROG acts like an endogenous antipsychotic, and an increased PROG during the early phase of the illness may be a counterregulatory mechanism that serves to restore normal functioning.

1.2.2.5 Summary of NAS Dysfunction in Schizophrenia

The investigation of NASs in schizophrenia is a relatively new field with many unknowns, and sometimes seemingly contradictory findings. Because NASs modulate neurotransmitter systems that are implicated in schizophrenia, studies examining NAS effects in the central nervous system and the role they play in schizophrenia are essential for understanding the neurochemistry of the illness.

Evidence implicating NASs in the pathology of schizophrenia is growing. Recent studies reveal NAS plasma, and possibly brain level fluctuations in the illness. However, the precise NAS level abnormalities are still unknown, and warrant further investigation. Furthermore, NASs may improve some symptoms of schizophrenia, but exacerbate others. It also appears that antipsychotic drugs, particularly atypical agents, exert their therapeutic effects partly by altering NAS levels. Although further investigation is

needed, the ever-growing evidence for a role of NASs in the pathophysiology of schizophrenia and in the efficacy of antipsychotic drugs may prove to be useful for future rational development of novel treatment options for schizophrenia.

1.2.3 Kynurenine (KYN) Metabolites in Schizophrenia

1.2.3.1 The KYN Pathway

Tryptophan (Trp) metabolism along the KYN pathway (Figure 1-3) produces the neuroactive compounds kynurenic acid (KYNA), which modulates NMDA and α_7 nicotinic acetylcholine receptors, and quinolinic acid (QUIN), which modulates NMDA receptors. KYNA is the only known endogenous Glu receptor antagonist in the human brain. At low μM concentrations, KYNA acts as an antagonist at the glycine co-agonist site of the NMDA receptor (IC_{50} = 8-15 μmol/L) (Birch et al 1988) and at the α_7 nicotinic acetylcholine receptor ($IC_{50} \approx$ 1 μmol/L) (Hilmas et al 2001). As α_7 receptor activation results in increased Glu neurotransmission, KYNA-induced α_7 receptor antagonism attenuates Glu function (Fu et al 2000; Gray et al 1996). Thus, at low μM concentrations KYNA inhibits NMDA and α_7 receptors, and decreases extracellular Glu levels. At high μM concentrations KYNA behaves as a competitive antagonist at the agonist recognition site of NMDA receptors (IC_{50} = 200-500 μmol/L) (Kessler et al 1989). QUIN, on the other hand, is a weak NMDA receptor agonist with neurotoxic properties that are potentiated by the KYN metabolite and QUIN precursor, 3-hydroxykynurenine (3-HKYN) (Klivenyi et al 2004).

It is unknown if peripheral levels of KYN metabolites reflect those found in the brain. Although QUIN and KYNA have limited ability to penetrate the BBB, KYN, the

precursor for KYNA and QUIN, and 3-HKYN, which is formed from KYN and is a QUIN precursor, are transported through the BBB by large neutral amino acid carriers (Klivenyi et al 2004). Furthermore, the CNS KYN pathway depends on KYN originating in the periphery, where an estimated 60% of brain KYN is derived from peripheral sources (Klivenyi et al 2004). Because both KYNA and QUIN brain levels are dependent on peripheral KYN and possibly 3-HKYN, and both of these precursors cross the BBB, peripheral levels of these neuroactive KYN metabolites may predict levels in the brain.

1.2.3.2 KYN Pathway Dysfunction in Schizophrenia

Very few studies have investigated KYN pathway intermediates and enzymes in schizophrenia. In a recent postmortem investigation Miller et al found higher than normal KYN and Trp 2,3-dioxygenase (TDO) mRNA levels in the anterior cingulate cortex of SZs, suggesting greater activation of the first enzymatic step of the KYN pathway (Miller et al 2006). While Miller et al did not find significantly different KYNA levels between patients and control subjects, higher than normal KYNA levels in the frontal and occipital cortices in schizophrenic patients were found in another postmortem study (Schwarcz et al 2001). In a more recent postmortem investigation, the same group of researchers also found higher than normal levels of KYN and KYNA, with increased TDO (+61%) and indolamine 2,3-dioxygenase (+83%), decreased KYN 3-hydroxylase (-26%), and normal KYN aminotransferases I and II, kynureninase, 3-hydroxyanthranillic acid oxygenase, and QUIN phosphoribosyltransferase enzymatic activity in the striatum of SZs (Schwarcz et al 2007). These metabolite and enzymatic abnormalities indicate that striatal KYN pathway metabolism is altered in schizophrenia in the direction of a net up-regulation of

KYN and KYNA synthesis with a down-regulation of KYN degradation (Figure 1-3) (Schwarcz et al 2007).

Increased CSF KYNA levels have also been found in SZs, and patients had greater than normal KYNA level variability that HCs (Erhardt et al 2001). A positive correlation between age and CSF KYNA levels in patients, but not in control subjects, was also found in this study, suggesting that KYNA may play a role in the pathogenic mechanism of late onset schizophrenia. It is unlikely that antipsychotics induced the increase in KYNA levels since most patients (25/28) in this study were drug-naïve FEPs (Erhardt et al 2001). Furthermore in a recent preclinical study investigating the effects of antipsychotics on brain KYNA levels in rats, Ceresoli-Borroni et al found that chronic (one month) typical (haloperidol or raclopride), or atypical (clozapine) antipsychotic administration caused significant reductions in KYNA levels in the striatum, hippocampus and frontal cortex, without affecting levels of other metabolites along the KYN pathway (Ceresoli-Borroni et al 2006). As the KYNA arm of KYN metabolism takes places mainly in the astrocytes while the QUIN arm takes place mainly in the microglia (Schwarcz and Pellicciari 2002), it appears that antipsychotic drugs preferentially affect astroglial KYN metabolism (Ceresoli-Borroni et al 2006). The reduction of KYNA levels and the resulting increase in glutamatergic neurotransmission via increased NMDA and α_7 receptor activation, and increased Glu release may be part of the therapeutic mechanism of action of antipsychotic drugs. However, this hypothesis has not been tested in humans, and postmortem studies, which examined chronically ill, medicated patients, negate this hypothesis, as similar abnormalities in KYN pathway metabolism were found in postmortem investigations as those found in FEPs. Thus, it

may be possible that KYN pathway abnormalities present at the onset of illness persist throughout the course of illness.

1.2.3.3 Animal Models of KYN Dysfunction

Animal models of schizophrenia further support the possibility that increased KYNA plays a pathophyisological role in schizophrenia. Two studies have implicated elevated KYNA in the hypothesized hyperdopaminergic state seen in schizophrenia, where pharmacologically elevated KYNA caused a dysregulation of the firing pattern of ventral tegmental area DA neurons in rats by increasing the firing rate and burst activity of these neurons (Erhardt and Engberg 2002; Schwieler and Erhardt 2003). These effects were likely mediated by the blockade of the glycine co-agonist site on NMDA receptors, since D-cycloserine, a partial NMDA glycine site agonist, blocked the effects of KYNA on DA neurons (Erhardt and Engberg 2002). Rat models of sensorimotor gating deficits that represent deficits seen in SZs, such as disruption of prepulse inhibition of the acoustic startle response, also implicate KYNA dysregulation in schizophrenia. For example, administration of the synthetic KYNA analogue 7-chloro-KYNA into the NAc disrupted prepulse inhibition in rats (Kretschmer and Koch 1997). Similarly, pharmacologically elevated KYNA levels via systemic KYN administration caused a disruption of prepulse inhibition, which was prevented by both clozapine and haloperidol (Erhardt et al 2004). These studies implicate elevated KYNA levels in the pathology of schizophrenia, and suggest that antipsychotics may exert some of their therapeutic effects by correcting KYNA dysfunction.

1.2.3.4 Summary of KYN Pathway Dysfunction

KYN pathway dysfunction is a new area in schizophrenia research. It appears that the KYNA arm of KYN metabolism is of particular relevance, where high KYNA levels may contribute to the glutamatergic dysfunction hypothesized in the illness. Furthermore, antipsychotic-induced alterations of KYNA levels may partially contribute to the therapeutic effects of these drugs. Further investigation of KYN pathway abnormalities may increase our understanding of the pathophysiological processes in schizophrenia.

1.3 THESIS AIMS AND OBJECTIVES

In the quest to better understand the complex neurochemistry of schizophrenia, this thesis investigates the role of NASs, KYN metabolites and Glu in the pathophysiology of early onset and chronic schizophrenia. In the first experiment, a crossover, randomized, PLA-controlled design was used to examine DHEA and PREG plasma concentration changes after a challenge with the panicogenic agent pentagastrin (CCK-5) in chronically ill SZs, patients with panic disorder (PDs) and HCs. This project was undertaken to explore the common pathophysiological processes in schizophrenia and panic disorder, and to help elucidate why anxiety disorders are more prevalent among SZs than among HCs.

This thesis then explores the role of NASs, which modulate Glu and GABA neurotransmission, and other compounds involved in Glu neurotransmission, including KYN metabolites and Glu in FEPs. The purpose of these experiments was to ascertain if these neuromodulating compounds are implicated in the disease process or in the mechanism of action of antipsychotic drugs. To address this question, a series of

longitudinal studies examining these compounds in FEPs was conducted, where plasma Glu, NASs and KYN metabolites, and brain Glu were quantified in unmedicated FEPs, and followed throughout the initial course of treatment with atypical antipsychotics. The relationship between peripheral compounds that modulate Glu neurotransmission and brain Glu levels was also explored in order to determine if blood levels of compounds involved in Glu neurotransmission can be used for investigating brain Glu abnormalities.

1.4 TABLES AND FIGURES

Table 1-1. NAS levels in schizophrenia.

Unmedicated	Medicated
Early psychosis	
High DHEA plasma/serum levels in males (Marx et al 2004)	High DHEAS plasma/serum levels in males (Oades and Schepker 1994)
High DHEA and DHEAS plasma/serum levels (Strous et al 2004)	Normal DHEAS plasma/serum levels in females (Oades and Schepker 1994)
Chronic SZ	
Low DHEA plasma/serum levels (Tourney and Erb 1979)	Normal DHEAS plasma/serum levels in males (Shiryama et al 2002)
	Normal DHEA and DHEAS plasma/serum levels (Ritsner et al 2004)
	High cortisol:DHEA and cortisol:DHEAS ratios in plasma/serum (Ritsner et al 2004)
	High DHEA and low DHEAS plasma/serum levels in males (Ritsner et al 2006)
	High DHEA plasma/serum levels (di Michele et al 2005; Gallagher et al 2007)
	High DHEA and PREG in posterior cingulate cortex (Marx et al 2006)
	High PREG, and a trend toward high DHEA in parietal cortex (Marx et al 2006)
	Low 3α,5α-THP in the parietal cortex (Marx et al 2006)

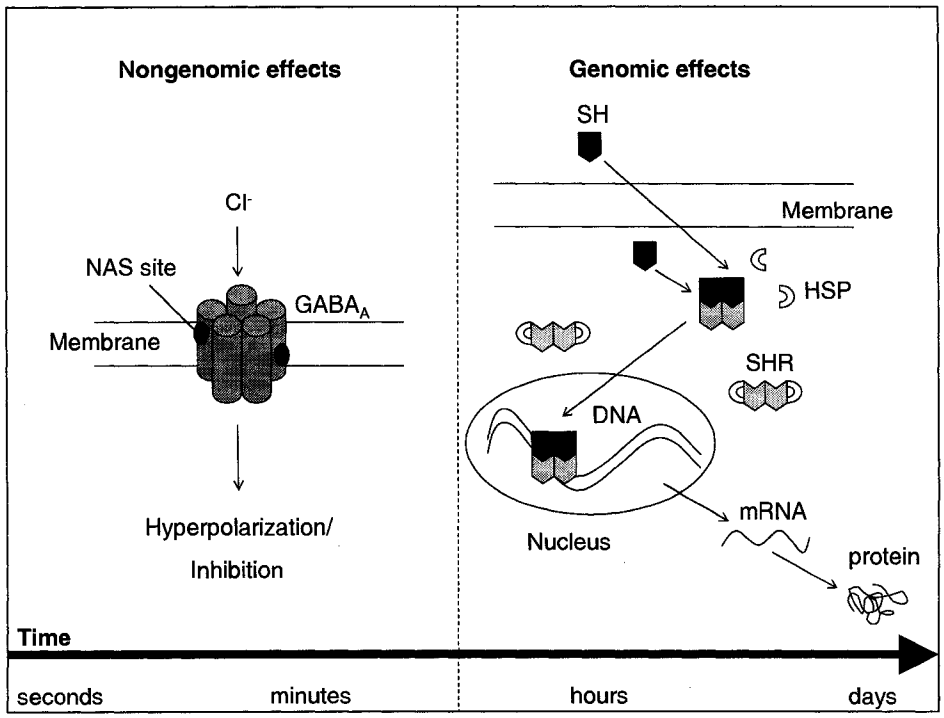

Figure 1-1. Genomic and nongenomic (neuroactive) steroidal effects. NASs alter neuronal excitability at the cell membrane by interacting with cell surface receptors, in this case a $GABA_A$ receptor. Steroid hormones (SH) diffuse though the cells membrane, bind to intracellular receptors, enter the nucleus, and control gene transcription, and in turn, protein synthesis. NASs exert their effects in seconds to minutes, while the effects of steroid hormones are limited by the rate of protein synthesis, and are not apparent for hours to days. HSP: heat shock protein; SHR: steroid hormone receptor (Shulman and Tibbo 2005).

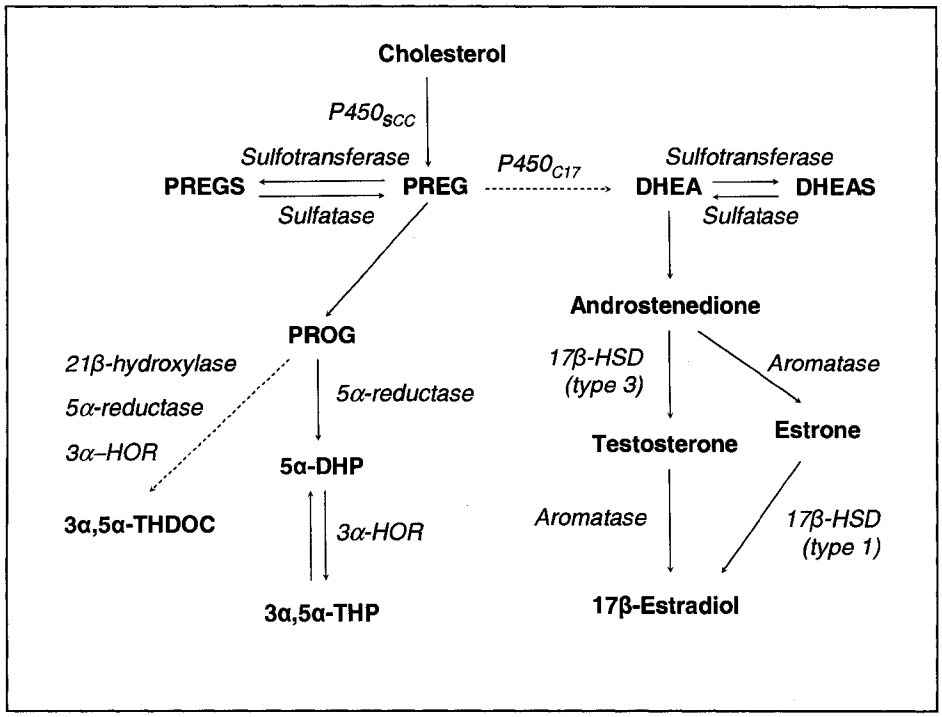

Figure 1-2. Biosynthesis of NASs. Solid arrows represent single step reactions. Dashed arrows represent multi-step reactions. Enzyme names (italicized), steroid names and intermediates are shown. DHEA: dehydroepiandrosterone; DHEAS dehydroepiandrosterone sulfate; 5α-DHP: 5α-dihydroprogesterone; HOR: hydroxysteroid oxidoreductase; HSD: hydroxysteroid dehydrogenase; PREG: pregnenolone; PREGS pregnenolone sulfate; PROG: progesterone; 3α,5α-THDOC: 3α,5α-tetrahydrodeoxycorticosterone; 3α,5α-THP: 3α,5α-tetrahydroprogesterone; SCC: side chain cleavage.

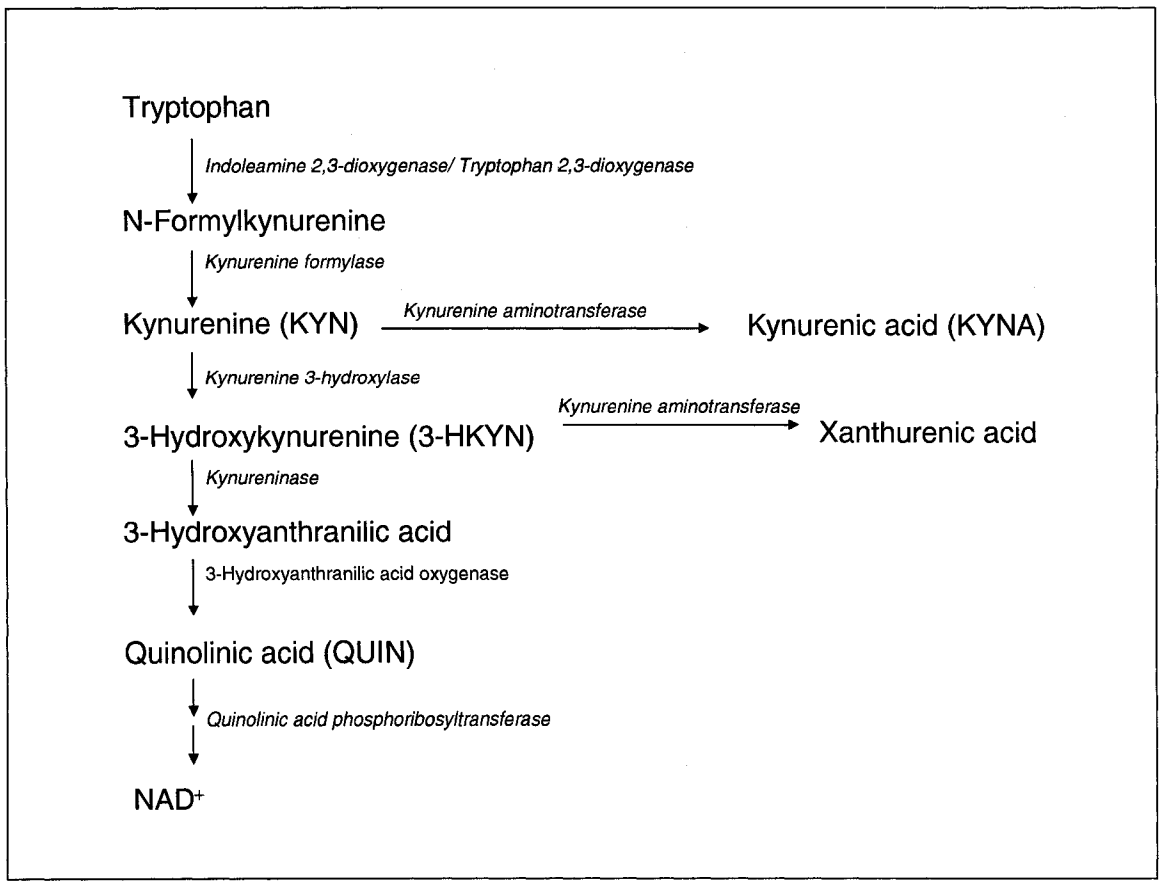

Figure 1-3. Trp metabolism via the KYN pathway. Enzyme names are italicized.

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Chapter 2

CCK-5-Induced Changes in Plasma DHEA and PREG Levels in Schizophrenia and Panic Disorder

2.1 INTRODUCTION

NASs have recently been implicated in the pathophysiology of both schizophrenia and panic disorder. 3α-reduced NASs, including the PROG metabolites 3α,5α-THP and 3α,5β-THP, as well as the deoxycorticosterone metabolite 3α,5α-THDOC, are positive allosteric $GABA_A$ receptor modulators (Zinder and Dar 1999), and as such, are thought to possess anxiolytic properties. Indeed, these NASs have been shown to exhibit anxiolytic effects, similar to those seen with benzodiazepines, in animal models of anxiety (Eser et al 2005b). Furthermore, Strohle et al found that a group of female and male PDs had elevated 3α-reduced NAS plasma levels and decreased levels of the 3β-reduced NAS and $GABA_A$ receptor functional antagonist, 3β,5α-THP (Strohle et al 2002). The authors suggested that these NAS level abnormalities may serve as a counterregulatory mechanism against the occurrence of spontaneous panic attacks in PDs (Strohle et al 2002). Similarly, Brambilla et al found increased 3α-reduced NASs in female (Brambilla et al 2003), but interestingly not in male (Brambilla et al 2005) PDs.

The role of other NASs, including DHEA, PREG and their sulfated forms, DHEAS and PREGS, in anxiety is more complex. At the molecular level PREGS acts as a mixed $GABA_A$ receptor agonist/antagonist, while PREG, DHEA and DHEAS act as antagonists. These NASs are also positive allosteric modulators at NMDA receptors (Baulieu and Robel 1998; Compagnone and Mellon 2000; Macdonald and Olsen 1994; Rupprecht and Holsboer 1999; Twyman and Macdonald 1992). Consistent with their molecular mechanisms of action, PREG displays anxiogenic effects, while PREGS exhibits anxiolytic effects at low doses, and anxiogenic effects at higher doses in animal models of anxiety (Eser et al 2005b). The effects of DHEA and DHEAS on anxiety

symptoms are not as well understood, as these NASs exhibit both anxiogenic and anxiolytic effects in animal models of anxiety (Eser et al 2005b). Clinical reports on DHEA in PDs have revealed normal DHEA plasma levels in female (Brambilla et al 2003), but high levels in male patients (Brambilla et al 2005), when compared to HCs.

The role of NASs in schizophrenia appears to be even more complex than in panic disorder. DHEA and DHEAS have been investigated in schizophrenia, but it is unclear if, and how plasma levels are altered in the illness, as high, low and normal levels have been reported (Shulman and Tibbo 2005). It is possible that DHEA has therapeutic effects, as it has been found to improve negative symptoms of schizophrenia (Strauss et al 1952; Strous 2005; Strous et al 2003), possibly via positive NMDA receptor modulation. DHEA has also been reported to improve anxiety symptoms in SZs (Strous et al 2003), but such a finding is inconsistent with its proposed molecular mechanism of action.

The current NAS literature on panic disorder and schizophrenia does not provide a clear understanding of NAS abnormalities in these illnesses since it is both incomplete and at times contradictory. Patient selection and inclusion criteria, such as medication and stage of illness, vary between studies, and a single measurement can be affected by a patient's state at the time of blood collection. As anxiety is a common symptom of both panic disorder and schizophrenia, the use of an anxiety-provoking challenge paradigm to measure NAS changes from before to after the challenge may be an effective way to investigate NAS abnormalities in the two disorders. This type of investigation may also help explain, in part, the high comorbidity rates between the two seemingly separate disorders, where up to 27 percent of SZs have a comorbid anxiety disorder (Tibbo et al 2003).

Cholecystokinin tetrapeptide (CCK-4) and CCK-5 are CCK type B (CCK-B) receptor agonists that induce a state of panic analogous to spontaneous panic attacks in PDs (Bradwejn and Koszycki 1991; Bradwejn et al 1990), and less intense anxiety symptoms in HCs (Bradwejn et al 1991b; de Montigny 1989) in a dose-dependent fashion (Bradwejn et al 1991a). The mechanism of anxiogenesis is thought to be via CCK-B receptor activation in the basolateral amygdala (Rotzinger and Vaccarino 2003). However, the precise mechanism of panic induction is not fully understood and may be caused by peripheral and/or central effects of CCK alone or via interaction of CCK with other neurotransmitter systems (Bradwejn and Koszycki 2001). In a non-PLA-controlled study, where plasma DHEA and 3α,5α-THP were quantified following a CCK-5 challenge in male PDs and in HCs, there was a statistically significant increase, followed by a delayed decrease, in DHEA levels, compared to baseline values in both PDs and in HCs, with no significant changes in 3α,5α-THP levels over time in either group (Tait et al 2002). In another study PLA had no effect, while CCK-4 administration caused a decrease in plasma 3α,5α-THP and 3α,5β-THP, and an increase in 3β,5α-THP levels in male and female PDs, but not in HCs (Strohle et al 2003). Also, plasma levels of these PROG-derived NASs did not change in HCs when they experienced comparable levels of anxiety as those experienced by PDs (Zwanzger et al 2004), indicating that alterations in the levels of these particular NASs are related to the pathophysiology of panic attacks in panic disorder, rather than simply reflecting the anxiety levels experienced by the individual (Strohle et al 2003; Zwanzger et al 2004). This, however, is not the case with 3α,5α-THDOC, as plasma 3α,5α-THDOC levels increase following CCK-4 administration in HCs (Eser et al 2005a). Unlike the PROG-derived NASs discussed

above, 3α,5α-THDOC is synthesized in the adrenal gland, and its synthesis is dependent on the availability of its precursor, deoxycorticosterone. Since deoxycorticosterone synthesis is induced by adrenocorticotropic hormone, which is released from the pituitary during stressful situations (e.g. during a panic attack), increased 3α,5α-THDOC during an experimentally induced panic attack may play a role in the regulation and termination of the endogenous stress response (Purdy et al 1991).

To the best of our knowledge, CCK-induced NAS level changes have not been investigated in schizophrenic patients. Furthermore, CCK-induced PREG level alterations have not been examined in panic disorder, while DHEA level changes have only been investigated in one study (Tait et al 2002), which was not PLA-controlled. We wished, therefore, to investigate CCK-induced DHEA and PREG plasma level changes in individuals with schizophrenia and in those with panic disorder. To improve on limitations of previous studies we used a crossover, randomized, PLA-controlled design to examine DHEA and PREG plasma concentration fluctuations after a challenge with the panicogenic agent CCK-5 in SZs, PDs and in HCs.

We predicted that all groups would report higher panic/anxiety symptoms post CCK-5 injection, compared to PLA, and that PDs would experience the most intense CCK-5-induced symptoms, followed by SZs and HCs, respectively. Consistent with previous results, we predicted that PDs would have higher baseline plasma DHEA (Brambilla et al 2005) and PREG concentrations than HCs. Higher levels of these putatively anxiogenic NASs may make PDs more susceptible to anxiety than HCs. Following the same logic, we expected to find higher plasma DHEA and PREG levels in SZs than in HCs. However, as DHEA and PREG are positive NMDA receptor

modulators, we predicted that plasma DHEA and PREG concentrations will be lower in SZs, in accordance with the NMDA receptor hypofunction hypothesis of the illness. We also expected greater changes in DHEA and PREG concentrations post CCK-5 injection, compared to PLA, with the greatest DHEA and PREG increases in PDs, followed by SZs and HCs, respectively. This hypothesis is based on the molecular mechanism of action of these NASs, where both DHEA and PREG are negative $GABA_A$ and/or positive NMDA receptor modulators, and as such, likely anxiogenic. We also predicted that HCs would have the greatest delayed decrease in DHEA and PREG concentrations, compared to baseline values, to serve as a compensatory mechanism to reduce anxiety and restore normal functioning following CCK-5-induced panic symptoms, with PDs having the least decrease in these NASs.

2.2 METHODS

2.2.1 Study Participants

Ethical approval for this study was obtained from the University of Alberta Health Research Ethics Board. Study participants were recruited from the Edmonton area via poster and radio advertisements. Thirty-two adult men, including SZs (n = 10), PDs (n = 10) and HCs (n = 12), participated in the study. Inclusion criteria for SZs included illness duration of at least one year, with no other current comorbid Axis-I disorders, and being on a stable dose of antipsychotic medication for at least one month prior to participating in the study (see Table 2-1). Inclusion criteria for PDs included presence of panic disorder (with or without agoraphobia), and absence of other Axis-I disorders. All PDs

were unmedicated. HCs included in the study had no past or present Axis-I disorder, and no first degree relatives with such disorders.

2.2.2 Visit 1

Following full description of all study procedures all participants gave informed, signed consent. Demographic and clinical data collected during this screening visit are summarized in Table 2-2. The Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (First et al 1994) was used to diagnose participants and determine eligibility. Other clinical data collected from SZs included duration of illness and current medications (see Tables 2-1 and 2-2). If SZs were taking benzodiazepines or zopiclone they were asked to refrain from taking these medications for five half lives of elimination of the drug prior to each of the injection visits, as these drugs act at $GABA_A$ receptors, and as such may affect NAS levels and/or action. The following clinical rating scales were administered to SZs: The PANSS for assessing psychiatric symptoms of schizophrenia (Kay et al 1987), Global Assessment of Function scale (GAF) to assess overall level of functioning, and the Calgary Depression Scale for Schizophrenia (CDSS) (Addington et al 1996) to assess depression. The Hamilton Anxiety Rating Scale (HAM-A) (Hall 1995) and State Trait Anxiety Inventory (STAI) (Spielberger et al 1970) were administered to all study participants to assess anxiety symptomatology.

2.2.3 Visits 2 and 3 (Injection Visits)

A double blind crossover PLA-controlled design was used to assess the effects of CCK-5 on panic symptom intensity and NAS plasma levels. Patients were randomly

assigned to either receive CCK-5 during visit 2 and PLA during visit 3, or vice versa. Following a 12 hour fast, and abstinence from alcohol and tobacco for 24 and 12 hours, respectively, subjects were seated in a reclining chair and an i.v. catheter, through which 0.09% sodium chloride solution was run at 125 mL/hour, was installed into the antecubital vein. Participants remained in this semi-supine position for the duration of the procedure. Arrival time, and, consequently, i.v. installation time (T) was recorded as T = -45 min (45 min from the time of CCK-5 or PLA administration). At T = 0 min 5 mL of either CCK-5 (50 μg) or PLA was introduced into the i.v. line via a five second bolus injection. At T = +5 min the subjects' panic symptoms were evaluated using an 18 item DSM-III-R-derived Panic Symptom Scale (PSS) (Bradwejn et al 1991b), and the subjects were asked to rate the maximum change in anxiety level they experienced as a result of the injection using the Visual Analogue Scale for maximum change in anxiety (VAS-A max), which uses a 10 point scale, with 0 representing no change and 10 representing the maximum change in anxiety level. Blood was collected by catheter at T = -10 min, +1 min, +10 min, +45 min, +90 min, +120 min, +150 min and +180 min before and after CCK-5/PLA injection and transferred into vacutainers containing the anticoagulant ethylenediamine tetraacetic acid. The plasma was immediately separated by centrifugation at 4°C and 3000 g, and stored at -80°C until analysis.

2.2.3 DHEA and PREG Analysis Using Gas Chromatography (GC) Combined with Mass Spectroscopy (MS)

NASs were isolated from plasma samples by solid-phase extraction (SPE) and then assayed by combined GC-MS with negative ion chemical ionization (NI-CI) using a

modification of the procedure of Kim et al (Kim et al 2000); heptafluorobutyrylimidazole (HFBI) was used for NAS derivatization prior to GC-MS analysis (Figure 2-1).

2.2.4 Statistical Analysis

SPSS 12.0 software was used for all statistical analysis. All results are expressed as mean ± standard deviation (SD). Paired-samples t-tests were used to compare post PLA and post CCK-5 PSS and VAS-A max scores (behavioral measures) in each group, separately. One-way analysis of variance (ANOVA) was used to analyze age differences between groups, and univariate ANOVA, with age as a covariate (analysis of covariance or ANCOVA), was used to analyze PSS and VAS-A max scores between groups.

Re-test reliability of pre-PLA and pre-CCK-5 injection (baseline) DHEA and PREG concentrations was assessed with intra-class correlations using Cronbach's α. For between-group comparisons of baseline DHEA and PREG concentrations, baseline concentrations of each NAS from pre-PLA and pre-CCK-5 injection were combined, and ANCOVA was performed (with age as a covariate) to account for effects of age on DHEA and PREG levels (Goncharova and Lapin 2000; Hill et al 1999; Orentreich et al 1984; Orentreich et al 1992; Vermeulen 1995). Bonferroni post hoc analysis was used for pair wise comparisons. In order to simplify statistical analysis and interpretation of injection-induced NAS concentration changes, only -10 min, +10 min and +180 min NAS measures were used, as these represented baseline, peak and lowest NAS concentrations, respectively (see Figure 2-2 and Figure 2-3). For statistical analysis of DHEA and PREG concentration changes from baseline to 10 and 180 min after CCK-5/PLA injection, a multivariate analysis of variance (MANOVA) with treatment and time as within-subject

factors, and diagnosis as a between subject factor was performed. In the case of a significant (or a trend toward a significant) treatment x time x group effect, changes (Δs) in DHEA and PREG concentrations for each group separately were calculated between baseline and +10 min, and between baseline and +180 min for PLA and CCK-5 injection days, separately. One-way ANOVA with Bonferroni post-hoc analysis was used to compare Δs between groups. For all statistical analyses, $\alpha = 0.05$ was set as the nominal level of significance.

2.3 RESULTS

2.3.1 Behavioral Measures and Age Differences

One way ANOVA revealed a statistically significant difference in age between groups ($F_{2, 29} = 4.47$, $p = 0.020$), attributed to PDs being significantly older than HCs ($p = 0.017$). All PDs and SZs, and 8 out of 12 HCs experienced a CCK-5-induced panic attack, as determined by the PSS (total PSS score > 4 and anxiety score > 2). One PD also experienced a panic attack post PLA injection. All groups reported higher PSS (Figure 2-4) and VAS-A max (Figure 2-5) scores post CCK-5, compared to post PLA administration [HCs: PSS ($t_{11} = 6.91$, $p < 0.001$), VAS-A max ($t_{11} = 10.20$, $p < 0.001$); PDs: PSS ($t_9 = 7.96$, $p < 0.001$), VAS-A max ($t_9 = 5.78$, $p < 0.001$); SZs: PSS ($t_9 = 7.77$, $p < 0.001$), VAS-A max ($t_9 = 7.47$, $p < 0.001$)]. PSS and VAS-A max scores were not significantly different between groups post PLA or post CCK-5 injection.

2.3.2 Baseline DHEA and PREG Concentrations

Re-test reliability of baseline DHEA and PREG concentrations assessed with intra-class correlations yielded Cronbach's α values of 0.77 and 0.70, respectively. Randomization (i.e. whether a subject received PLA during visit 2 and CCK-5 during visit 3, or vice versa) had no effect on baseline DHEAS measures (data not shown). ANCOVA, with age as a covariate, revealed a statistically significant difference in baseline DHEA (Figure 2-6) concentrations ($F_{2, 62} = 4.35$, $p = 0.017$) and PREG (Figure 2-7) concentrations ($F_{2, 63} = 11.78$, $p < 0.001$) between groups. This difference was attributed to lower DHEA and PREG concentrations in PDs and SZs, compared to HCs (DHEA: $p < 0.001$; PREG: $p < 0.001$ and DHEA: $p = 0.002$; PREG: $p < 0.001$, respectively). Baseline DHEA and PREG levels were not significantly different between PDs and SZs.

2.3.3 DHEA Changes Over Time

MANOVA showed a significant time effect (Wilks' multivariate tests of significance: $F_{2, 26} = 21.66$, $p < 0.001$), a significant time x group effect (Wilks' multivariate tests of significance: $F_{4, 52} = 3.71$, $p = 0.010$) and a significant treatment x time x group effect (Wilks' multivariate tests of significance: $F_{4, 52} = 3.71$, $p = 0.020$) on DHEA concentration (Figure 2-8).

One way ANOVA revealed a statistically significant difference in ΔDHEA from baseline to +10 min post CCK-5 injection ($F_{2, 28} = 3.42$, $p = 0.047$), attributed to a greater increase of plasma DHEA in PDs, compared to HCs ($p = 0.045$) (Figure 2-9). There was also a statistically significant difference in ΔDHEA from baseline to +180 min post CCK-5 injection ($F_{2, 28} = 4.92$, $p = 0.015$), attributed to a smaller decrease of plasma DHEA in

SZs, compared to HCs ($p = 0.025$), with a trend toward a smaller decrease of plasma DHEA in PDs, compared to HCs ($p = 0.053$) (Figure 2-10).

2.3.4 PREG Changes Over Time

MANOVA showed a significant time effect (Wilks' multivariate tests of significance: $F_{2, 27} = 20.99$, $p < 0.001$), a significant time x group effect (Wilks' multivariate tests of significance: $F_{4, 54} = 3.79$, $p = 0.009$) and a trend toward a significant treatment x time x group effect (Wilks' multivariate tests of significance: $F_{4, 54} = 2.38$, $p = 0.063$) on PREG concentration (Figure 2-11).

There were no statistically significant differences in ΔPREG concentrations from baseline to +10 min between groups post PLA or post CCK-5 injection (Figure 2-12). Oneway ANOVA revealed a statistically significant difference in ΔPREG from baseline to +180 min post CCK-5 injection ($F_{2, 29} = 5.56$, $p = 0.009$), attributed to a smaller decrease of plasma PREG in PDs ($p = 0.025$) and SZs ($p = 0.023$), compared to HCs (Figure 2-13). There was also a statistically significant difference in ΔPREG from baseline to +180 min post PLA injection ($F_{2, 29} = 4.02$, $p = 0.029$), attributed to a smaller decrease of plasma PREG in SZs, compared to HCs ($p = 0.045$) (Figure 2-13).

2.4 DISCUSSION

In this study we examined, for the first time, CCK-5-induced NAS changes in SZs. This was also, to our knowledge, the first PLA-controlled study examining plasma DHEA changes following CCK-5 injection, and the first investigation of CCK-5-induced plasma PREG changes in PDs. We found that both PDs and SZs have lower DHEA and

PREG plasma levels than HCs at baseline, and that there is a greater increase in DHEA plasma concentrations from baseline to +10 min following CCK-5 injection in PDs, compared to HCs. There was also a smaller decrease of plasma DHEA and PREG concentrations from baseline to +180 min post CCK-5 injection in SZs and PDs, compared to HCs, and a smaller delayed decrease in PREG plasma concentration from baseline to +180 min post PLA injection in SZs, compared to HCs.

In our study, PDs had lower baseline DHEA and PREG plasma levels, compared to HCs. PREG and DHEA have similar molecular mechanisms of action, where these putative anxiogenic NASs are negative $GABA_A$ receptor and positive NMDA receptor modulators (Baulieu and Robel 1998; Compagnone and Mellon 2000; Macdonald and Olsen 1994; Rupprecht and Holsboer 1999; Twyman and Macdonald 1992). Lower DHEA levels found in our study are inconsistent with Brambilla's finding (Brambilla et al 2005). Based on their pharmacological effects, it would be expected that higher DHEA levels would increase the patients' anxiety and susceptibility to spontaneous panic attacks, while lower DHEA levels would have an opposite effect. Thus, similar to higher 3α-reduced NAS plasma levels found in PDs, the lower DHEA and PREG levels found in our study may serve as a counterregulatory mechanism against the occurrence of spontaneous panic attacks (Strohle et al 2002). The opposing results of Brambilla's and our studies calls for further investigation of whether these NAS changes contribute to the primary pathophysiology of, or if they are secondary compensatory changes in panic disorder.

As hypothesized, SZs had lower DHEA and PREG levels than HCs. To the best of our knowledge this is the first investigation of plasma PREG levels in schizophrenia,

and low PREG and DHEA may contribute to NMDA receptor hypofunction thought to be present in the illness. This theory is supported by reports of the clinically beneficial effects of DHEA augmentation therapy in schizophrenia (Nachshoni et al 2005; Shulman and Tibbo 2005). In agreement with our finding, low DHEA levels in SZs have been reported (Tourney and Erb 1979). However, normal (Ritsner et al 2004) and high (di Michele et al 2005; Gallagher et al 2007; Marx et al 2004; Ritsner et al 2005; Ritsner et al 2006; Strous et al 2004) DHEA or DHEAS levels have also been found. Thus, the precise role of and changes in DHEA and PREG remain elusive and require further investigation. It is possible that the lower NAS levels found in our SZs sample may have been due to medication effects. In a preclinical investigation it was found that the atypical antipsychotic clozapine, but not the typical agent haloperidol, decreased cortical DHEA and DHEAS following eight days of administration to rats (Nechmad et al 2003). However, clozapine was also found to increase PREG in rat hippocampus, cerebral cortex and plasma (Marx et al 2006), and sulpiride, a typical antipsychotic, increased DHEA plasma levels in HCs (Oseko et al 1986). In a recent investigation of DHEA and DHEAS serum level fluctuations in medicated SZs, there were no associations between NAS changes and the type or dose of antipsychotic that the patients used (Ritsner et al 2006). As it is currently unknown how antipsychotics alter NAS levels in humans, and as we did not include an unmedicated schizophrenic group in our study, we cannot exclude the possibility that the low DHEA and PREG levels found in our SZs sample were caused by antipsychotics. This matter warrants further investigation.

As expected, all groups reported higher panic/anxiety symptoms following CCK-5 injections, compared to post-PLA injection. However, we did not find significant

differences in anxiety symptom severity between groups following either CCK-5 or PLA injection, which was both unexpected and inconsistent with previous reports (Abelson and Nesse 1994; Tait et al 2002; van Megen et al 1994). This lack of between-group differences may reflect the small sample sizes in our study. Alternatively, our findings may be a consequence of the higher dose of CCK-5 used in our investigation compared to previous studies. Dose response evaluations of CCK-5 indicate that doses greater than 0.6 μg/kg induce anxiety symptoms in HCs (McCann et al 1994; van Megen et al 1994). The dose of CCK-5 used in our study was 50 μg, which is equivalent to 0.71 μg/kg in an average 70 kg man. This higher dose may have caused a ceiling effect, resulting in comparable induction of panic/anxiety symptoms in the three study groups. If this is the case, then in this study we compared, for the first time, DHEA and PREG concentration changes during equivalently intense panic/anxiety symptoms between the three groups.

In accordance with our hypothesis, there was a higher increase in DHEA concentration from baseline to 10 min post CCK-5 injection in PDs, compared to HCs. Because DHEA is likely anxiogenic, a lower increase in HCs may be a protective mechanism during acutely anxious situations that is not present, or is blunted in PDs. However, as there were no significant differences on PSS and VAS-A max scores post CCK-5 injections between HCs and PDs, this smaller increase in DHEA concentration may reflect our finding that a smaller fraction of HCs (8/12) than PDs (10/10) experienced a CCK-5-induced panic attack. There were no statistically significant differences between SZs and either of the other groups in DHEA or PREG level changes from baseline to 10 min post injection, but mean DHEA and PREG level changes in SZs

were between those found in HCs and PDs. This likely reflects the clinical picture of schizophrenia, where anxiety is a common, but not a primary, symptom of the illness.

HCs had a greater decrease in DHEA and PREG concentrations from baseline to 180 min, compared to PDs and SZs, and a greater decrease in PREG concentrations than SZs post PLA injection. The ability to decrease these putatively anxiogenic NASs may be a mechanism to help restore normal functioning after anticipatory (post PLA) or provoked (post CCK-5) acute anxiety. Thus, healthy individuals may have a greater capacity to restore normal functioning following acute anxiety than either of the patient groups.

In our study we found that PLA injection increased DHEA and PREG concentrations with minimal effect on behavioral measures of panic/anxiety in HCs and in PDs. These NAS levels changes are likely due to nonspecific stress associated with the procedure. It is therefore essential to use a PLA control in panic-challenge paradigms in order to establish whether NAS levels changes are due to provoked anxiety or due to other nonspecific factors. Interestingly, PLA-induced increase in NASs was not seen in SZs. This may be because SZs are more accustomed to hospital environment and medical procedures, including injections, and as such may be less likely to experience nonspecific injection stress.

In this study we found that PDs and SZs have similar baseline DHEA and PREG plasma concentrations, as well as similar NAS concentration fluctuations following panic induction with CCK-5. These similarities may partially explain why SZs have a high rate of comorbid anxiety disorders (Tibbo et al 2003). Further investigation is needed to establish if these similarities are coincidental or are part of a common pathophysiological

phenomenon in the two psychiatric illnesses. Future research should also investigate if other common neurochemical abnormalities exist in the two disorders.

2.5 TABLES AND FIGURES

Table 2-1. Antipsychotic medication taken by SZs.

Antipsychotic Medication	**n**	**Mean Dose (mg/day)**	**Mean Dose (mg/injection)**
quetiapine	4	418.75	N/A
clozapine	4	310	N/A
risperidone	1	1.5	N/A
olanzapine	1	10	N/A
flupentixol decanoate	2	N/A	26
trihexyphenidyl	1	6	N/A
fluphenazine decanoate	1	N/A	20

Table 2-2. Clinical and demographic data.

	HC (n=12)	PD (n=10)	SZ (n=10)
Age in years (mean±S.D)	27.58±9.87	40.90±12.22*	33.30±8.99
HAM-A (mean±S.D)	1.00±1.04	11.5±5.84	9.80±8.12
STAI (mean±S.D)	28.08±7.44	40.90±13.28	39.10±10.57
Panic disorder with agoraphobia (n)	0	7	1
Panic disorder without agoraphobia (n)	0	3	0
Social anxiety disorder (n)	0	0	1
Generalized anxiety disorder (n)	0	0	1
Schizophrenia (n)	0	0	10
Duration of schizophrenia (years)	N/A	N/A	11.40±8.37
PANSS positive (mean±S.D)	N/A	N/A	16.00±4.32
PANSS negative (mean±S.D)	N/A	N/A	14.60±5.28
PANSS general (mean±S.D)	N/A	N/A	28.60±3.78
PANSS total (mean±S.D)	N/A	N/A	59.20±10.79
GAF (mean±S.D)	N/A	N/A	49.30±15.15
CDSS (mean±S.D)	N/A	N/A	1.90±1.45

*One way ANOVA revealed a statistically significant difference in age between groups ($F_{2, 29} = 4.47$, $p = 0.020$), attributed to PDs being older than HCs ($p = 0.017$).

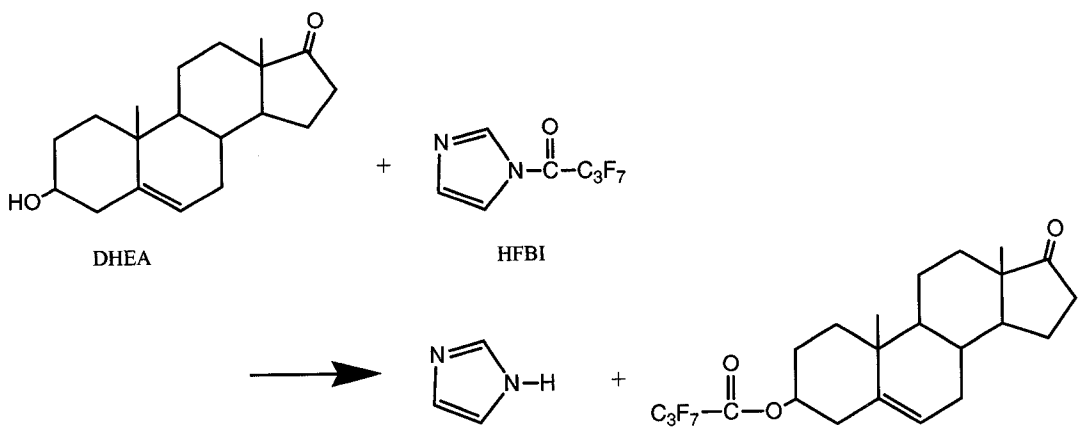

Figure 2-1. Derivatization of DHEA with HFBI.

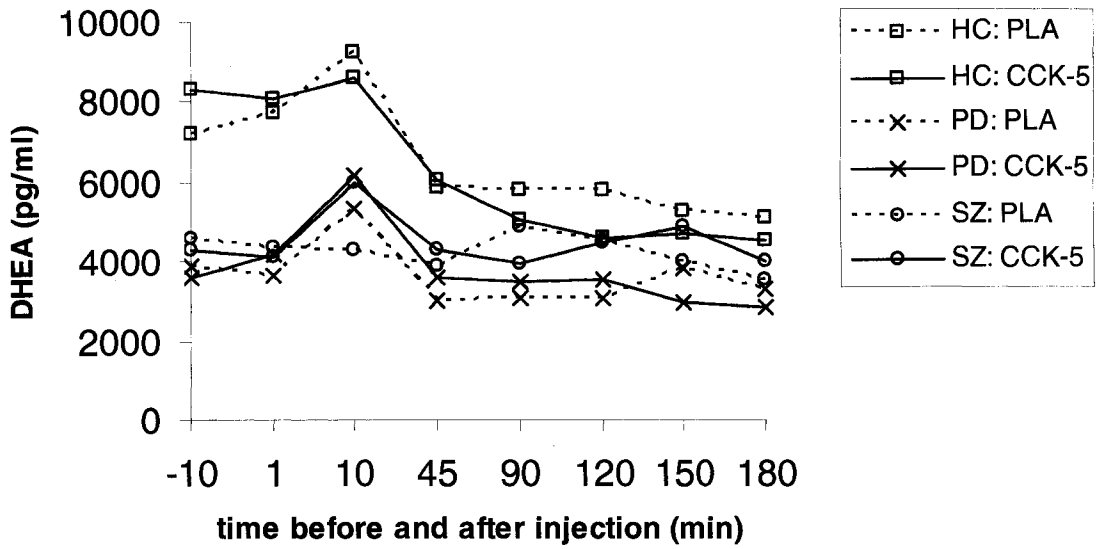

Figure 2-2. Mean DHEA plasma concentrations before and after PLA and CCK-5 injection in PDs, SZs and HCs.

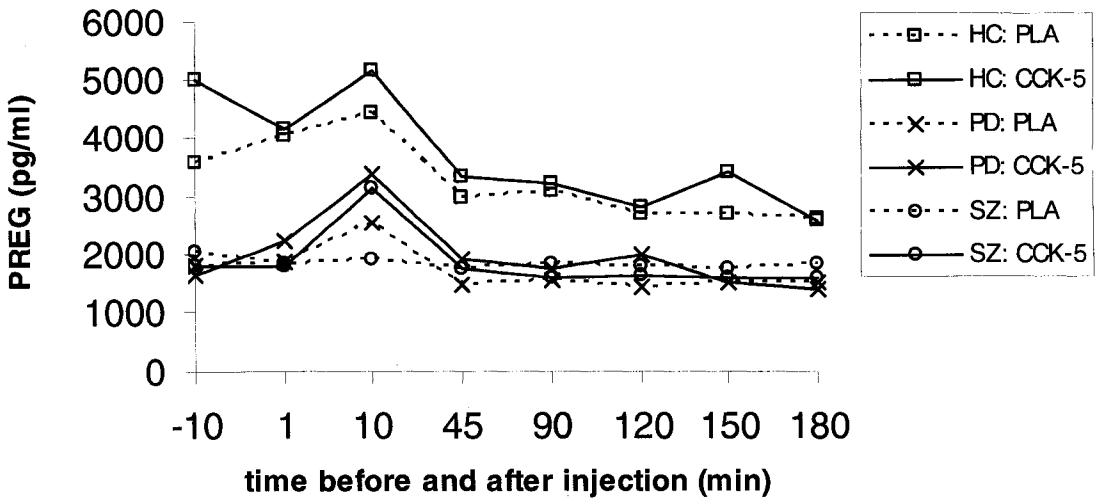

Figure 2-3. Mean PREG plasma concentrations before and after PLA and CCK-5 injection in PDs, SZs and HCs.

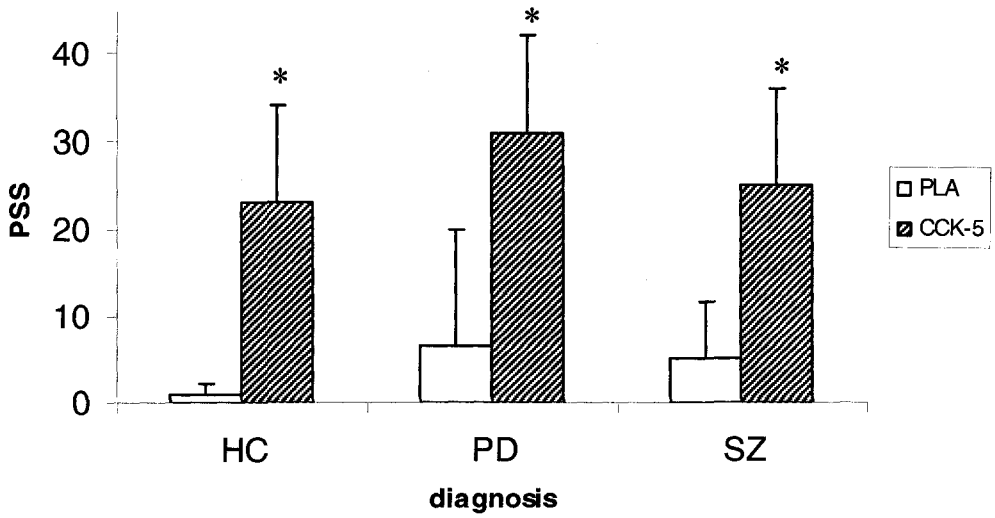

Figure 2-4. Mean PSS sum of scores (+SD) post PLA and post CCK-5 injection in HCs, PDs and SZs. All groups reported higher PSS scores post CCK-5, compared to post PLA administration (*$p < 0.001$).

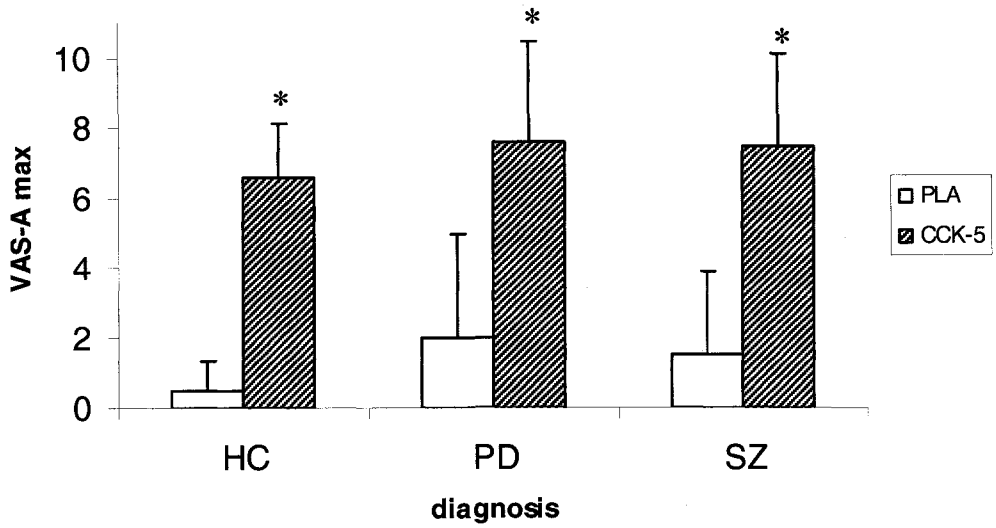

Figure 2-5. Mean VAS-A max scores (+ SD) post PLA and post CCK-5 injection in HCs, PDs and SZs. All groups reported higher VAS-A max scores post CCK-5, compared to post PLA administration (*$p < 0.001$).

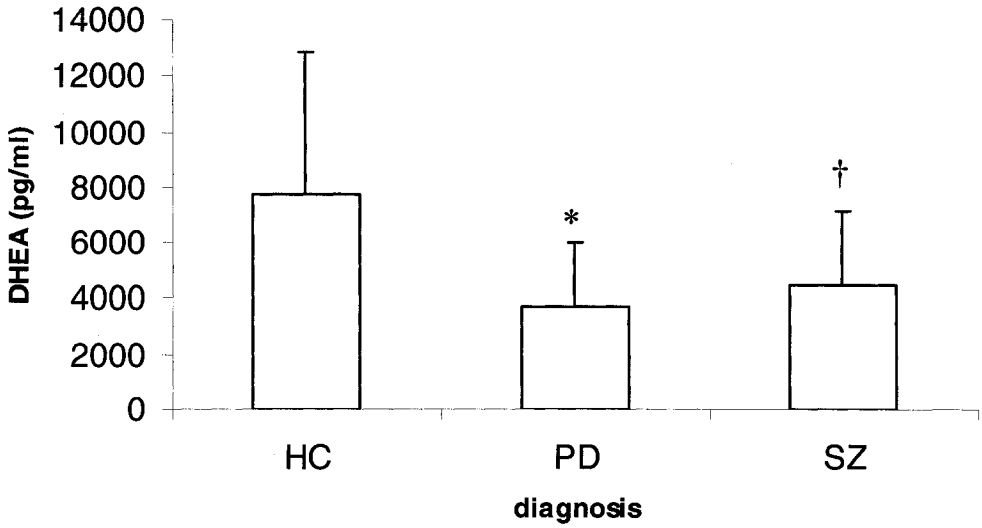

Figure 2-6. Mean DHEA concentrations (+ SD) at baseline in HCs, PDs and SZs. ANCOVA, with age as a covariate, revealed a statistically significant difference in baseline DHEA concentrations between groups ($F_{2, 62} = 4.35$, $p = 0.017$). This difference was attributed to SZs ($^{\dagger}p = 0.002$) and PDs ($^{*}p < 0.001$) having lower DHEA concentrations than HCs.

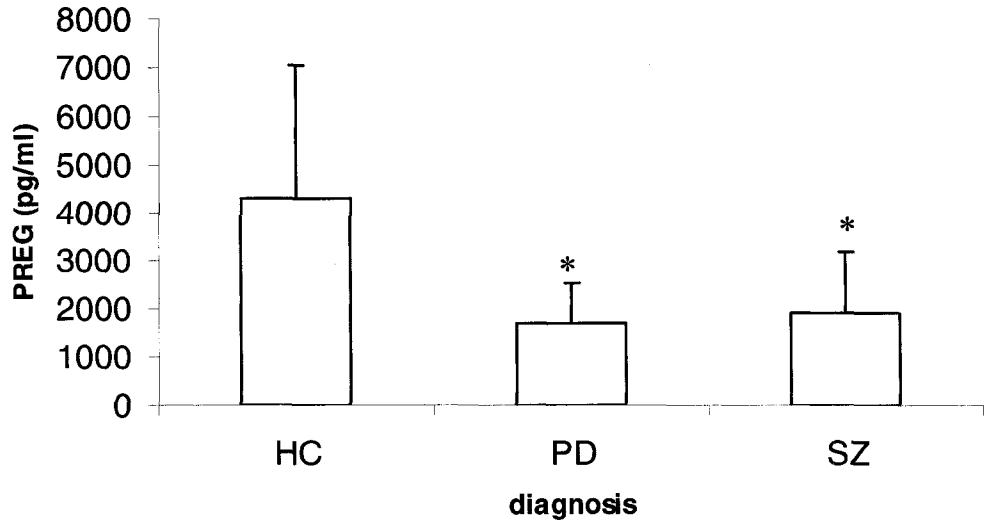

Figure 2-7. Mean PREG concentrations (+ SD) at baseline in HCs, PDs and SZs. ANCOVA, with age as a covariate, revealed a statistically significant difference in baseline PREG concentrations between groups ($F_{2, 63} = 11.78$, $p < 0.001$). This difference was attributed to SZs and PDs having lower PREG concentrations than HCs (*$p < 0.001$).

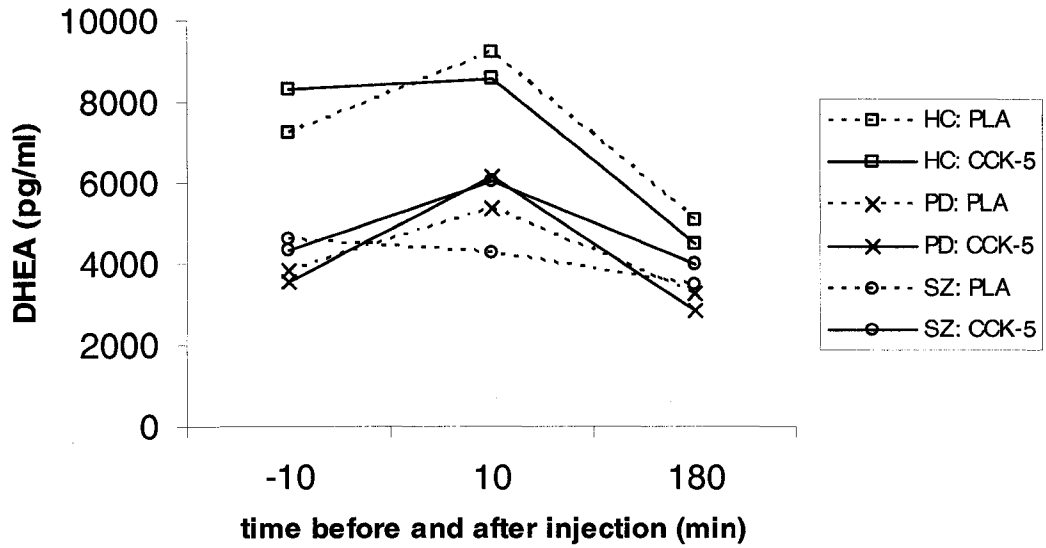

Figure 2-8. DHEA concentrations before (-10 min) and after (+10 min and +180 min) PLA and CCK-5 injections in HCs, PDs and SZs. MANOVA showed a significant time effect (Wilks' multivariate tests of significance: $F_{2, 26} = 21.66$, $p < 0.001$), a significant time x group effect (Wilks' multivariate tests of significance: $F_{4, 52} = 3.71$, $p = 0.010$) and a significant treatment x time x group effect (Wilks' multivariate tests of significance: $F_{4,52} = 3.71$, $p = 0.020$) on DHEA concentration.

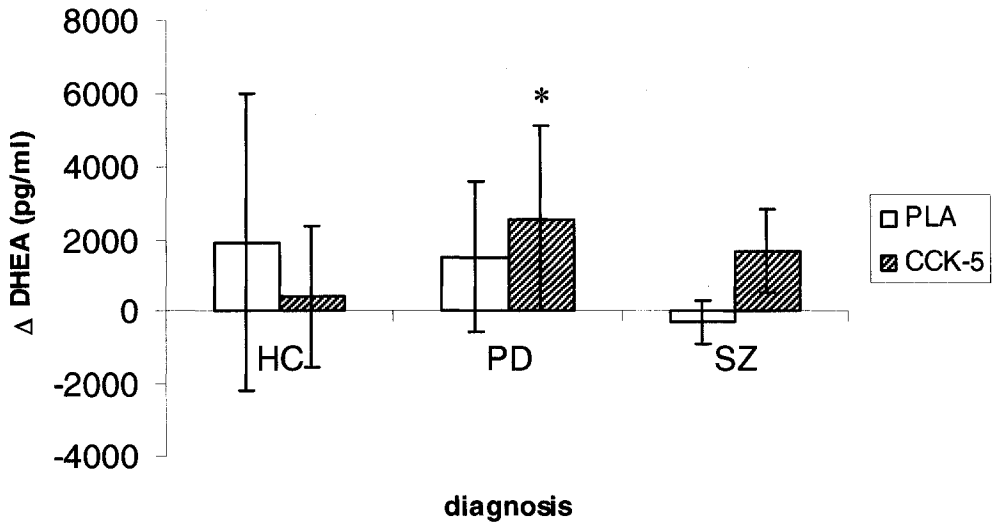

Figure 2-9. Mean change (Δ) in DHEA concentration from baseline to +10 min post injection (± SD) in HCs, PDs and SZs. One way ANOVA revealed a statistically significant difference in ΔDHEA from baseline to +10 min post CCK-5 injection ($F_{2, 28}$ = 3.42, p = 0.047), attributed to a greater increase of plasma DHEA in PDs, compared to HCs (*p < 0.05).

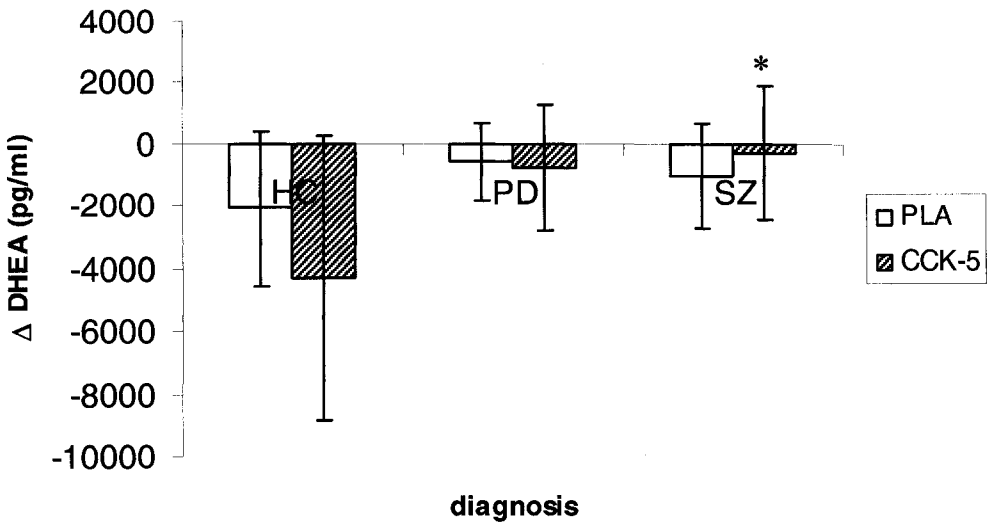

Figure 2-10. Mean change (Δ) in DHEA concentration from baseline to +180 min post injection (± SD) in HCs, PDs and SZs. One way ANOVA revealed a statistically significant difference in ΔDHEA from baseline to +180 min post CCK-5 injection ($F_{2, 28} = 4.92$, $p = 0.015$), attributed to a smaller decrease of plasma DHEA in SZs, compared to HCs (*$p = 0.025$).

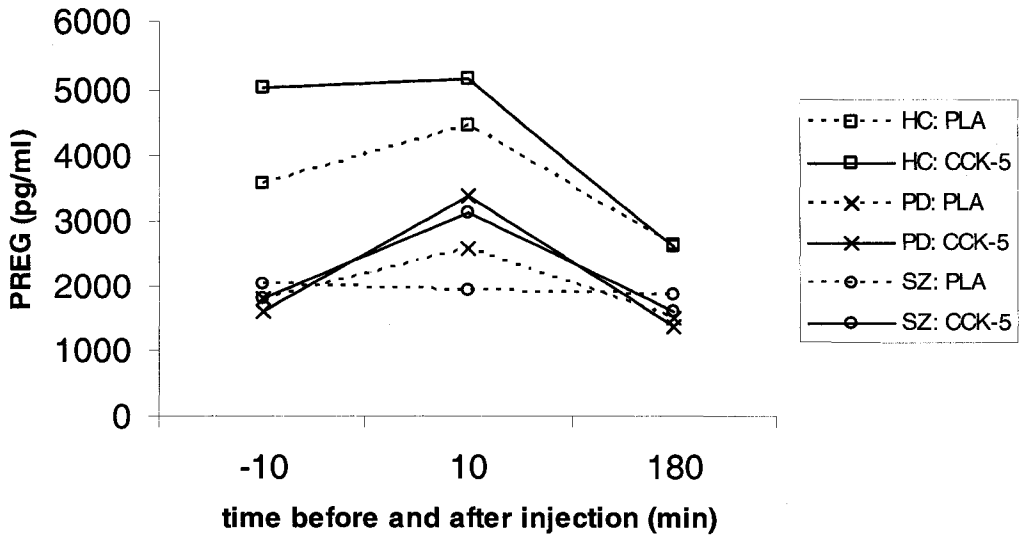

Figure 2-11. PREG concentrations before (-10 min) and after (+10 min and +180 min) PLA and CCK-5 injection in HCs, PDs and SZs. MANOVA showed a significant time effect (Wilks' multivariate tests of significance: $F_{2, 27} = 20.99$, $p < 0.001$), a significant time x group effect (Wilks' multivariate tests of significance: $F_{4, 54} = 3.79$, $p = 0.009$) and a trend toward a significant treatment x time x group effect (Wilks' multivariate tests of significance: $F_{4, 54} = 2.38$, $p = 0.063$) on PREG concentration.

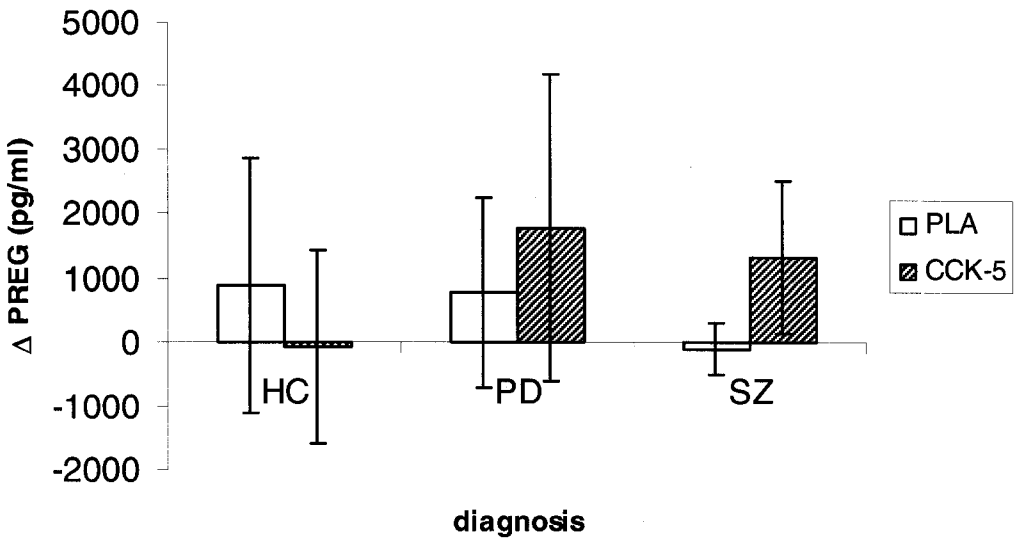

Figure 2-12. Mean change (Δ) in PREG concentration from baseline to +10 min post injection (± SD) in HCs, PDs and SZs. There were no statistically significant differences between groups.

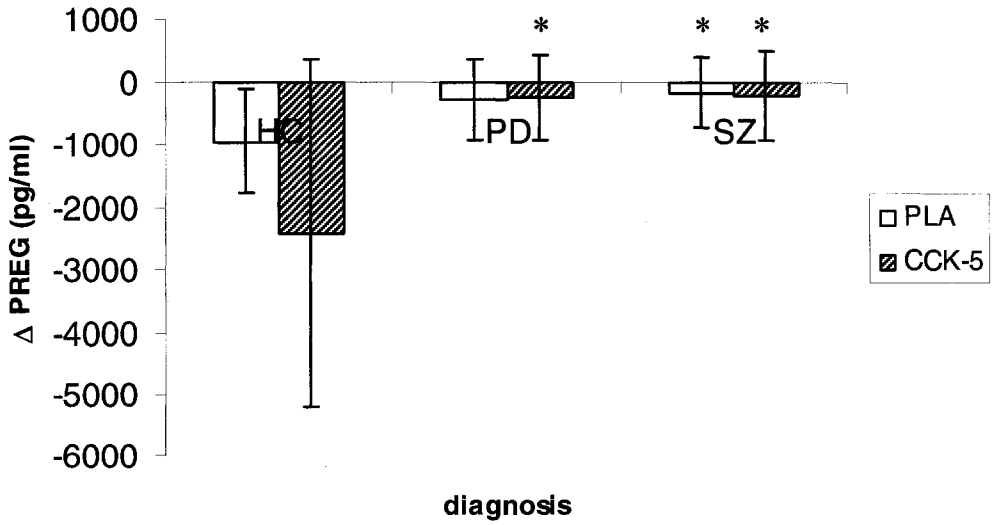

Figure 2-13. Mean change (Δ) in PREG concentration from baseline to +180 min post injection (± SD) in HCs, PDs and SZs. One way ANOVA revealed a statistically significant difference in ΔPREG from baseline to +180 min post CCK-5 injection ($F_{2, 29}$ = 5.56, p = 0.009), attributed to a smaller decrease of plasma PREG in SZs and PDs, compared to HCs (*p < 0.05). There was also a statistically significant difference in ΔPREG from baseline to +180 min post PLA injection ($F_{2, 29}$ = 4.02, p = 0.029), attributed to a smaller decrease of plasma PREG in SZs, compared to HCs (*p < 0.05).

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Chapter 3

Longitudinal Investigation of NASs in First Episode Psychosis

3.1 INTRODUCTION

NASs are steroids that affect neuronal excitability by interacting with, and modulating the activity of, cell surface ligand-gated ion channel receptors, including $GABA_A$ and NMDA receptors (Paul and Purdy 1992; Rupprecht and Holsboer 1999). The 3α-reduced NASs 3α,5α-THP, 3α,5β-THP and 3α,5α-THDOC are among the most selective, potent and efficacious positive allosteric modulators of the $GABA_A$ receptor complex (Follesa et al 2001). At nM concentrations these NASs positively modulate $GABA_A$ receptors by increasing both the probability and the frequency of chloride channel opening (Macdonald and Olsen 1994; Twyman and Macdonald 1992). Other NASs such as DHEA, PREG and/or their sulfated metabolites, DHEAS and PREGS, are negative $GABA_A$ and/or positive NMDA receptor modulators (Rupprecht and Holsboer 1999; Twyman and Macdonald 1992). At low μM concentrations PREGS and DHEAS negatively modulate $GABA_A$ receptors (Imamura and Prasad 1998; Park-Chung et al 1999); PREGS acts as a mixed $GABA_A$ receptor agonist/antagonist and DHEAS behaves solely as an antagonist. DHEA also inhibits $GABA_A$ receptors; however, it is less potent than DHEAS (Baulieu and Robel 1998; Imamura and Prasad 1998; Park-Chung et al 1999). PREGS, DHEAS and DHEA are also positive allosteric modulators at NMDA receptors (Baulieu and Robel 1998; Macdonald and Olsen 1994).

As abnormalities in the glutamatergic and GABAergic systems are implicated in schizophrenia, NASs are also thought to play a role in the pathophysiology of the illness. Few studies have investigated NAS abnormalities in schizophrenia. While some of these found normal NAS plasma levels in medicated FEPs and chronic patients, specifically, normal DHEA and/or DHEAS levels (Oades and Schepker 1994; Ritsner et al 2004;

Shirayama et al 2002), reports of abnormal NAS plasma levels can also be found in the literature. These include abnormal plasma levels in FEPs, such as high DHEA and DHEAS, with trends toward low PREG and 3α,5α-THP in unmedicated (Marx et al 2004; Strous et al 2004), and high DHEAS in medicated (Oades and Schepker 1994) male patients, and abnormal NAS plasma levels in chronic patients, including low DHEA in unmedicated (Tourney and Erb 1979), and high DHEA (di Michele et al 2005; Gallagher et al 2007; Ritsner et al 2006), low DHEAS (Ritsner et al 2006) and high cortisol/DHEA and cortisol/DHEAS ratios in medicated patients (Ritsner et al 2004). Analysis of brain tissue collected postmortem from chronically ill medicated patients, in which higher that normal PREG and DHEA levels in the posterior cingulate cortex, and higher than normal PREG, lower 3α,5α-THP, and a trend toward higher DHEA levels in the parietal cortex were found, also implicate NASs in schizophrenia (Marx et al 2006b).

There are also several reports of statistically significant linear relationships between NAS plasma levels and various symptom domains. In a study conducted by Ritsner et al, plasma cortisol/DHEA ratios were directly correlated with depression, anxiety, anger and hostility (Ritsner et al 2004). In a later investigation by the same group, the researchers found that both DHEA and DHEAS were positively associated with dysphoric mood and positive symptom severity (Ritsner et al 2006). However, an inverse correlation between DHEA and Brief Psychiatric Rating Scale scores has also been found (Harris et al 2001). DHEAS levels and the ratio of DHEAS/cortisol in the plasma were also found to be directly correlated with cognitive function in one study (Silver et al 2005), while an inverse relationship between DHEA and memory function was found in another study (Harris et al 2001). Marx et al found that DHEA and PREG

plasma levels were inversely correlated with negative symptom severity (Marx et al 2004), and Stous et al found that DHEAS serum levels were inversely correlated with illness severity and aggressive behavior (Strous et al 2004). In a later study, Marx et al also found that in chronic patients who took PREG as an adjunct to antipsychotics, serum PREG levels were positively correlated with cognitive improvement (Marx et al 2007).

Although there is evidence to suggest that NASs may be implicated in schizophrenia, the number of studies conducted in this area is small and the results from these studies have not been consistent. One reason for this may be that most of these studies measured NAS levels in patients who were taking antipsychotic drugs at the time of phlebotomy, making it unclear if plasma level abnormalities are due to the disease process, or if they are secondary to the effects of medication. Indeed, some atypical antipsychotics have been found to increase PREG, 3α,5α-THP and 3α,5α-THDOC, and decrease DHEA and DHEAS levels in rat brains and/or plasma (Barbaccia et al 2001; Marx et al 2000; Marx et al 2006a; Marx et al 2003; Nechmad et al 2003). However, it is unknown if antipsychotics affect NAS levels in humans in the same way as they do in rats, as only one small study, where six weeks of clozapine treatment improved symptoms but had no effect on plasma 3α,5α-THP and 3α,5α-THDOC, addressed this issue (Monteleone et al 2004). The effects of antipsychotics on NAS levels in humans remain elusive due to the small sample size examined in this study (n = 9; five men and four women). Furthermore, although the patients discontinued all medication (except for typical antipsychotics) for at least two weeks before commencing treatment with clozapine, the effects of long-term exposure to antipsychotics prior to the study may have affected the experimental results.

To address these issues, this longitudinal investigation of NASs was designed to determine which NASs are altered in schizophrenia, establish if NAS level alterations are part of the disease process or are secondary to medication effects, and determine if there is a relationship between NAS level abnormalities and various symptom domains. This was accomplished by measuring NAS plasma concentrations in FEPs in an unmedicated state and after two months of treatment with atypical antipsychotics, comparing these levels to those found in individuals with no psychopathology, and examining how levels correlate with symptom severity.

3.2 METHODS

3.2.1 Study Participants

Ethical approval for this study was obtained from the University of Alberta Health Research Ethics Board, and written informed consent was obtained from all participants after all procedures had been fully explained. Twenty-one male FEPs, who were recruited from the Edmonton Early Psychosis Intervention Clinic (EEPIC) and met the current EEPIC inclusion/exclusion criteria (Table 3-1), participated in the study. Thirty age-matched, male HCs with no psychopathology [as determined by the SCID-I (First et al 1994)] also participated in the study, and were recruited from the community via poster advertisement. Exclusion criteria for HCs included major neurological/medical illness, history of substance/alcohol abuse, history of significant head injury (loss of consciousness > 29 minutes), and a positive family history of Axis-I disorders in first or second degree relatives.

3.2.2 Clinical Assessment

Demographic and diagnosis data are shown in Table 3-2, clinical rating scores are shown in Table 3-3, and medications taken by study participants are shown in Table 3-4. Of the 21 EEPIC patients included in the study, nine were antipsychotic-naïve, and the remaining 12 patients had a mean lifetime exposure to antipsychotics prior to entering EEPIC of 10.25 days. If a patient was taking any psychotropic medication it was discontinued for a minimum of two to seven days, as determined by the half-life of elimination of the specific drug. All FEPs completed a clinical assessment in an unmedicated state (baseline), as required by the EEPIC protocol. During this assessment the SCID-I was administered to all FEPs to determine diagnosis, the PANSS, subdivided into positive, negative and general symptom clusters (Kay et al 1987) was used to quantify psychopathology, the STAI (Spielberger et al 1970) and the Beck Anxiety Inventory (BAI) (Beck and Steer 1990) were used to assess symptoms of anxiety, the Magical Ideation Scale (MIS) (Eckblad and Chapman 1983) was used to assess schizotypy and delusional thinking, and the Psychological General Well-Being Index (PGWB) (McDowell and Newell 1996) was used to assess subjective well-being. As per EEPIC protocol, following baseline assessment, all FEPs began taking one of three atypical antipsychotics (olanzapine, risperidone, or quetiapine), and were followed longitudinally. The treating EEPIC psychiatrists prescribe concomitant medication as necessary for standard patient care. Symptom rating scales described above were administered to FEPs two and six months post baseline assessment in order to track symptom changes throughout the course of treatment. All symptom rating scales, with the

exception of the PANSS, were administered to HCs at baseline and two months after initial assessment. The HCs were unmedicated throughout the study.

3.2.3 Plasma Collection

All study participants followed an overnight fast prior to blood collection, which was done between 8 and 10 am using vacutainers containing the anticoagulant ethylenediamine tetraacetic acid. Blood was collected from FEPs using the same regimen as that used for PANSS administration [i.e. at baseline (unmedicated state), and following two and six months of treatment with antipsychotic medication]. Blood was also collected twice from unmedicated HCs: at baseline and two months after initial collection. Following blood collection the plasma was immediately separated by centrifugation, transferred into plastic tubes and stored at the Neurochemical Research Unit, University of Alberta, in a -80°C freezer until analysis.

3.2.4 DHEAS and PREGS Analysis with High Performance Liquid Chromatography (HPLC) Coupled with MS

Plasma samples were analyzed using a procedure developed in the Neurochemical Research Unit (Rauw et al, manuscript in preparation). Samples (250 μL) were prepared by precipitating the protein with 200 μL methanol, and adding 200 ng of deuterated PREGS (D4 PREGS), which served as an internal standard. After sitting on ice for 10 min, the samples were centrifuged at 4°C to precipitate the protein, and the resultant supernatants were subjected to solid-phase extraction (SPE) using Oasis® HLB cartridges. The eluents were transferred into HPLC vials with polypropylene inserts for

analysis on a Waters Alliance HPLC system coupled to a Waters Micromass ZQ single quad mass spectrometer. Single ion recording was used with negative electrospray.

Standard curves consisting of varying amounts of DHEAS and PREGS and a fixed amount of D4 PREGS (internal standard) were constructed and used to quantify plasma NAS levels. The standards were subjected to the same extraction and analysis procedures as the plasma samples. Identification and quantification of DHEAS and PREGS in the samples were achieved by comparison of retention times and response of authentic standards in the calibration curves (Figure 3-1).

3.2.5 DHEA, PREG, 3α,5α-THP and 3α,5β-THP Analysis with GC-MS

Plasma samples (1 mL) were prepared by precipitating the protein with 800 μL methanol, and adding 4 ng of D4 PREG, which served as an internal standard. After sitting on ice for 10 min, the samples were centrifuged for 4 min at 4°C to precipitate the protein. The supernatants were then analyzed using the procedure of Kim et al. (Kim et al 2000) as modified at the Neurochemical Research Unit (Rauw et al, manuscript in preparation). The method involves SPE (Oasis® HLB cartridges) followed by derivatization with HFBI and analysis using GC combined with NI-CI MS. Analysis was performed using an Agilent 6890 GC system and an Agilent 59373N mass selective detector.

Standard curves consisting of varying amounts of the NASs of interest and a fixed amount of D4 PREG (internal standard) were constructed and used for quantifying plasma NAS levels. The standards were subjected to the same extraction, derivatization, and analysis procedures as the plasma samples. Identification and quantification of

DHEA, PREG, 3α,5α-THP, and 3α,5β-THP in the samples were achieved by comparison of retention times and response of authentic standards in a calibration curves (Figure 3-2).

3.2.6 Statistical Analysis

SPSS 12.0 software was used for all statistical analysis. All results are expressed as mean ± SD. For all statistical analyses, $\alpha = 0.05$ was set as the nominal level of significance.

MANOVA, with time (baseline and two months) as a within-subject factors and group (FEP and HC) as a between-subject factor, was used to analyze changes in plasma NAS concentrations.

Independent samples t-tests were also done to determine if there were any significant between-group differences in plasma concentrations at baseline and at two months, separately, and paired samples t-tests were done to determine if there were significant differences in plasma concentrations between baseline and two months within each group separately. MANOVA, with time (baseline, two months, six months) as a within-subject factor, was also used to determine any significant changes in plasma concentrations between baseline, two months and six months in FEPs.

In order to determine if a linear relationship exists between plasma NAS concentrations and clinical rating scores, correlation analyses between each NAS concentration and each clinical rating score were done for baseline and two month measures, separately, in each group separately. The clinical rating scales included in correlation analyses in FEPs were PANSS-P, PANSS-N, PANSS-G, MIS, STAI and

BAI. As the PANSS was not administered to HCs, only MIS, STAI and BAI were included in correlation analyses in HCs.

3.3 RESULTS

3.3.1 Plasma DHEAS Concentration Changes

MANOVA showed a significant time effect (Wilks' multivariate tests of significance: $F_{1, 37} = 7.581$, $p = 0.009$) and a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 37} = 6.666$, $p = 0.014$) on plasma DHEAS concentration (Figure 3-3).

Independent samples t-tests did not reveal significant between-group differences in plasma DHEAS concentrations at baseline ($t_{49} = 1.788$, $p = 0.080$), but did reveal a statistically significant difference at two months ($t_{37} = 2.606$, $p = 0.013$) (Figure 3-3).

Paired samples t-tests examining differences in DHEAS concentrations between baseline and two months in each group separately did not show significantly different DHEAS plasma concentrations in HCs ($t_{28} = 0.158$, $p = 0.876$), but did show significantly different DHEAS plasma concentrations in FEPs ($t_9 = 4.224$, $p = 0.002$) (Figure 3-3).

MANOVA, with time (baseline, two months, six months) as a within-subject factor, did not reveal significant changes in plasma DHEAS concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2, 4} = 2.164$, $p = 0.231$) (Figure 3-4).

3.3.2 Plasma PREGS Concentration Changes

MANOVA showed a significant time effect (Wilks' multivariate tests of significance: $F_{1, 37} = 9.255$, $p = 0.004$) and a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 37} = 14.978$, $p < 0.001$) on plasma PREGS concentration (Figure 3-5).

Independent samples t-tests did not reveal significant between group differences in plasma PREGS concentrations at baseline ($t_{49} = 0.863$, $p = 0.393$), but did reveal a statistically significant difference at two months ($t_{37} = 2.962$, $p = 0.011$) (Figure 3-5).

Paired samples t-tests examining differences in PREGS concentrations between baseline and two months in each group separately did not show significantly different PREGS plasma concentrations in HCs ($t_{28} = 1.016$, $p = 0.318$), but did show significantly different PREGS plasma concentrations in FEPs ($t_9 = 2.767$, $p = 0.022$) (Figure 3-5).

MANOVA, with time (baseline, two months, six months) as a within-subject factor, did not reveal significant changes in plasma PREGS concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2, 4} = 4.615$, $p = 0.091$) (Figure 3-6).

3.3.3 Plasma DHEA Concentration Changes

MANOVA did not show a significant time effect (Wilks' multivariate tests of significance: $F_{1, 37} = 1.794$, $p = 0.189$), or a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 37} = 0.145$, $p = 0.705$) on plasma DHEA concentration (Figure 3-7).

Independent samples t-tests did not reveal significant between group differences in plasma DHEA concentrations at baseline ($t_{49} = 0.432$, $p = 0.667$), or at two months ($t_{37} = 0.843$, $p = 0.405$) (Figure 3-7).

Paired samples t-tests examining differences in DHEA concentrations between baseline and two months in each group separately did not show significantly different DHEA plasma concentrations in HCs ($t_{28} = 1.008$, $p = 0.322$), or in FEPs ($t_9 = 0.631$, $p = 0.416$) (Figure 3-7).

MANOVA, with time (baseline, two months, six months) as a within-subject factor, did not reveal significant changes in plasma DHEA concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2,4} = 2.176$, $p = 0.229$) (Figure 3-8).

3.3.4 Plasma PREG Concentration Changes

MANOVA did not show a significant time effect (Wilks' multivariate tests of significance: $F_{1, 37} = 3.342$, $p = 0.076$) or a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 37} = 0.449$, $p = 0.507$) on plasma PREG concentration (Figure 3-9).

Independent samples t-tests did not reveal significant between group differences in plasma PREG concentrations at baseline ($t_{49} = 0.769$, $p = 0.446$), or at two months ($t_{37} = 0.973$, $p = 0.337$) (Figure 3-9).

Paired samples t-tests examining differences in PREG concentrations between baseline and two months in each group separately did not show significantly different

DHEA plasma concentrations in HCs ($t_{28} = 1.390$, $p = 0.176$), or in FEPs ($t_9 = 1.023$, $p = 0.333$) (Figure 3-9).

MANOVA, with time (baseline, two months, six months) as a within-subject factor, did not reveal significant changes in plasma PREG concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2,4} = 1.648$, $p = 0.301$) (Figure 3-10).

3.3.5 Plasma 3α,5α-THP Concentration Changes

MANOVA did not show a significant time effect (Wilks' multivariate tests of significance: $F_{1,36} = 1.779$, $p = 0.191$) or a significant time x group effect (Wilks' multivariate tests of significance: $F_{1,36} = 0.923$, $p = 0.343$) on plasma 3α,5α-THP concentration (Figure 3-11).

Independent samples t-tests did not reveal significant between group differences in plasma 3α,5α-THP concentrations at baseline ($t_{49} = 0.808$, $p = 0.423$), but did reveal a statistically significant difference at two months ($t_{36} = 2.846$, $p = 0.007$) (Figure 3-11).

Paired samples t-tests examining differences in 3α,5α-THP concentrations between baseline and two months in each group separately did not show significantly different 3α,5α-THP plasma concentrations in FEPs ($t_8 = 0.177$, $p = 0.846$), but did show significantly different 3α,5α-THP plasma concentrations in HCs ($t_{28} = 2.531$, $p = 0.017$) (Figure 3-11).

MANOVA, with time (baseline, two months, six months) as a within-subject factor, did not reveal significant changes in plasma 3α,5α-THP concentrations between

baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2,2} = 0.346$, $p = 0.743$) (Figure 3-12).

3.3.6 Plasma 3α,5β-THP Concentration Changes

MANOVA showed a significant time effect (Wilks' multivariate tests of significance: $F_{1,36} = 12.630$, $p = 0.001$) and a significant time x group effect (Wilks' multivariate tests of significance: $F_{1,36} = 9.697$, $p = 0.004$) on plasma 3α,5β-THP concentration (Figure 3-13).

Independent samples t-tests revealed a significant between group difference in plasma 3α,5β-THP concentrations at baseline ($t_{49} = 2.806$, $p = 0.007$), but not at two months ($t_{36} = 0.397$, $p = 0.694$) (Figure 3-13).

Paired samples t-tests examining differences in 3α,5β-THP concentrations between baseline and two months in each group separately revealed significantly different 3α,5β-THP plasma concentrations in FEPs ($t_9 = 2.547$, $p = 0.031$), but not in HCs ($t_{27} = 0.574$, $p = 0.571$) (Figure 3-13).

MANOVA, with time (baseline, two months, six months) as a within-subject factor, did not reveal significant changes in plasma 3α,5β-THP concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2,4} = 2.651$, $p = 0.185$) (Figure 3-14).

3.3.7 Relationship Between Plasma NAS Concentrations and Clinical Rating Scores

Table 3-5 shows results from correlation analyses investigating the linear relationship between each NAS plasma concentration and PANSS scores in FEPs at baseline and at two months. There was a statistically significant positive correlation between plasma DHEAS concentrations and PANSS-N scores at baseline and at two months ($R_{1,19} = 0.499$, $p = 0.021$ and $R_{1,8} = 0.662$, $p = 0.037$, respectively), and plasma DHEAS and DHEA concentrations were positively correlated with PANSS-G scores at baseline ($R_{1,19} = 0.496$, $p = 0.022$ and $R_{1,19} = 0.520$, $p = 0.016$, respectively). No other statistically significant linear relationships between plasma NAS concentrations and PANSS scores were found.

Results from correlation analyses investigating the linear relationship between each NAS plasma concentration and STAI, BAI and MIS scores in FEPs and HCs at baseline and at two months are presented in Table 3-6. There was a statistically significant positive correlation between plasma 3α,5β-THP concentrations and BAI scores in FEPs and in HCs at two months ($R_{1,8} = 0.763$, $p = 0.010$ and $R_{1,26} = 0.475$, $p = 0.011$, respectively). No other statistically significant linear relationships between plasma NAS concentrations and STAI, BAI and MIS scores were found.

3.4 DISCUSSION

This study was, to our knowledge, the first longitudinal investigation of NASs in FEPs, and the first investigation of 3α,5β-THP and PREGS in the pathophysiology of psychosis. In this study we examined NAS plasma concentrations in FEPs in an unmedicated state, examined how these concentrations changed following treatment with atypical antipsychotics, compared these concentrations to those found in HCs, and

established if a relationship exists between NAS plasma concentrations and symptom severity. Furthermore, we were able to examine and quantify six NASs that are thought to play a modulatory role in the GABAergic and glutamatergic neurotransmitter systems, thus enabling us to better understand dysfunctions in these systems in schizophrenia.

We found that plasma 3α,5β-THP concentrations in unmedicated FEPs were significantly higher than those found in HCs, and that two months of treatment with atypical antipsychotics normalized these concentrations. DHEAS and PREGS plasma concentrations were normal (i.e. not significantly different from HCs) when FEPs were unmedicated, but declined after two months of treatment with atypical antipsychotics, while DHEA and PREG concentrations were comparable to normal throughout the study in FEPs. 3α,5α-THP concentrations did not change in FEPs throughout the study, and were comparable to those found in HCs during their first visit (i.e. baseline). Unexpectedly, 3α,5α-THP concentrations declined in HCs from their baseline to their two month visit.

We also found that some NAS concentrations were correlated with symptom severity. Plasma DHEAS concentrations were directly correlated with PANSS-N scores at baseline and at two months. DHEA and DHEAS concentrations were also positively correlated with PANSS-G scores at baseline, but not at two months. Also, at two months, but not at baseline, 3α,5β-THP concentrations were positively correlated with BAI scores in both FEPs and HCs.

It appears that 3α,5β-THP is the only NAS examined in this study that may be implicated in both the pathophysiology of psychosis and in the mechanism of action of antipsychotic drugs, as it is the only NAS whose levels were abnormal in FEPs in an

unmedicated state, and were affected by antipsychotics. At baseline FEPs had higher than normal 3α,5β-THP concentrations. Since 3α,5β-THP is a positive $GABA_A$ receptor modulator, an increase in 3α,5β-THP in unmedicated FEPs may serve to compensate for the hypothesized hypoGABAergic state in schizophrenia. Thus increased 3α,5β-THP may be an endogenous antipsychotic mechanism. After two months of treatment with atypical antipsychotics 3α,5β-THP concentrations decreased in FEPs, reaching concentrations comparable to those found in HCs. This can be interpreted in two ways: 1) atypical antipsychotics decrease 3α,5β-THP levels; or 2) levels decline because the endogenous antipsychotic mechanism of increasing 3α,5β-THP is no longer necessary after atypical antipsychotic administration. As this is the first investigation of 3α,5β-THP in psychosis, and there are currently no reports on how antipsychotic drugs affect 3α,5β-THP levels, it is difficult to speculate which explanation is more likely. In order to resolve this issue, studies examining 3α,5β-THP levels changes following atypical antipsychotic administration in rats and HCs would need to be conducted.

Interestingly, we found that at two months, but not at baseline, 3α,5β-THP plasma levels were positively correlated with BAI scores in both FEPs and HCs. BAI scores reflect anxiety levels experienced by an individual throughout the previous week, and the presence of a correlation between 3α-reduced NAS levels and anxiety is not surprising since 3α-reduced NASs have been implicated in anxiety. Indeed, these NASs exert anxiolytic effects similar to those seen with benzodiazepines (Eser et al 2005). Thus, an increase in 3α,5β-THP levels when anxiety is high may be an endogenous anxiolytic mechanism, and supports the hypothesis that increased 3α-reduced NAS levels serve as a counterregulatory mechanism against anxiety (Strohle et al 2002). Oddly, there was no

relationship between 3α,5β-THP and BAI scored at baseline in FEPs or HCs. This may be because study participants rate their own level of anxiety when completing the BAI (i.e. the BAI is a self-report anxiety scale), and were not as comfortable with the interviewer, and therefore less forthcoming about their anxiety symptoms at their baseline, compared to their two month visits. Another unforeseen finding was the lack of a correlation between 3α,5α-THP, a NAS with a similar molecular mechanism of action as 3α,5β-THP, and BAI scores. This may indicate that when using the BAI as an anxiety assessment tool, 3α,5β-THP may be a better predictor of anxiety symptom severity than 3α,5α-THP. Also, 3α-reduced NASs were not correlated with STAI scores in our study. As STAI scores reflect an individual's current anxiety level, the lack of a relationship may have been because the STAI was not administered at the same time as phlebotomy. Since both anxiety and alterations in plasma NAS concentrations in response to anxiety are time-dependent, if a study participant was feeling more anxious during clinical assessment than during phlebotomy, or vice versa, and the STAI measured the level of anxiety during clinical assessment, the relationship between STAI scores and NAS levels would be obscured. In future studies that examine the relationship between plasma metabolite levels and STAI scores, the STAI should be administered immediately before phlebotomy.

In our study, 3α,5α-THP plasma levels fluctuated only in HCs. We found that while 3α,5α-THP decreased from baseline to two months in HCs, levels found throughout the study in FEPs were comparable to levels in HCs at baseline. 3α,5α-THP levels have only been investigated in schizophrenia in two other studies, and only one study examined 3α,5α-THP levels in a similar patient sample as the one used in our study

(unmedicated FEPs) (Marx et al 2004). In this study, although Marx et al found a trend toward lower 3α,5α-THP in the patient group, the results are in agreement with ours since this difference was not statistically significant (Marx et al 2004). In the other study, 3α,5α-THP plasma levels in parietal cortical brain tissue collected postmortem from chronically ill, medicated patients were examined, and it was found that 3α,5α-THP levels were significantly lower in patients than in HCs (Marx et al 2006b). Although these results are inconsistent with ours, the many differences between the patient samples, including stage of illness (chronic vs. recent onset), medication status (medicated vs. unmedicated), age (older vs. younger), and mortality status (postmortem vs. living sample) likely impacted the results to a great extent, making it fruitless to compare results from the two studies.

To the best of our knowledge, this is the first time that 3α,5α-THP was investigated in HCs longitudinally. Although our finding that plasma 3α,5α-THP decreased from baseline to two months in HCs is surprising, it indicates that when conducting longitudinal investigations, data collection for HCs should follow the same schedule as that used for patients in order to control for unexpected fluctuations. As only FEPs took antipsychotics in our study, it is possible that antipsychotics prevented the natural decline in 3α,5α-THP levels that may occur in this age group. This explanation is consistent with studies that found that atypical antipsychotics increase 3α,5α-THP levels in rats (Barbaccia et al 2001; Marx et al 2000; Marx et al 2003).

Examination of other NASs in our study revealed that DHEAS and PREGS plasma concentrations were normal when FEPs were unmedicated, and declined after two months of treatment with atypical antipsychotics, and DHEA and PREG concentrations

were normal throughout the study in FEPs. While normal, low and high DHEA and DHEAS, and normal and high PREG have been found in patients with schizophrenia (di Michele et al 2005; Gallagher et al 2007; Marx et al 2004; Oades and Schepker 1994; Ritsner et al 2006; Ritsner et al 2004; Strous et al 2004; Tourney and Erb 1979), only two of these studies examined DHEA, DHEAS and/or PREG levels in unmedicated FEPs. Findings from these studies include higher than normal DHEA and DHEAS, and a trend toward lower PREG levels in patients, compared to HCs (Marx et al 2004; Strous et al 2004). Consistent with our results, PREG levels were similar in patients and HCs; however, the elevated DHEA and DHEAS levels found in these studies are inconsistent with our results. This discrepancy may be accounted for by the different DHEA and DHEAS quantification methods used in our and their studies, where we used chromatography to quantify DHEA and DHEAS plasma levels, and they used radioimmunoassay (RIA). Although RIA is a sensitive method for detecting NASs, but the specificity of RIA is poor due to antibody cross-reactions (Appelblad and Irgum 2002). Thus, the higher than normal DHEA and DHEAS levels found in these studies may actually reflect an increase in some other plasma metabolite present in the patient group that could have cross-reacted with the antibodies used for quantifying these NASs.

Interestingly, both DHEAS and PREGS decreased in FEPs after two months of treatment with atypical antipsychotics. Although atypical antipsychotic-induced reduction in DHEAS levels has been demonstrated in rats (Nechmad et al 2003), this is, to the best of our knowledge, the first time that a similar pattern has been demonstrated in humans. Furthermore, PREGS has a similar molecular mechanism of action as DHEAS, and a similar pattern in PREGS fluctuations was also demonstrated in our study. The similar

level fluctuation patterns of NASs with similar mechanisms of action, and the previous demonstration of this pattern in rats, makes us confident to conclude that atypical antipsychotics reduce DHEAS and PREGS.

We also found significant linear relationships between DHEA and DHEAS and some symptom domains in FEPs. The most robust relationship found in our study was a direct linear correlation between DHEAS levels and negative symptom severity as measured by PANSS-N scores, because this relationship was statistically significant both at baseline and at two months. We also found that DHEAS and DHEA levels were positively correlated with PANSS-G scores at baseline, but these relationships were not statistically significant at two months. Perhaps treatment with atypical antipsychotics changes the relationship between NAS plasma levels and symptom severity.

Despite the methodological improvements made in our study, including using a longitudinal design and GC-MS as a quantification tool for investigating NAS plasma level in FEPs, there are some methodological concerns in our study that need to be addressed. As shown in Table 3-2, the FEPs are a heterogeneous group of patients, with only 6/21 having a DSM-IV diagnosis of schizophrenia. Due to the duration criteria when diagnosing schizophrenia, a person has to be assessed six months after they experience their first psychotic episode before a diagnosis of schizophrenia can be established, and only five patients completed their six month assessment (three with schizophrenia and two with substance-induced psychosis). Furthermore, as there are currently no laboratory diagnostic tests for schizophrenia, the diagnosis is subjective and dependent on the treating psychiatrist. Thus, the diagnosis may change throughout a patient's involvement in EEPIC and throughout their lifetime. This issue is not restricted to our study and is a

concern in all studies that investigate schizophrenia pathology, as well as the pathology of other psychiatric disorders. The only way to resolve this issue is to improve diagnostic procedures. The FEPs included in our study also took different atypical antipsychotics, which may have affected NAS plasma levels differently. This has been demonstrated in rat studies that found that olanzapine increases brain 3α,5α-THP levels (Barbaccia et al 2001; Marx et al 2000; Marx et al 2003), but risperidone does not (Marx et al 2000). Unfortunately, the two month FEP sample size in our study was too small to statistically examine the differential effects of different antipsychotics on NAS levels, and some of our patients took more than one type of antipsychotic, thus further complicating the relationship between antipsychotic drugs and NAS level changes. In the future, as our patient sample size increases, we will examine if different antipsychotics affect NAS levels differently.

Another concern in our study that was mentioned previously is that clinical assessment and phlebotomy were not done simultaneously, but rather hours-days apart. This makes it difficult to examine the relationship between some symptoms and NAS plasma levels because some of the symptoms rating scores, particularly STAI scores, are time-dependent. However, scores from other clinical rating scales used in our study, including the MIS, PANSS, and BAI, remain stable for at least a week. Therefore we were effectively able to examine the relationship between plasma NAS levels and scores derived using these symptom rating scales.

As with all studies that use peripheral metabolite levels to investigate the neurochemistry of brain disorders, we cannot be certain that peripheral levels reflect those found in the brain. However, due to their lipophilic nature, NASs are likely able to

cross the BBB. O'Dell et al demonstrated that brain and plasma NASs increase in parallel after acute ethanol administration in rats, and adrenalectomy/gonadectomy prevents the increase in NASs (O'Dell et al 2004). These findings implicate peripheral endocrine glands, rather than the brain, as the source of some NASs following ethanol administration, indicating that peripheral NASs can penetrate the BBB. Whether a similar pattern exists in humans has only been investigated in one postmortem study, where Marx et al found that brain PREG and 3α,5α-THP concentrations exceeded, while brain DHEA levels were comparable to those found in serum or plasma (Marx et al 2006b). The researchers did not examine other NASs in this study, and it is possible that postmortem changes affected the distribution of the NASs in various body compartments. Until an *in vivo* technique that allows for quantification of NASs in the brain is available, this issue cannot be resolved with certainty.

This study was the first longitudinal investigation of NASs in first episode psychosis. We established which NAS level alterations may be implicated in the pathophysiology of psychosis, and which may be secondary to the effects of atypical antipsychotics. Our results indicate that 3α,5β-THP may be implicated in both the pathophysiology of psychosis and in the mechanism of action of antipsychotic drugs, and that 3α,5α-THP, DHEAS and PREGS levels may be affected by atypical antipsychotics. Furthermore, we found that DHEAS may be a good predictor of negative symptom severity. In the future we want to establish which antipsychotics affect NAS levels, and if antipsychotic-induced NAS level alterations are beneficial (i.e. part of the therapeutic mechanism of action of these drugs) or if they are simply a side effect of the drug. NAS investigation presents an exciting new avenue of research that may further our

understanding of the neurochemical abnormalities that contribute to the complex pathophysiology of schizophrenia and other psychotic disorders.

3.5 TABLES AND FIGURES

Table 3-1. EEPIC inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
Age 16-35 years	Subjects requiring mood stabilizers at study entry
Diagnosis of schizophrenia or schizophreniform disorder	Head injury with > 29 minutes loss of consciousness
Less than 1 year of active-phase symptoms	Active substance abuse or dependence disorder (i.e. within past 30 days)
Less than 3 months of lifetime prior exposure to antipsychotic medication	Exposure to long acting depot neuroleptic medication
Current psychotic symptoms requiring long term antipsychotic treatment	Known sensitivity to olanzapine, risperidone, or quetiapine
	Serious past or current CNS/medical illness

Table 3-2. Diagnostic and demographic data for FEPs and HCs.

		FEP n=21	HC n=30
Age	Mean ± SD (years)	22.97±3.28	21.18±3.36
DSM-IV diagnosis	Schizophrenia	n=6	n=0
	Schizophreniform disorder	n=3	n=0
	Substance-induced psychotic disorder	n=5	n=0
	Brief psychotic disorder	n=1	n=0
	Psychotic disorder not otherwise specified	n=5	n=0
	Psychotic disorder due to a general medical condition	n=1	n=0
First degree family history	No history	n=14	n=30
	Schizophrenia	n=4	n=0
	Bipolar disorder	n=2	n=0
	Major depressive disorder	n=1	n=0
Handedness	Right handed	n=17	n=25
	Left handed	n=4	n=5
Marital status	Married or common law	n=0	n=6
	Single	n=21	n=24
Number of children	No children	n=21	n=29
	Two children	n=0	n=1
Occupation	Administrative/minor professional	n=0	n=4
	Clerical/sales/technician/farmer	n=0	n=1
	Skilled manual employee	n=2	n=0
	Unskilled employee	n=6	n=0
	Student	n=5	n=25
	Unemployed	n=8	n=1
Highest level of education achieved	Graduated professional/graduate school	n=0	n=1
	Part graduate/professional school	n=0	n=2
	Graduated four year college school	n=1	n=7
	Graduated two year college/ technical school	n=1	n=0
	Part college	n=7	n=19
	Graduated high school	n=5	n=1
	Attended grade 7-12 without graduating	n=7	n=0

Table 3-3. Clinical rating scores for FEPs and HCs.

Clinical rating scales	FEP *baseline* n=21	*2 months* n=11	*6 months* n=5	HC *baseline* n=30	*2 months* n=29
PANSS-P	19.76±5.88	13.40±5.06	10.60±1.52	N/A	N/A
PANSS-N	20.38±6.00	17.90±7.46	16.40±4.67	N/A	N/A
PANSS-G	40.62±10.60	34.70±10.89	31.20±4.21	N/A	N/A
PANSS-T	80.76±20.23	66.00±22.36	58.20±9.01	N/A	N/A
MIS	11.05±5.99	9.70±6.83	8.00±7.46	2.53±2.16	2.07±2.09
STAI	44.70±11.07	42.10±6.90	40.40±10.43	26.80±5.68	25.55±5.08
BAI	15.80±10.54	6.20±5.73	3.83±2.99	2.93±2.94	4.17±5.70
PGWB	57.33±18.45	68.30±7.10	66.00±8.88	90.93±9.55	90.28±10.11

All results are expressed as mean ± SD. PANSS-P, PANSS-N, PANSS-G, and PANSS-T refer to the positive, negative, general, and total PANSS scores, respectively.

Table 3-4. Medications taken by FEPs and HCs.

Time	Anti-psychotics	Group	n	Other psychotropic medication	Group	n	Other medication	Group	n
Baseline							minocycline	FEP	1
							fluticasone	HC	1
							salbutamol	HC	2
							celecoxib	HC	1
							budesonide	HC	1
							formoterol	HC	1
							isotretinoid	HC	1
2	olanzapine	FEP	4	mirtazapine	FEP	1	minocycline	FEP	1
months	quetiapine	FEP	1	divalproax	FEP	1	fluticasone	HC	1
	risperidone	FEP	3	lorazepam	FEP	1	salbutamol	HC	2
	olanzapine+ quetiapine	FEP	1	venlafaxine	FEP	1	celecoxib	HC	1
	risperidone+ quetiapine	FEP	1				budesonide	HC	1
							formoterol	HC	1
							isotretinoid	HC	1
6	olanzapine	FEP	1	miratazapine	FEP	1	minocycline	FEP	1
months	quetiapine	FEP	1	venlafaxine	FEP	1			
	risperidone	FEP	3						

All FEPs were unmedicated (not taking psychotropic medication) at baseline, and all HCs were unmedicated (not taking psychotropic medication) throughout the duration of the study. The daily doses for antipsychotic medication ranged between 5-20 mg, 150-500 mg, and 1.5-4 mg for olanzapine, quetiapine, and risperidone, respectively. The daily doses for other psychotropic medications were 15 mg, 750 mg, 1 mg, and 150 mg for mirtazapine, divalproax, lorazepam, and venlafaxine, respectively.

Table 3-5. Relationship between plasma NAS concentrations and PANSS scores, subdivided into PANSS-P, PANSS-N and PANSS-G in FEPs at baseline and at two months. R and p values are displayed.

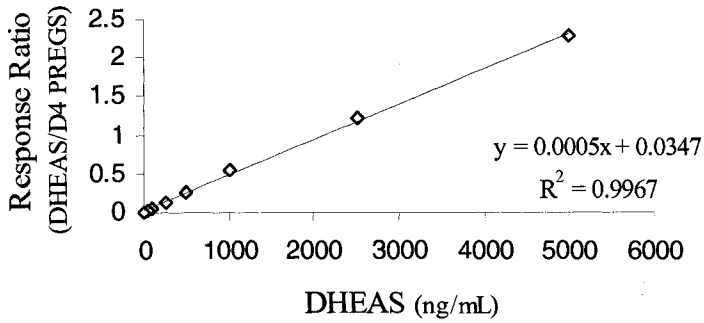
		PANSS-P		**PANSS-N**		**PANSS-G**	
Time	**NAS**	R	p	R	p	R	p
Baseline	DHEAS	0.327	0.147	***0.499***	***0.021****	***0.496***	***0.022****
	PREGS	0.029	0.902	0.367	0.102	0.321	0.156
	DHEA	0.321	0.156	0.329	0.145	***0.520***	***0.016****
	PREG	0.194	0.399	0.225	0.372	0.403	0.070
	3α,5α -THP	0.162	0.483	0.409	0.066	0.416	0.061
	3α,5β-THP	0.099	0.669	0.254	0.267	0.338	0.133
2 months	DHEAS	0.318	0.830	***0.662***	***0.037****	0.532	0.113
	PREGS	0.079	0.829	0.093	0.779	0.238	0.508
	DHEA	0.283	0.428	0.394	0.260	0.461	0.180
	PREG	0.476	0.164	0.396	0.257	0.502	0.140
	3α,5α -THP	0.150	0.700	0.137	0.725	0.011	0.978
	3α,5β-THP	0.480	0.160	0.517	0.126	0.502	0.139

* $p < 0.05$

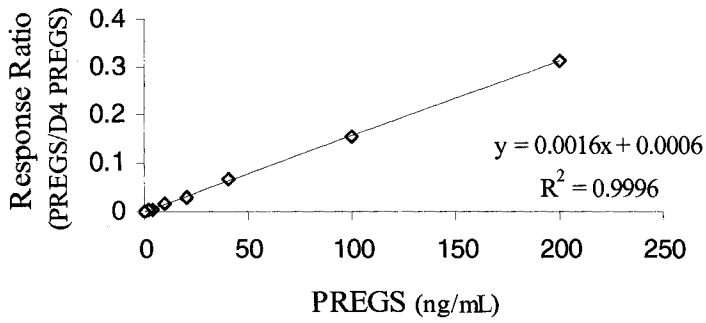
Table 3-6. Relationship between plasma NAS concentrations and STAI, BAI and MIS scores in FEPs and HCs at baseline and at two months. R and p values are displayed.

			STAI		BAI		MIS	
Time	**Group**	**NAS**	R	p	R	p	R	p
Baseline	FEP	DHEAS	0.174	0.463	0.366	0.112	0.041	0.860
		PREGS	0.201	0.395	0.016	0.946	0.230	0.317
		DHEA	0.113	0.636	0.238	0.312	0.189	0.412
		PREG	0.053	0.825	0.072	0.764	0.312	0.169
		3α,5α -THP	0.026	0.915	0.181	0.444	0.170	0.461
		3α,5β-THP	0.159	0.502	0.123	0.592	0.260	0.254
	HC	DHEAS	0.018	0.923	0.084	0.658	0.115	0.546
		PREGS	0.311	0.095	0.157	0.408	0.300	0.107
		DHEA	0.141	0.458	0.120	0.529	0.304	0.102
		PREG	0.253	0.177	0.069	0.718	0.018	0.925
		3α,5α -THP	0.035	0.058	0.054	0.775	0.124	0.512
		3α,5β-THP	0.122	0.520	0.067	0.727	0.048	0.801
2 months	FEP	DHEAS	0.099	0.785	0.335	0.344	0.617	0.058
		PREGS	0.114	0.754	0.350	0.321	0.065	0.859
		DHEA	0.083	0.820	0.222	0.538	0.591	0.072
		PREG	0.542	0.105	0.253	0.480	0.154	0.670
		3α,5α -THP	0.416	0.265	0.436	0.241	0.263	0.494
		3α,5β-THP	0.016	0.965	***0.763***	***0.010****	0.531	0.115
	HC	DHEAS	0.287	0.131	0.106	0.585	0.167	0.386
		PREGS	0.248	0.195	0.020	0.919	0.048	0.803
		DHEA	0.072	0.710	0.149	0.441	0.188	0.329
		PREG	0.237	0.215	0.094	0.629	0.133	0.490
		3α,5α -THP	0.166	0.389	0.094	0.627	0.103	0.595
		3α,5β-THP	0.109	0.590	***0.475***	***0.011****	0.065	0.744

* $p < 0.05$

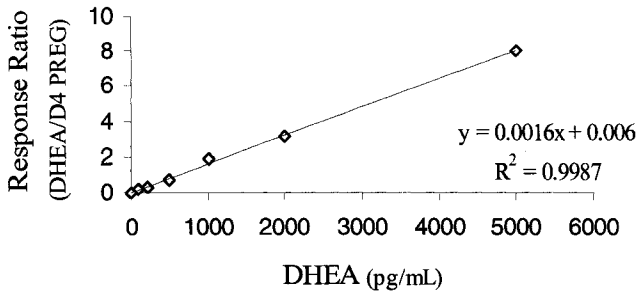


A



B

Figure 3-1. Calibration curves used to quantify plasma DHEAS (A) and PREGS (B).



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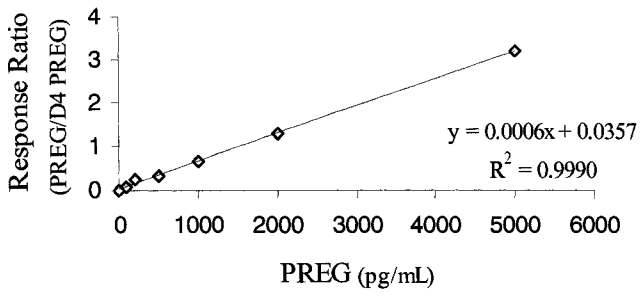

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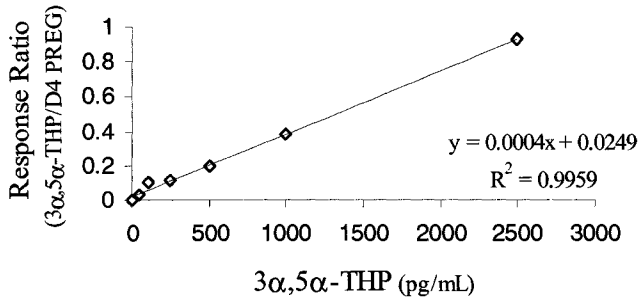
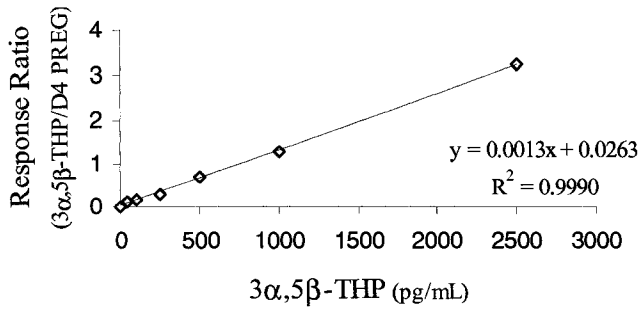


C



D

Figure 3-2. Calibration curves used to quantify plasma DHEA (A), PREG (B), 3α,5α-THP (C), and 3α,5β-THP (D).

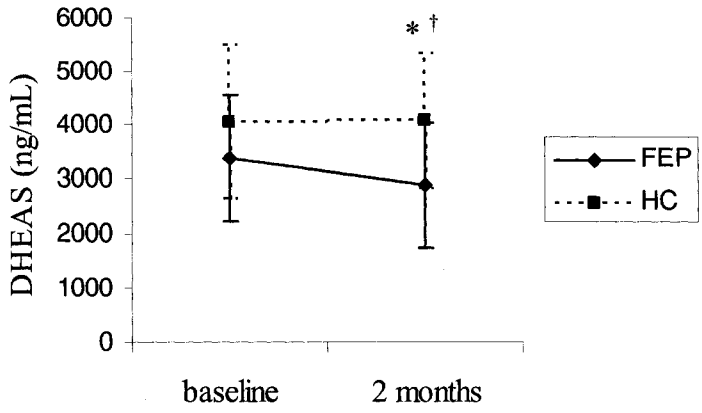


Figure 3-3. Mean (± SD) DHEAS concentrations at baseline and at two months in FEPs (unmedicated and medicated, respectively) and HCs (unmedicated at both time intervals). MANOVA showed a significant time effect (Wilks' multivariate tests of significance: $F_{1, 37} = 7.581$, $p = 0.009$) and a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 37} = 6.666$, $p = 0.014$) on plasma DHEAS concentration. Plasma DHEAS concentrations were not significantly different between groups at baseline ($t_{49} = 1.788$, $p = 0.080$), but at two months FEPs had significantly lower plasma DHEAS concentrations than HCs ($t_{37} = 2.606$, $^{*}p = 0.013$). There were no significant differences between baseline and two month plasma DHEAS concentrations in HCs ($t_{28} = 0.158$, $p = 0.876$); however, in FEPs DHEAS concentrations were significantly lower at two months, compared to baseline ($t_9 = 4.224$, $^{\dagger}p = 0.002$).

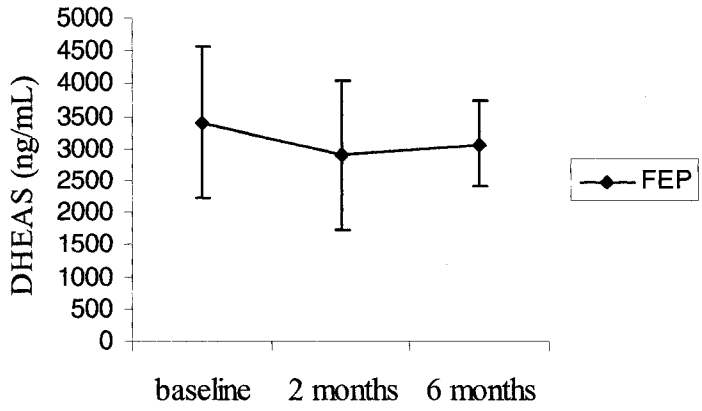


Figure 3-4. Mean (± SD) DHEAS concentrations at baseline, at two months and at six months in FEPs. MANOVA did not reveal significant changes in plasma DHEAS concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2,4} = 2.164$, $p = 0.231$).

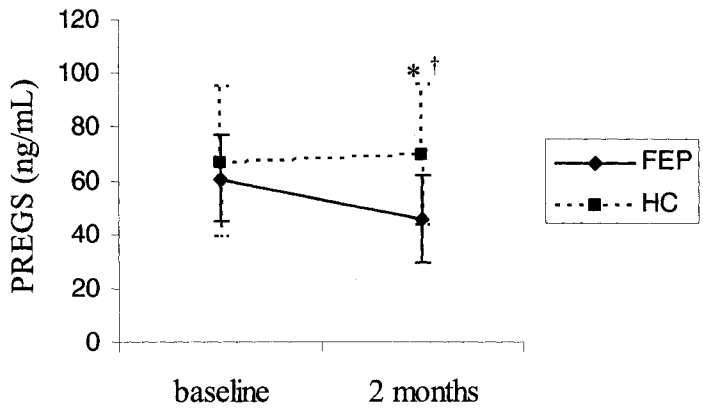


Figure 3-5. Mean (± SD) PREGS concentrations at baseline and at two months in FEPs (unmedicated and medicated, respectively) and HCs (unmedicated at both time intervals). MANOVA showed a significant time effect (Wilks' multivariate tests of significance: $F_{1, 37} = 9.255$, $p = 0.004$) and a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 37} = 14.978$, $p < 0.001$) on plasma PREGS concentration. Plasma PREGS concentrations were not significantly different between groups at baseline ($t_{49} = 0.863$, $p = 0.393$), but at two months FEPs had significantly lower plasma PREGS concentrations than HCs ($t_{37} = 2.962$, $^{*}p = 0.011$). There were no significant differences between baseline and two month plasma PREGS concentrations in HCs ($t_{28} = 1.016$, $p = 0.318$); however, in FEPs PREGS concentrations were significantly lower at two months, compared to baseline ($t_9 = 2.767$, $^{\dagger}p = 0.022$).

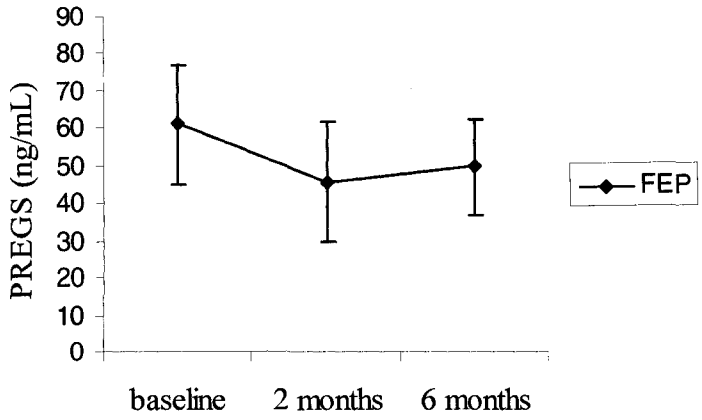


Figure 3-6. Mean (± SD) PREGS concentrations at baseline, at two months and at six months in FEPs. MANOVA did not reveal significant changes in plasma PREGS concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2,4} = 4.615$, $p = 0.091$).

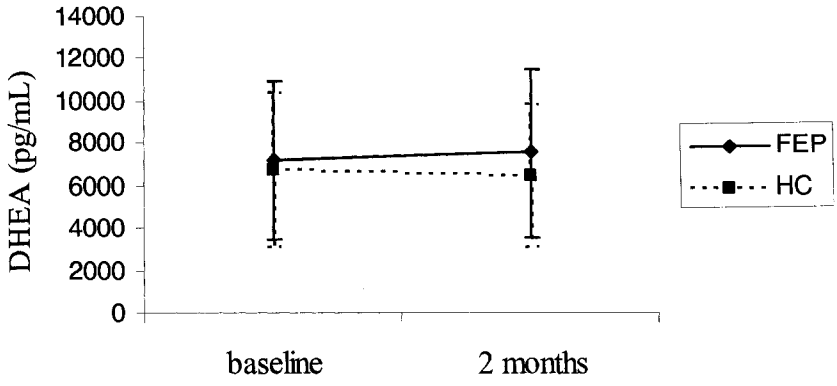


Figure 3-7. Mean (± SD) DHEA concentrations at baseline and at two months in FEPs (unmedicated and medicated, respectively) and HCs (unmedicated at both time intervals). MANOVA did not show a significant time effect (Wilks' multivariate tests of significance: $F_{1, 37} = 1.794$, $p = 0.189$) or a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 37} = 0.145$, $p = 0.705$) on plasma DHEA concentration. Plasma DHEA concentrations were not significantly different between groups at baseline ($t_{49} = 0.432$, $p = 0.667$), or at two months ($t_{39} = 1.449$, $p = 0.155$). There were no significant differences between baseline and two month plasma DHEA concentrations within each group separately (FEPs: $t_{11} = 0.631$, $p = 0.541$; HCs: $t_{28} = 1.008$, $p = 0.322$).

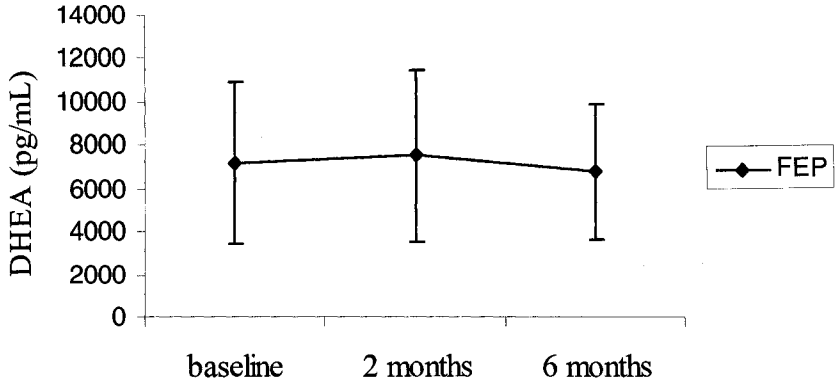


Figure 3-8. Mean (± SD) DHEA concentrations at baseline, at two months and at six months in FEPs. MANOVA did not reveal significant changes in plasma DHEA concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2,4} = 2.176$, $p = 0.229$).

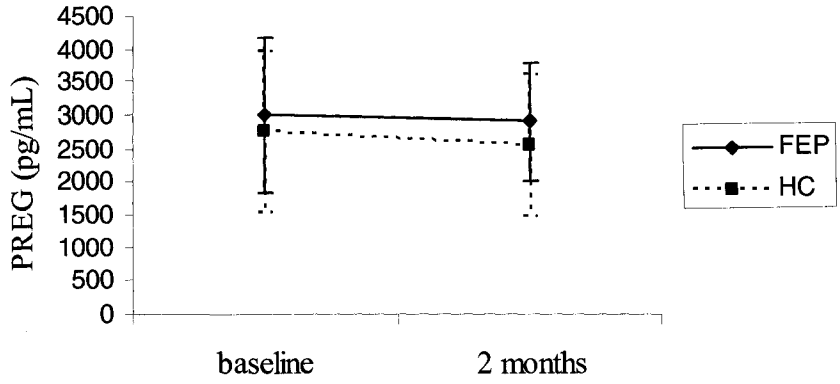


Figure 3-9. Mean (± SD) PREG concentrations at baseline and at two months in FEPs (unmedicated and medicated, respectively) and HCs (unmedicated at both time intervals). MANOVA did not show a significant time effect (Wilks' multivariate tests of significance: $F_{1, 37} = 3.342$, $p = 0.076$) or a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 37} = 0.446$, $p = 0.507$) on plasma PREG concentration. Plasma PREG concentrations were not significantly different between groups at baseline ($t_{49} = 0.769$, $p = 0.446$), or at two months ($t_{37} = 0.973$, $p = 0.337$). There were no significant differences between baseline and two month plasma PREG concentrations within each group separately (FEPs: $t_9 = 1.023$, $p = 0.333$; HCs: $t_{28} = 1.390$, $p = 0.176$).

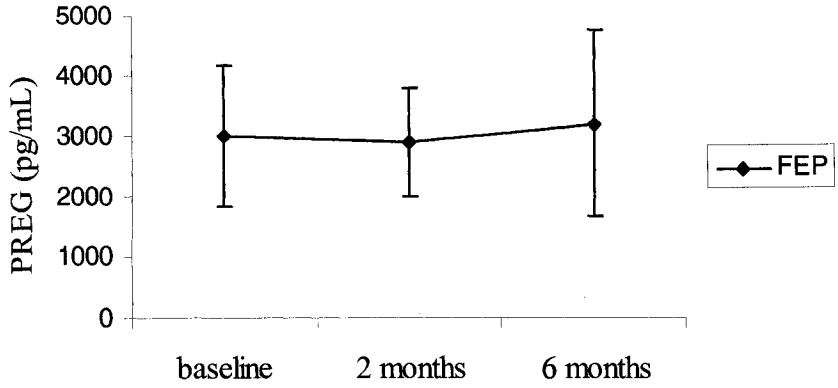


Figure 3-10. Mean (± SD) PREG concentrations at baseline, at two months and at six months in FEPs. MANOVA did not reveal significant changes in plasma PREG concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2,4} = 1.648$, $p = 0.301$).

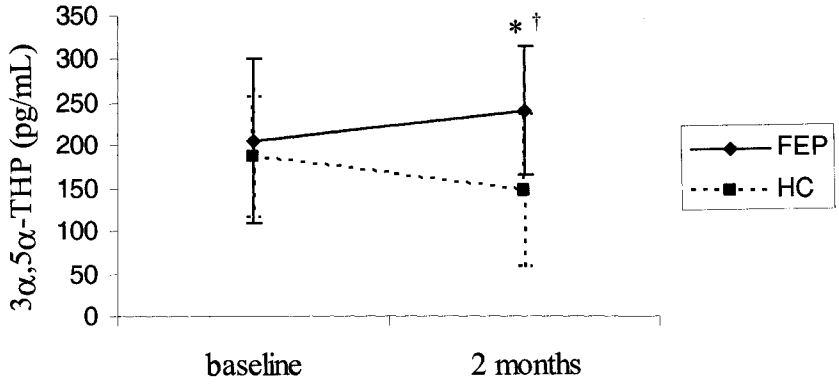


Figure 3-11. Mean (± SD) 3α,5α-THP concentrations at baseline and at two months in FEPs (unmedicated and medicated, respectively) and HCs (unmedicated at both time intervals). MANOVA did not show a significant time effect (Wilks' multivariate tests of significance: $F_{1, 36} = 1.779$, $p = 0.191$) or a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 36} = 0.923$, $p = 0.343$) on plasma 3α,5α-THP concentration. Plasma 3α,5α-THP concentrations were not significantly different between groups at baseline ($t_{49} = 0.808$, $p = 0.423$), but at two months FEPs had significantly higher plasma 3α,5α-THP concentrations than HCs ($t_{36} = 2.846$, $^{*}p = 0.007$). There were no significant differences between baseline and two month plasma 3α,5α-THP concentrations in FEPs ($t_8 = 0.177$, $p = 0.846$); however, in HCs 3α,5α-THP concentrations were significantly lower at two months, compared to baseline ($t_{28} = 2.531$, $^{\dagger}p = 0.017$).

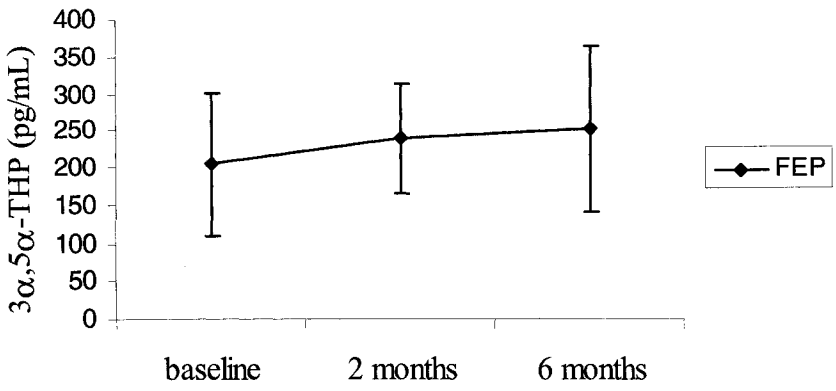


Figure 3-12. Mean (± SD) 3α,5α-THP concentrations at baseline, at two months and at six months in FEPs. MANOVA did not reveal significant changes in plasma 3α,5α-THP concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2,2} = 0.346$, $p = 0.743$).

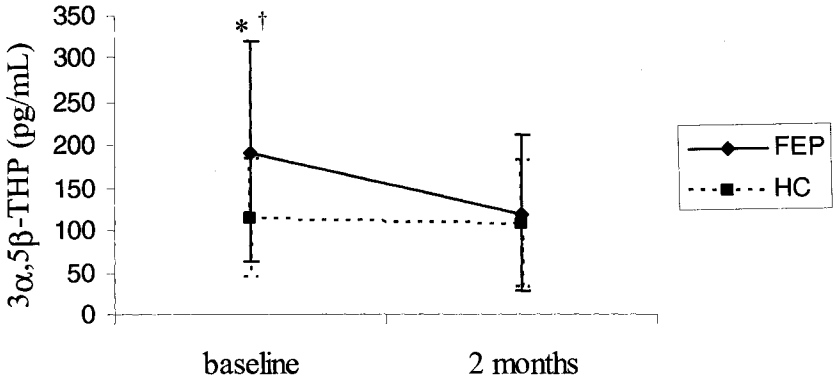


Figure 3-13. Mean (± SD) 3α,5β-THP concentrations at baseline and at two months in FEPs (unmedicated and medicated, respectively) and HCs (unmedicated at both time intervals). MANOVA showed a significant time effect (Wilks' multivariate tests of significance: $F_{1, 36} = 12.630$, $p = 0.001$) and a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 36} = 9.697$, $p = 0.004$) on plasma 3α,5β-THP concentration. Plasma 3α,5β-THP concentrations were significantly higher in FEPs, compared to HCs at baseline ($t_{49} = 2.806$, $^{*}p = 0.007$), but not at two months ($t_{36} = 0.397$, $p = 0.694$). FEPs has significantly higher 3α,5β-THP plasma concentrations at baseline, compared to two months ($t_9 = 2.547$, $^{\dagger}p = 0.031$), while HCs did not have significantly different 3α,5β-THP plasma concentrations between the two time intervals ($t_{27} = 0.574$, $p = 0.571$).

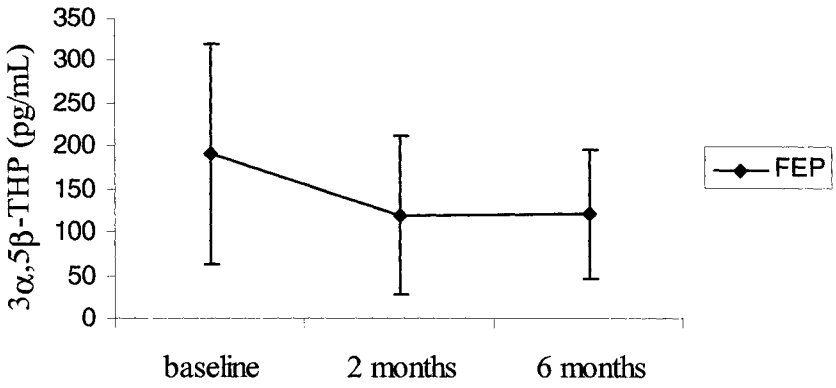


Figure 3-14. Mean (± SD) 3α,5β-THP concentrations at baseline, at two months and at six months in FEPs. MANOVA did not reveal significant changes in plasma 3α,5β-THP concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2,4} = 2.651$, $p = 0.185$).

3.6 REFERENCES

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Chapter 4

Longitudinal Investigation of the KYN Pathway in First Episode Psychosis

4.1 INTRODUCTION

The KYN arm of Trp metabolism produces the neuroactive compound KYNA which modulates glutamatergic neurotransmission by inhibiting NMDA receptors at the glycine co-agonist site (Birch et al 1988), and inhibiting α_7 nicotinic acetylcholine receptor activity and reducing Glu release. Since glutamatergic dysfunction is implicated in schizophrenia, it is likely that KYNA, which modulates Glu neurotransmission, also plays a role in the pathophysiology of the illness. Furthermore, like the NMDA receptor antagonists PCP and ketamine (French 1994), KYNA increases midbrain DA neuronal activity (Erhardt and Engberg 2002; Schwieler and Erhardt 2003). Thus, KYNA may be implicated in both the hypoglutamatergic and hyperdopaminergic states hypothesized in schizophrenia.

Trp is the precursor for the KYN pathway, and although normal plasma Trp levels have been reported in SZs (van der Heijden et al 2005), lower than normal Trp plasma levels have also been found in drug-naïve, in unmedicated and in medicated SZs (Rao et al 1990; Tortorella et al 2001). As indicated by a study conducted by van der Heijden et al, Trp plasma levels may also be affected by antipsychotic administration. In this study 3-14 weeks of atypical antipsychotic treatment decreased plasma Trp levels in those patients who either did not respond to, or had a poor response to antipsychotic treatment, while Trp levels in those patients who responded well to treatment did not change (van der Heijden et al 2005). Since 99% of Trp is metabolized along the KYN pathway (Russo et al 2003), abnormal Trp levels found in SZs may translate to abnormal KYN metabolism. Indeed, although very few studies have investigated KYN pathway intermediates and enzymes in schizophrenia, findings from these studies suggest that

KYN pathway dysfunction may be implicated in the illness. Postmortem studies revealed that brain KYN and KYNA levels are significantly higher in SZs, compared to control subjects (Miller et al 2006; Schwarcz et al 2007; Schwarcz et al 2001). Also, SZs were found to have increased activity and/or levels of enzymes that catalyze the formation of KYN and KYNA and decreased activity of enzymes that catalyze KYN degradation (Miller et al 2006; Schwarcz et al 2007). Since the brain tissue examined in these studies was collected from chronically ill medicated patients, it is possible that these changes in KYN pathway metabolism were induced by antemortem antipsychotic use. However, evidence from both clinical and preclinical studies suggest that KYN pathway dysfunction is part of the disease process rather than antipsychotic-induced. In a clinical sample, Erhardt et al found higher than normal CSF KYNA levels in SZs, most of whom were drug-naïve FEPs, indicating that KYN pathway dysfunction is present prior to commencing treatment with antipsychotic drugs (Erhardt et al 2001). Furthermore, in a preclinical study Ceresoli-Borroni et al found that chronic antipsychotic administration to rats caused significant reductions in brain KYNA levels (Ceresoli-Borroni et al 2006). Since this study suggests that antipsychotics decrease, rather than increase KYNA levels, it is unlikely that the increased KYNA levels found in postmortem schizophrenia studies were caused by antemortem antipsychotic use.

It is possible that the reduction of KYNA levels and the resulting increase in glutamatergic neurotransmission via NMDA receptor activation, and increased α_7 receptor activation with subsequent increase in Glu release may be part of the therapeutic mechanism of action of antipsychotic drugs. However, this hypothesis has not been tested in humans. Furthermore, postmortem studies, which examine chronically ill, medicated

patients, negate this hypothesis, as similar abnormalities in KYN pathway metabolism are found in postmortem investigations as those seen in FEPs.

To date, only one study has examined KYN pathway metabolite levels in a live patient sample, but the effects of antipsychotics on these levels have only been examined in preclinical studies. Furthermore, it is unknown if KYN pathway metabolite levels correlate with symptom severity. In order to address these issues, this longitudinal investigation measures KYN, its precursor Trp, and its neruoactive metabolite KYNA in unmedicated FEPs, examines if treatment with atypical antipsychotics alters these KYN pathway metabolites, compares these levels to those found in individuals with no psychopathology, and determines if levels of these metabolites correlate with symptom severity.

4.2 METHODS

4.2.1 Study Participants

Ethical approval for this study was obtained from the University of Alberta Health Research Ethics Board, and written informed consent was obtained from all participants after the nature of all procedures had been fully explained. Twenty-one male FEPs, who were recruited from EEPIC and met the current EEPIC inclusion/exclusion criteria (Table 4-1), participated in the study. Thirty age-matched, male HCs with no psychopathology [as determined by the SCID-I (First et al 1994)] also participated in the study, and were recruited from the community via poster advertisement. Exclusion criteria for HCs included major neurological/medical illness, history of substance/alcohol abuse, history

of significant head injury (loss of consciousness > 29 minutes), and a positive family history of Axis-I disorders in first or second degree relatives.

4.2.2 Clinical Assessment

Demographic and diagnosis data are shown in Table 4-2, clinical rating scores are shown in Table 4-3, and medications taken by study participants are shown in Table 4-4. Of the 21 EEPIC patients included in the study, nine were antipsychotic-naïve, and the remaining 12 patients had a mean lifetime exposure to antipsychotics prior to entering EEPIC of 10.25 days. If a patient was taking any psychotropic medication it was discontinued for a minimum of two to seven days, as determined by the half-life of elimination of the specific drug. All FEPs completed a clinical assessment in an unmedicated state (baseline), as required by the EEPIC protocol. During this assessment the SCID-I was administered to all FEPs to determine diagnosis, the PANSS, subdivided into positive, negative and general symptom clusters (Kay et al 1987) was used to quantify psychopathology, the STAI (Spielberger et al 1970) and the BAI (Beck and Steer 1990) were used to assess symptoms of anxiety, the MIS (Eckblad and Chapman 1983) was used to assess magical thinking, and the PGWB (McDowell and Newell 1996) was used to assess subjective well-being. As per EEPIC protocol, following baseline assessment, all FEPs began taking one of three atypical antipsychotics (olanzapine, risperidone, or quetiapine), and were followed longitudinally. The treating EEPIC psychiatrists prescribe concomitant medication as necessary for standard patient care. Symptom rating scales described above were administered to FEPs two and six months post baseline assessment in order to track symptom changes throughout the course of

treatment. All symptom rating scales, with the exception of the PANSS, were administered to HCs at baseline and two months after initial assessment. The HCs were unmedicated throughout the study.

4.2.3 Plasma Collection

All study participants followed an overnight fast prior to blood collection, done between 8 and 10 am using vacutainers containing the anticoagulant ethylenediamine tetraacetic acid. Blood was collected from FEPs using the same regimen as that used for PANSS administration [i.e. at baseline (unmedicated state), and following two and six months of treatment with antipsychotic medication]. Blood was also collected twice from unmedicated HCs: at baseline and two months after initial collection. Following blood collection the plasma was immediately separated by centrifugation, transferred into plastic tubes and stored at the Neurochemical Research Unit, University of Alberta, in a -80°C freezer until analysis.

4.2.4 Trp, KYN and KYNA Analysis with HPLC

Plasma samples (500 μL) were prepared by precipitating the protein with 1 mL methanol. After sitting on ice for 20 min, the samples were centrifuged for 5 min at 4°C and 12000 g, and the resultant supernatants were analyzed using a procedure developed at the Neurochemical Research Unit (MacKenzie et al, unpublished). The method consists of SPE using Oasis® HLB cartridges followed by separation and analysis using Waters Alliance HPLC system with UV detection. Identification and quantification of Trp, KYN

and KYNA in the samples were achieved by comparison of retention times and response of authentic standards in a calibration curves (Figure 4-1).

4.2.5 Statistical Analysis

SPSS 12.0 software was used for all statistical analysis. All results are expressed as mean ± SD. For all statistical analyses, $\alpha = 0.05$ was set as the nominal level of significance.

MANOVA, with time (baseline and two months) as a within-subject factors and group (FEP and HC) as a between-subject factor, was used to analyze changes in plasma Trp, KYN and KYNA concentrations.

Independent samples t-tests were also done to determine if there were any significant between-group differences in plasma concentrations at baseline and at two months, separately, and paired samples t-tests were done to determine if there were significant differences in plasma concentrations between baseline and two month within each group separately. MANOVA, with time (baseline, two months, six months) as a within-subject factor, was also used to determine any significant changes in plasma concentrations between baseline, two months and six months in FEPs.

In order to determine if a linear relationship exists between plasma concentrations of KYN metabolites and clinical rating scores, correlation analyses between each KYN metabolite concentration and each clinical rating score were done for baseline and two month measures, separately, in each group separately. The clinical rating scales included in correlation analyses in FEPs were PANSS-P, PANSS-N, PANSS-G, MIS, STAI and

BAI. As the PANSS was not administered to HCs, only MIS, STAI and BAI were included in correlation analyses in HCs.

4.3 RESULTS

4.3.1 Plasma Trp Concentration Changes

MANOVA did not show a significant time effect (Wilks' multivariate tests of significance: $F_{1, 36} = 0.004$, $p = 0.950$) or a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 36} = 2.081$, $p = 0.245$) on plasma Trp concentration (Figure 4-2).

Independent samples t-tests did not reveal significant between group differences in plasma Trp concentrations at baseline ($t_{48} = 1.777$, $p = 0.082$), or at two months ($t_{36} = 0.498$, $p = 0.622$) (Figure 4-2).

Paired samples t-tests did not show a significant difference in plasma Trp concentrations between baseline and two months within each group separately (FEPs: $t_8 = 0.683$, $p = 0.514$; HCs: $t_{28} = 1.163$, $p = 0.255$) (Figure 4-2).

MANOVA, with time (baseline, two months, six months) as a within-subject factor, did not reveal significant changes in plasma Trp concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2, 3} = 0.321$, $p = 0.747$) (Figure 4-3).

4.3.2 Plasma KYN Concentration Changes

MANOVA did not show a significant time effect (Wilks' multivariate tests of significance: $F_{1, 36} = 2.074$, $p = 0.158$) or a significant time x group effect (Wilks'

multivariate tests of significance: $F_{1, 36} = 0.089$, $p = 0.768$) on plasma KYN concentration (Figure 4-4).

Independent samples t-tests did not reveal significant between group differences in plasma KYN concentrations at baseline ($t_{48} = 0.469$, $p = 0.641$), or at two months ($t_{36} = 1.295$, $p = 0.204$) (Figure 4-4).

Paired samples t-tests did not show a significant difference in plasma KYN concentrations between baseline and two months within each group separately (FEPs: $t_8 = 1.590$, $p = 0.150$; HCs: $t_{28} = 1.084$, $p = 0.288$) (Figure 4-4).

MANOVA, with time (baseline, two months, six months) as a within-subject factor, did not reveal significant changes in plasma KYN concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2, 4} = 0.567$, $p = 0.607$) (Figure 4-5).

4.3.3 Plasma KYNA Concentration Changes

MANOVA did not show a significant time effect (Wilks' multivariate tests of significance: $F_{1, 35} = 0.194$, $p = 0.662$) or a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 35} = 0.391$, $p = 0.536$) on plasma KYNA concentration (Figure 4-6).

Independent samples t-tests did not reveal a significant between group difference in plasma KYNA concentrations at baseline ($t_{46} = 0.648$, $p = 0.520$), but did reveal a statistically significant difference at two months ($t_{36} = 2.073$, $p = 0.045$) (Figure 4-6).

Paired samples t-tests did not show a significant difference in KYNA plasma concentrations between baseline and two months within each group separately (FEPs: t_8 = 0.117, p = 0.909; HCs: t_{27} = 1.053, p = 0.302) (Figure 4-6).

MANOVA, with time (baseline, two months, six months) as a within-subject factor, did not reveal significant changes in plasma KYNA concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2,4}$ = 2.010, p = 0.249) (Figure 4-7).

4.3.4 Relationship Between Plasma KYN Metabolite Concentrations and Clinical Rating Scores

Table 4-5 shows results from correlation analyses investigating the linear relationship between each KYN metabolite plasma concentration and PANSS scores in FEPs at baseline and at two months. There were no statistically significant linear relationships between plasma KYN metabolite concentrations and PANSS scores.

Results from correlation analyses investigating the linear relationship between each KYN metabolite plasma concentration and STAI, BAI and MIS scores in FEPs and HCs at baseline and at two months are presented in Table 4-6. There were no statistically significant linear relationships between plasma KYN metabolite concentrations and STAI, BAI and MIS scores.

4.4 DISCUSSION

This study was, to our knowledge, the first longitudinal investigation of the KYN pathway of Trp metabolism, and first investigation of the relationship between KYN

pathway metabolite levels and symptom severity in FEPs. In this study we examined plasma concentrations of KYN pathway metabolites in FEPs in an unmedicated state, examined how these concentrations changed following treatment with atypical antipsychotics, compared these concentrations to those found in HCs, and established if a relationship exists between KYN pathway metabolite plasma concentrations and symptom severity.

We found that while plasma KYNA concentrations were not significantly different between groups at baseline (i.e. unmedicated state), after two months of treatment with atypical antipsychotics FEPs had significantly lower plasma KYNA concentrations than unmedicated HCs. We did not find any other significant between or within-group differences in plasma KYN metabolite concentrations. Also, we did not find any significant correlations between plasma KYN metabolite levels and symptom severity.

Our finding of normal KYNA plasma concentrations in FEPs at baseline is inconsistent with those of Erhardt et al, who found higher than normal KYNA CSF levels in unmedicated SZs (Erhardt et al 2001). It is possible that the higher than normal CSF KYNA levels found in schizophrenic patients was caused by the age gap between chronic patients and FEPs, as 25 of the patients examined were drug-naïve FEPs, while three were chronically ill older patients (Erhardt et al 2001). Indeed, KYNA levels in CSF, but not in plasma, were found to be directly correlated with age in one study (Kepplinger et al 2005), and Erhardt et al found a positive correlation between KYNA levels and age in SZs, but not in HCs, which skewed the distribution of CSF KYNA levels in the patient sample (Erhardt et al 2001). In order to resolve this issue and determine if CSF KYNA

levels differ between FEPs and HCs, the authors need to exclude the three chronic patients from statistical analysis.

The discrepant results may also be attributed to the different body fluids examined in the two studies (plasma vs. CSF). The relationship between CSF and plasma KYNA levels is not entirely clear. While the increase in KYNA levels in both CSF and plasma following KYN administration to nonhuman primates was found to be proportional to the KYN dose administered, plasma levels rose more quickly than CSF levels (i.e. plasma levels plateaued after 10 min, while CSF levels plateaued four hours after KYN administration) (Jauch et al 1993). Furthermore, CSF, but not plasma, KYNA levels have been found to increase with age (Kepplinger et al 2005). The relationships between CSF and brain and between plasma and brain KYNA levels are also unclear. KYNA is a polar molecule and has limited ability to penetrate the BBB, but KYN, the precursor for KYNA, crosses the BBB via large neutral amino acid carriers (Fukui et al 1991; Klivenyi et al 2004). The CNS KYN pathway is dependent on KYN originating in the periphery (Jauch et al 1993), and it is estimated that 60% of brain KYN is derived from peripheral sources (Klivenyi et al 2004). After entering the brain, KYN is converted into KYNA in astrocytes and into other KYN metabolites in microglia (Schwarcz and Pellicciari 2002). Because KYNA brain levels are dependent on peripheral KYN, which can cross the BBB, peripheral KYNA levels may predict levels found in the brain. Indeed, plasma KYNA levels found in our study (nM range) are similar to those reported in the human brain (Moroni et al 1988; Turski et al 1988). However, until an *in vivo* technique that allows for quantification of brain NASs and KYN metabolites is available, we cannot

determine with certainty whether plasma levels of these compounds reflect those found in the brain.

In our study we found that after two months of treatment with atypical antipsychotics FEPs had significantly lower KYNA levels than unmedicated HCs. These results are consistent with results from a study conducted by Ceresoli-Borroni et al, who found that chronic antipsychotic administration to rats caused significant reductions in brain KYNA levels (Ceresoli-Borroni et al 2006). While it is tempting to conclude that atypical antipsychotics partly exert their therapeutic effects by decreasing KYNA levels, we do not know if this occurs in the human brain. Furthermore, in our study, the difference between baseline and two month KYNA levels in FEPs was not statistically significant, and the statistically significant difference in KYNA levels found at two months between FEPs and HCs may be attributed to the slight, but not statistically significant increases and decreases of plasma KYNA levels from baseline to two months in HCs and FEPs, respectively. Thus, the effects of antipsychotics on KYNA levels require further investigation.

A major drawback to our study is that the FEP group examined is heterogenous in terms of diagnoses and type of antipsychotic use. Only 6/21 of FEPs included in our study had a DSM-IV diagnosis of schizophrenia. Due to the duration criteria when diagnosing schizophrenia, a person has to be assessed six months after they experience their first psychotic episode before a diagnosis of schizophrenia can be established, and only five patients completed their six month assessment (three with schizophrenia and two with substance-induced psychosis). Furthermore, as there are currently no laboratory diagnostic tests for schizophrenia, the diagnosis is subjective and dependent on the

treating psychiatrist. Thus, the diagnosis may change throughout a patient's involvement in EEPIC and throughout their lifetime. This issue is not restricted to our study and is a concern in all studies that investigate schizophrenia pathology, as well as the pathology other psychiatric disorders. The only way to resolve this issue is to improve diagnostic procedures. The FEPs included in our study also took different atypical antipsychotics, which may have affected KYN metabolite levels differently. Unfortunately, the two month FEP sample size in our study was too small to statistically examine the differential effects of different antipsychotics on KYN metabolite levels, and some of our patients took more than one type of antipsychotic, thus further complicating the relationship between antipsychotic drugs and KYN metabolite level changes. In the future, as our patient sample size increases, we will examine how different antipsychotics affect KYN metabolite levels.

This study was the first longitudinal investigation of KYN metabolites in first episode psychosis. We examined if KYN metabolite plasma level alterations are implicated in the pathophysiology of psychosis, if they are secondary to the effects of atypical antipsychotics, and if they correlate with symptom severity. Our results indicate that a decrease in plasma KYNA levels may be implicated in the mechanism of action of atypical antipsychotic drugs; however, this needs to be examined further. In the future we want to establish if antipsychotics alter KYN metabolite levels, which antipsychotics affect these levels, and if antipsychotic-induced KYN metabolite level alterations are beneficial (i.e. part of the therapeutic mechanism of action of these drugs) or if they are simply a side effect of the drug. In order to conclusively determine if the KYN pathway is implicated in schizophrenia pathophysiology, we need to develop a technique that

allows for the investigation of brain KYN metabolism *in vivo*. KYN metabolite investigation presents an exciting new avenue of research that may further our understanding of the neurochemical abnormalities that contribute to the complex pathophysiology of schizophrenia.

4.5 TABLES AND FIGURES

Table 4-1. EEPIC inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
Age 16-35 years	Subjects requiring mood stabilizers at study entry
Diagnosis of schizophrenia or schizophreniform disorder	Head injury with > 29 minutes loss of consciousness
Less than 1 year of active-phase symptoms	Active substance abuse or dependence disorder (i.e. within past 30 days)
Less than 3 months of lifetime prior exposure to antipsychotic medication	Exposure to long acting depot neuroleptic medication
Current psychotic symptoms requiring long term antipsychotic treatment	Known sensitivity to olanzapine, risperidone, or quetiapine
	Serious past or current CNS/medical illness

Table 4-2. Diagnostic and demographic data for FEPs and HCs.

		FEP n=21	HC n=30
Age	Mean ± SD (years)	22.97±3.28	21.18±3.36
DSM-IV diagnosis	Schizophrenia	n=6	n=0
	Schizophreniform disorder	n=3	n=0
	Substance-induced psychotic disorder	n=5	n=0
	Brief psychotic disorder	n=1	n=0
	Psychotic disorder not otherwise specified	n=5	n=0
	Psychotic disorder due to a general medical condition	n=1	n=0
First degree family history	No history	n=14	n=30
	Schizophrenia	n=4	n=0
	Bipolar disorder	n=2	n=0
	Major depressive disorder	n=1	n=0
Handedness	Right handed	n=17	n=25
	Left handed	n=4	n=5
Marital status	Married or common law	n=0	n=6
	Single	n=21	n=24
Number of children	No children	n=21	n=29
	Two children	n=0	n=1
Occupation	Administrative/minor professional	n=0	n=4
	Clerical/sales/technician/farmer	n=0	n=1
	Skilled manual employee	n=2	n=0
	Unskilled employee	n=6	n=0
	Student	n=5	n=25
	Unemployed	n=8	n=1
Highest level of education achieved	Graduated professional/graduate school	n=0	n=1
	Part graduate/professional school	n=0	n=2
	Graduated four year college school	n=1	n=7
	Graduated two year college/ technical school	n=1	n=0
	Part college	n=7	n=19
	Graduated high school	n=5	n=1
	Attended grade 7-12 without graduating	n=7	n=0

Table 4-3. Clinical rating scores for FEPs and HCs.

Clinical rating scales	FEP *baseline* n=21	*2 months* n=11	*6 months* n=5	HC *baseline* n=30	*2 months* n=29
PANSS-P	19.76±5.88	13.40±5.06	10.60±1.52	N/A	N/A
PANSS-N	20.38±6.00	17.90±7.46	16.40±4.67	N/A	N/A
PANSS-G	40.62±10.60	34.70±10.89	31.20±4.21	N/A	N/A
PANSS-T	80.76±20.23	66.00±22.36	58.20±9.01	N/A	N/A
MIS	11.05±5.99	9.70±6.83	8.00±7.46	2.53±2.16	2.07±2.09
STAI	44.70±11.07	42.10±6.90	40.40±10.43	26.80±5.68	25.55±5.08
BAI	15.80±10.54	6.20±5.73	3.83±2.99	2.93±2.94	4.17±5.70
PGWB	57.33±18.45	68.30±7.10	66.00±8.88	90.93±9.55	90.28±10.11

All results are expressed as mean ± SD. PANSS-P, PANSS-N, PANSS-G, and PANSS-T refer to the positive, negative, general, and total PANSS scores, respectively.

Table 4-4. Medications taken by FEPs and HCs.

Time	Anti-psychotics	Group	n	Other psychotropic medication	Group	n	Other medication	Group	n
Baseline							minocycline	FEP	1
							fluticasone	HC	1
							salbutamol	HC	2
							celecoxib	HC	1
							budesonide	HC	1
							formoterol	HC	1
							isotretinoid	HC	1
2	olanzapine	FEP	4	mirtazapine	FEP	1	minocycline	FEP	1
months	quetiapine	FEP	1	divalproax	FEP	1	fluticasone	HC	1
	risperidone	FEP	3	lorazepam	FEP	1	salbutamol	HC	2
	olanzapine+ quetiapine	FEP	1	venlafaxine	FEP	1	celecoxib	HC	1
	risperidone+ quetiapine	FEP	1				budesonide	HC	1
							formoterol	HC	1
							isotretinoid	HC	1
6	olanzapine	FEP	1	miratazapine	FEP	1	minocycline	FEP	1
months	quetiapine	FEP	1	venlafaxine	FEP	1			
	risperidone	FEP	3						

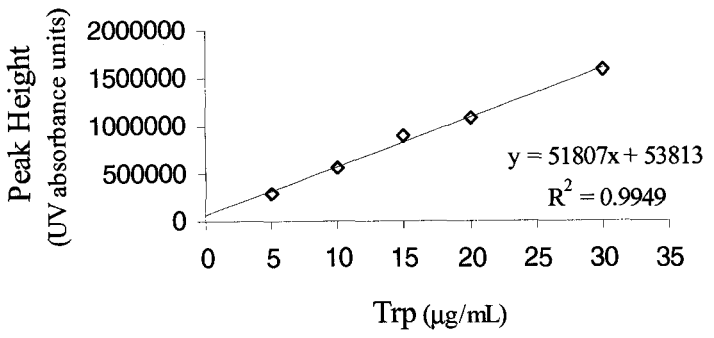
All FEPs were unmedicated (not taking psychotropic medication) at baseline, and all HCs were unmedicated (not taking psychotropic medication) throughout the duration of the study. The daily doses for antipsychotic medication ranged between 5-20 mg, 150-500 mg, and 1.5-4 mg for olanzapine, quetiapine, and risperidone, respectively. The daily doses for other psychotropic medications were 15 mg, 750 mg, 1 mg, and 150 mg for mirtazapine, divalproax, lorazepam, and venlafaxine, respectively.

Table 4-5. Relationship between plasma KYN metabolite concentrations and PANSS scores, subdivided into PANSS-P, PANSS-N and PANSS-G in FEPs at baseline and at two months. R and p values are displayed.

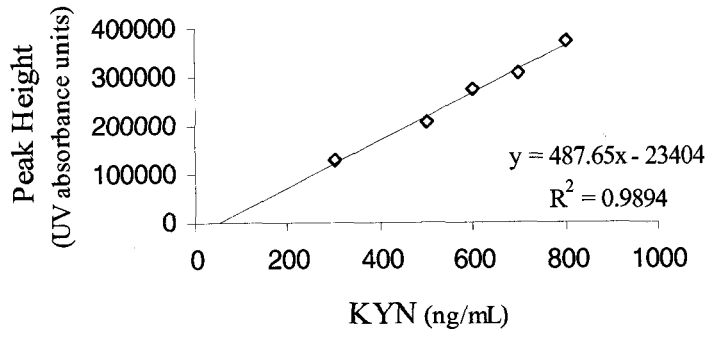
	KYN	**PANSS-P**		**PANSS-N**		**PANSS-G**	
Time	**metabolite**	R	p	R	p	R	p
Baseline	Trp	0.019	0.937	0.157	0.508	0.207	0.382
	KYN	0.088	0.713	0.065	0.786	0.311	0.182
	KYNA	0.141	0.565	0.055	0.824	0.250	0.303
2 months	Trp	0.059	0.881	0.603	0.085	0.342	0.367
	KYN	0.243	0.529	0.009	0.982	0.007	0.985
	KYNA	0.517	0.154	0.095	0.807	0.224	0.562

Table 4-6. Relationship between plasma KYN metabolite concentrations and STAI, BAI and MIS scores in FEPs and HCs at baseline and at two months. R and p values are displayed.

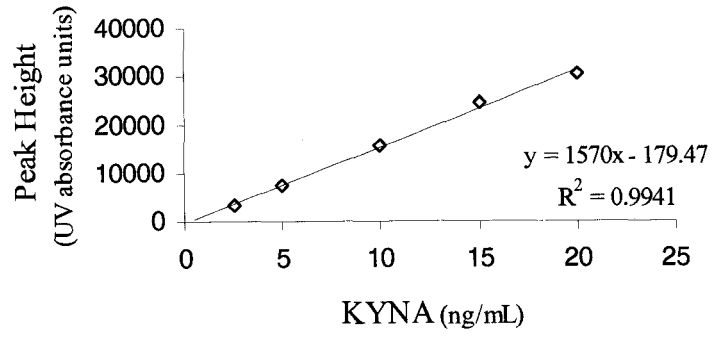
Time	Group	KYN metabolite	STAI R	STAI p	BAI R	BAI p	MIS R	MIS p
Baseline	FEP	Trp	0.135	0.582	0.029	0.906	0.219	0.353
		KYN	0.058	0.813	0.298	0.215	0.210	0.374
		KYNA	0.172	0.495	0.174	0.489	0.097	0.694
	HC	Trp	0.287	0.123	0.166	0.381	0.311	0.094
		KYN	0.181	0.338	0.154	0.415	0.068	0.723
		KYNA	0.222	0.247	0.070	0.718	0.006	0.975
2 months	FEP	Trp	0.212	0.584	0.284	0.460	0.269	0.484
		KYN	0.069	0.860	0.080	0.838	0.036	0.972
		KYNA	0.174	0.654	0.536	0.137	0.290	0.449
	HC	Trp	0.172	0.373	0.115	0.552	0.078	0.688
		KYN	0.008	0.969	0.209	0.277	0.263	0.167
		KYNA	0.105	0.587	0.087	0.654	0.014	0.941



A



B



C

Figure 4-1. Calibration curves used to quantify plasma Trp (A), KYN (B) and KYNA (C).

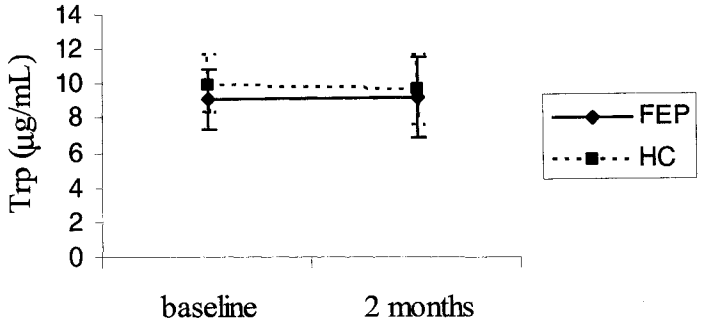


Figure 4-2. Mean (± SD) Trp concentrations at baseline and at two months in FEPs (unmedicated and medicated, respectively) and HCs (unmedicated at both time intervals). MANOVA did not show a significant time effect (Wilks' multivariate tests of significance: $F_{1, 36} = 0.004$, $p = 0.950$) or a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 36} = 2.081$, $p = 0.245$) on plasma Trp concentration. Plasma Trp concentrations were not significantly different between groups at baseline ($t_{48} = 1.777$, $p = 0.082$), or at two months ($t_{36} = 0.498$, $p = 0.622$). There were no significant differences between baseline and two month plasma Trp concentrations within each group separately (FEPs: $t_8 = 0.683$, $p = 0.514$; HCs: $t_{28} = 1.163$, $p = 0.255$).

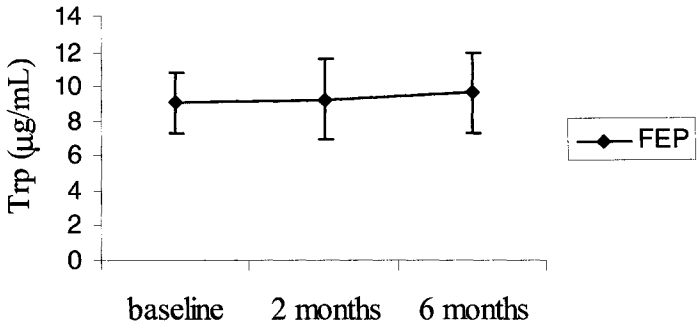


Figure 4-3. Mean (± SD) Trp concentrations at baseline, at two months and at six months. MANOVA did not reveal significant changes in plasma Trp concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2,3} = 0.321$, $p = 0.747$).

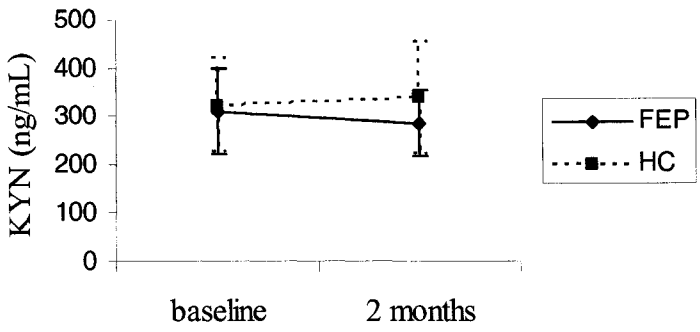


Figure 4-4. Mean (± SD) KYN concentrations at baseline and at two months in FEPs (unmedicated and medicated, respectively) and HCs (unmedicated at both time intervals). MANOVA did not show a significant time effect (Wilks' multivariate tests of significance: $F_{1, 36} = 2.074$, $p = 0.158$) or a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 36} = 0.089$, $p = 0.768$) on plasma KYN concentration. Plasma KYN concentrations were not significantly different between groups at baseline ($t_{48} = 0.469$, $p = 0.641$), or at two months ($t_{36} = 1.295$, $p = 0.204$). There were no significant differences between baseline and two month plasma KYN concentrations within each group separately (FEPs: $t_8 = 1.590$, $p = 0.150$; HCs: $t_{28} = 1.084$, $p = 0.288$).

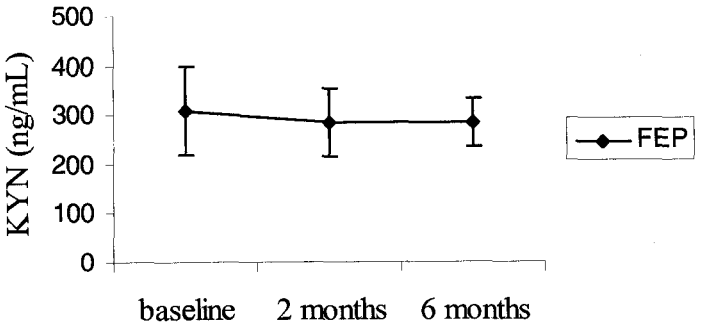


Figure 4-5. Mean (± SD) KYN concentrations at baseline, at two months and at six months. MANOVA did not reveal significant changes in plasma KYN concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2,4} = 0.567$, $p = 0.607$).

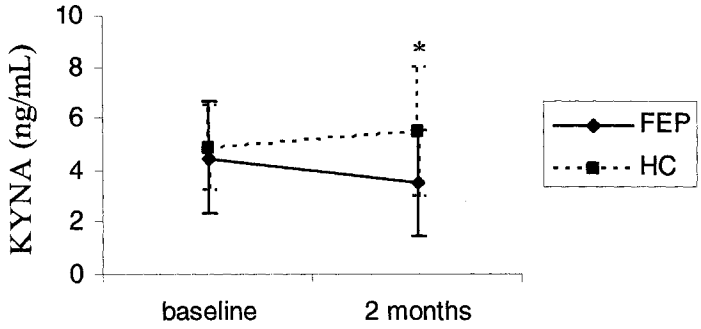


Figure 4-6. Mean (± SD) KYNA concentrations at baseline and at two months in FEPs (unmedicated and medicated, respectively) and HCs (unmedicated at both time intervals). MANOVA did not show a significant time effect (Wilks' multivariate tests of significance: $F_{1, 35} = 0.194$, $p = 0.662$) or a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 35} = 0.391$, $p = 0.536$) on plasma KYNA concentration. Plasma KYNA concentrations were not significantly different between groups at baseline ($t_{46} = 0.648$, $p = 0.520$), but at two months FEPs had significantly lower plasma KYNA concentrations than HCs ($t_{36} = 2.073$, $*p = 0.045$). There were no significant differences between baseline and two month plasma KYNA concentrations within each group separately (FEPs: $t_8 = 0.117$, $p = 0.909$; HCs: $t_{27} = 1.053$, $p = 0.302$).

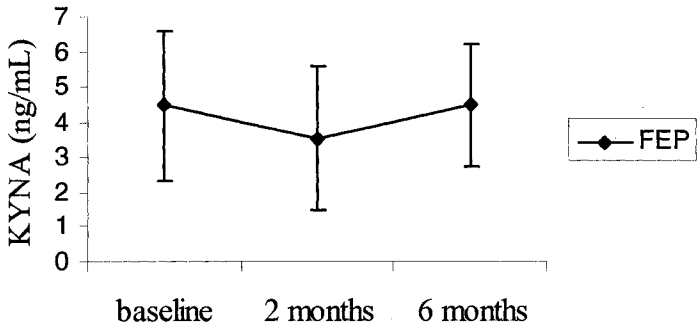


Figure 4-7. Mean (± SD) KYNA concentrations at baseline, at two months and at six months in FEPs. MANOVA did not reveal significant changes in plasma KYNA concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2,4} = 2.010$, $p = 0.249$).

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Chapter 5

The Relationship Between Peripheral and Central Glu and Gln in Healthy Male Volunteers

A version of this chapter has been published (Shulman Y et al (2006), *JPN* 31:406-10)

5.1 INTRODUCTION

Glu dysfunction is an emerging hypothesis of schizophrenia, with NMDA Glu receptor hypofunction potentially being a major contributor to the pathophysiology of the illness (Krystal et al 1994). Studies of *in vivo* brain Glu and Gln levels with high field strength ^{1}H MRS support the glutamatergic dysfunction hypothesis, with reports of increased Gln in the left dorsolateral PFC (Stanley et al 1996), left anterior cingulate cortex and thalamus (Theberge et al 2002; Williamson et al 2003) in FEPs, compared to HCs. Glu and Gln decreased in the thalamus of FEPs following 30 months, but not nine months of treatment, a finding that is consistent with low Glu and Gln found in the left anterior cingulate cortex in chronically ill medicated patients (Theberge et al 2003; Williamson et al 2003). However, findings of high Gln in the thalamus of medicated (Theberge et al 2003; Williamson et al 2003), and in the left medial PFC of never-treated (Bartha et al 1997; Williamson et al 1999) chronically ill patients have also been reported. At lower field strength (< 1.5 Tesla) Glu and Gln are difficult to separate due to the overlap of resonances (generally not an issue at higher field strengths of ≥ 3 Tesla). As a result, most reliable lower field strength MRS studies report Glx. A reduction in Glx in the PFC of chronic patients, compared to drug-naïve FEPs (Ohrmann et al 2006; Ohrmann et al 2005) and HCs (Choe et al 1994; Ohrmann et al 2006; Ohrmann et al 2005), as well as findings of lateralized abnormalities, with left sided deficits of Glx in the hippocampus of SZs, compared to HCs have been reported (Kegeles et al 2000). Other studies, however, showed no differences in Glx in the medial temporal lobe (Bartha et al 1999), dorsolateral PFC (Stanley et al 1995), the left frontal lobe (Block et al 2000), and anterior cingulate cortex (Wood et al 2007) in schizophrenia. In a study linking

NMDA receptor hypofunction and abnormalilies in the Glu-Gln cycle, an acute increase in Gln in the anterior cingulate cortex was found following ketamine administration in HCs (Rowland et al 2005).

In the few schizophrenia studies examining peripherally circulating (serum/plasma) Glu and Gln levels, abnormalities such as high Glu levels (Tortorella et al 2001; van der Heijden et al 2005) and low Gln levels (Rao et al 1990) in unmedicated chronic patients, and low Glu levels in drug-naïve FEPs (Palomino et al 2007) have been reported, while other studies found no differences in Glu or Gln in acutely ill patients, compared to HCs (Alfredsson and Wiesel 1989). Some studies also suggest that atypical (Evins et al 1997; Goff et al 2002; Maeshima et al 2007; Palomino et al 2007; Tortorella et al 2001), and possibly typical (Palomino et al 2007) antipsychotics exert some of their therapeutic effects by altering peripheral Glu levels. What is unknown is if a relationship exists between the peripheral Glu/Gln and the central Glu/Gln measured by high field strength ^{1}H MRS. This relationship remains unknown despite the fact that Glu and Gln can cross the BBB via active transport at the abluminal, and facilitative transport at the luminal endothelial membranes (Hawkins et al 2002). To the best of our knowledge only four studies have investigated central and peripheral Glu and/or Gln levels simultaneously. Three of these analyzed serum and CSF levels and found significant positive correlations between serum and CSF Glu (Alfredsson et al 1988a; Alfredsson et al 1988b; McGale et al 1977) and Gln concentrations (Alfredsson et al 1988a; McGale et al 1977). *In vivo* ^{1}H MRS-derived brain measures and serum levels have only been investigated simultaneously in one study, with reports of a significant increase in serum Glu concentrations, but no change in anterior cingulate Glx following a switch from

conventional antipsychotics to olanzapine in SZs (Goff et al 2002). While the authors did not directly examine the correlation between peripheral Glu and central Glx, the discordance between peripheral and central changes suggests a lack of a relationship between the two compartments.

Investigations of neurochemical abnormalities in schizophrenia are crucial for understanding the pathophysiology of the illness and directing future pharmacotherapy development. Currently, high field strength ^{1}H MRS is one of the best methods for investigating *in vivo* Glu and Gln abnormalities. However, the availability of high field strength ^{1}H MRS is limited, and the methodologies between groups who have access can vary greatly. Given the relative ease and greater feasibility of using peripheral circulating measures clinically, it is important to determine if peripheral measures reflect those found centrally with MRS. To this end, the present study investigates the relationship between plasma and medial prefrontal cortical (mPFC) measures of Glu and Gln in HCs.

5.2 METHODS

5.2.1 Study Participants

Following approval of the study by the University of Alberta Health Research Ethics Board, healthy male volunteers (n = 17, mean age ± SD = 21.9 ± 2.9 years, age range: 18-29 years, 13/17 right handed) were recruited from the community, via poster advertisement. Written informed consent was obtained from all participants after the procedures had been fully explained. All subjects were administered the SCID-I (First et al 1994) to rule out psychopathology. Other exclusion criteria were major neurological/medical illness, history of substance/alcohol abuse, history of significant

head injury (loss of consciousness > 29 minutes), and a positive family history of Axis-I disorders in first or second degree relatives.

5.2.2 mPFC Glu and Glx Analysis with ^{1}H MRS

^{1}H MRS was performed using a 3-Tesla magnet (Magnex Scientific, Concord, CA) equipped with actively shielded gradient and spectrometer (Surrey Medical Imaging System, Surrey, U.K.), and with a quadrature birdcage resonator. A 2 x 3 x 3 cm voxel (for segmentation and spectroscopy) was positioned such that the 2 cm dimension was centered on, and parallel to the midline, using both transverse and coronal gradient echo image series (TE = 20 ms, TR = 500 ms, 5 mm slice thickness, 256 x 256 point resolution). The center sagittal slice was then used to first register the voxel such that the posterior edge touched the corpus callosum and inferior edge lay along the anterior commissure-posterior commissure (AC-PC) line. The voxel was then rotated until the corners of the anterior edge were equidistant from the brain surface, while maintaining one corner contacting the AC-PC line, and an edge contacting the corpus callosum (Figure 5-1).

An optimal *in vivo* Glu contrast to background was used, with (TE, TM) = (240, 27 ms), evaluated using numerical simulation (Thompson and Allen 2001). These timing conditions were applied *in vivo*, with a TR = 3 s. The long TE time reduced the signal contamination from macromolecules due to their short T_2 relaxation time. Each spectrum was the sum of 512 averages, acquired in 16 blocks of 32 averages. This allowed each of the 16 sub-spectra to be examined for spectral artifacts due to subject movement or hardware fluctuations prior to their final summing. It also allowed re-registering each of

the 16 sub-spectra to the same frequency reference prior to summing. Estimation of the peak areas for the MRS data used the LCModel (version 6.0-1) analysis program, which gave measures of Glu, Glx, N-acetylaspartate (NAA) and creatine plus phosphocreatine (Cr), with typical SD of the fit for both Glu and Glx < 15%, for NAA < 5% and for Cr < 10%. Gln measures were not reliable, with SD of the fit typically > 30%, and therefore not used for statistical analysis. A representative spectrum used for analysis is shown in Figure 5-2. All measures derived by MRS are expressed as a ratio to Cr.

Segmentation of the frontal brain region was performed using the method described previously (Hanstock and Allen 2000). In brief, a PRESS volume was co-registered to the same region of brain used for the STEAM acquisitions. Two inversion pulses preceded the PRESS pulse sequence. The delay time between the two inversion pulses and between the last inversion pulse and the PRESS sequence were optimized to selectively suppress CSF and either gray matter (GM) or white matter (WM). Ten GM or WM 1D-projections were acquired, TR = 9 s, TE = 120 ms, and in each case were the sum of two averages. An additional ten CSF-only 1D-projections were acquired with no inversion pulses and with TE of 500 ms. These conditions minimized the signal contamination from GM and WM while maintaining significant signal from CSF. Scaled 1D-projections were calculated, eliminating relaxation effects, which when integrated allowed estimation of the compartment volumes.

All computations necessary for calculating experimental timings prior to acquisition, and for the data analysis, were performed using the MATLAB program environment.

5.2.3 Plasma Collection

Blood was collected from all participants between 8 and 10 am following an overnight fast, within one week of ^{1}H MRS analysis. Plasma was immediately separated with centrifugation and stored at -80°C until analysis using HPLC with fluorescence detection.

5.2.4 Glu and Gln Analysis with HPLC

Plasma samples (100 µL) were prepared by precipitating the protein with 300 µL methanol. Following centrifugation, the supernatants were used to quantify Glu and Gln using a modification of a procedure by Hashimoto et al (Hashimoto et al 1992), as described by Grant et al (Grant et al 2006). Briefly, HPLC was performed on a Waters Alliance 2690XE instrument coupled to a Waters 474 programmable fluorescence detector (Waters Corporation, Milford MA). Amino acids were subjected to pre-column derivatization with o-phthaldialdehyde (OPA) and *N*-isobutyryl-L-cysteine (IBLC) (Figure 5-3). A 5 µL aliquot of standard or sample solution was mixed with 5 µL of reagent solution and held in the injection loop 5 min prior to injection. Separation was carried out on a Symmetry C_{18} column (4.6 x 150 mm, 3.5 µm) from Waters with a methanolic phosphate buffer mobile phase (pH 6.2). The fluorescent amino acid derivatives were monitored at an excitation wavelength of 260 nm and an emission wavelength of 455 nm. Identification and quantification of amino acids in the samples were achieved by comparison of retention times and response of authentic standards in a calibration curve (Figure 5-4).

5.2.5 Statistical Analysis

Correlation analyses between MRS and HPLC measurements of Glu and Gln (or Glx), with $\alpha = 0.05$ set as the nominal level of significance, were conducted using SPSS 12.0 for Windows.

5.3 RESULTS

5.3.1 Relationship Between mPFC Glu and Glx and Plasma Glu and Gln

Mean ± SD plasma Glu and Gln concentrations were 4.21 ± 2.49 μg/ml and 68.42 ± 11.30 μg/ml, respectively. Mean ± SD mPFC Glu/Cr and Glx/Cr were 2.27 ± 0.68 and 3.91 ± 1.74, respectively. There was no correlation between plasma Glu and either mPFC Glu or Glx ($R_{1,\ 15} = 0.019$, $p = 0.944$, and $R_{1,\ 15} = 0.081$, $p = 0.757$, respectively). Similarly, there was no correlation between plasma Gln and either mPFC Glu or Glx ($R_{1,\ 15} = 0.029$, $p = 0.911$ and $R_{1,\ 15} = 0.025$, $p = 0.925$, respectively).

5.3.2 Plasma Glu and Gln Stability and the Effects of Fasting on mPFC Glu and Glx

In our study plasma collection and MRS analysis were conducted on different days and under different conditions (fasting and non-fasting, respectively). We therefore determined whether fasting has an effect on mPFC Glu and Gln levels and if plasma Glu and Gln levels are stable over time. In order to examine if fasting affects mPFC Glu and Glx measures, six participants were rescanned following an overnight fast, and the results were compared to those from their non-fasting scans using paired samples t-tests. There were no significant differences in either Glu or Glx measures between fasting and non-

fasting conditions ($t_5 = 1.463$, $p = 0.203$ and $t_5 = 1.118$, $p = 0.314$, respectively). In order to determine if plasma Glu and Gln levels are stable over time, we collected and analyzed plasma samples from 16 participants for Glu and Gln levels two months after the initial analysis. Using paired samples t-tests, we found no significant differences in Glu or Gln levels between samples ($t_{15} = 0.865$, $p = 0.400$ and $t_{15} = 0.147$, $p = 0.885$, respectively).

5.4 DISCUSSION

We report, to our knowledge, the first study that directly examined the relationship between high field strength ^{1}H MRS-derived central Glu and Glx and circulating Glu and Gln. We found no relationship between mPFC and plasma levels in young healthy male volunteers. Our results are in agreement with a previous study reporting a discordant change in serum Glu and anterior cingulate Glx levels following pharmacological intervention (Goff et al 2002), although this study did not investigate correlations between the two compartments. In their study, Goff et al also found that following a switch from typical antipsychotics to olanzapine, brain Glx levels increased in those patients who showed improvement in negative symptoms (Goff et al 2002), providing preliminary evidence that MRS may be a clinically useful tool for assessing neurochemical abnormalities and medication effects in psychiatric disorders.

A potential limitation of our study is that phlebotomy and MRS were conducted on different days and under different conditions (fasting and non-fasting, respectively), which may account for the non-significant correlations between peripheral and central measures. In order to examine if fasting affects mPFC Glu and Glx measures, six participants were rescanned following an overnight fast, and the results were compared to

those from their non-fasting scans. There were no significant differences in either Glu or Glx measures between fasting and non-fasting conditions, indicating that fasting does not affect glutamatergic neurotransmission in the brain. Furthermore, Glaeser and colleagues showed that in rats fasted overnight, Glu serum levels were higher, while brain levels were the same in rats fed a protein containing meal, compared to unfed rats (Glaeser et al 1983), indicating that fasting affects peripheral circulating Glu levels without significantly altering brain levels. We also collected and analyzed plasma samples from 16 participants for Glu and Gln levels nine weeks after the initial analysis and found no significant differences in Glu or Gln levels between these samples and the ones collected originally, indicating that under fasting conditions Glu and Gln plasma levels remain stable over time.

In combination with our results, findings of significant correlations between serum and CSF Glu and Gln (Alfredsson et al 1988a; Alfredsson et al 1988b) suggest that CSF levels may not correlate with those found in GM and/or WM, thus indicating that segmentation is essential for achieving accurate results with MRS.

This study demonstrates that in HCs a simple blood test is not appropriate for investigating glutamatergic neurotransmission in the brain, and provides strong support for using a more direct approach, such as high field strength MRS, for such investigations. Our group is currently investigating both peripheral and central markers of glutamatergic neurotransmission in FEPs, and examining the effects of medication on these systems. This will help determine if peripheral and/or central measures are clinically useful for investigating glutamatergic dysfunction in schizophrenia, and for examining the neurochemical effects of medical intervention.

5.5 FIGURES

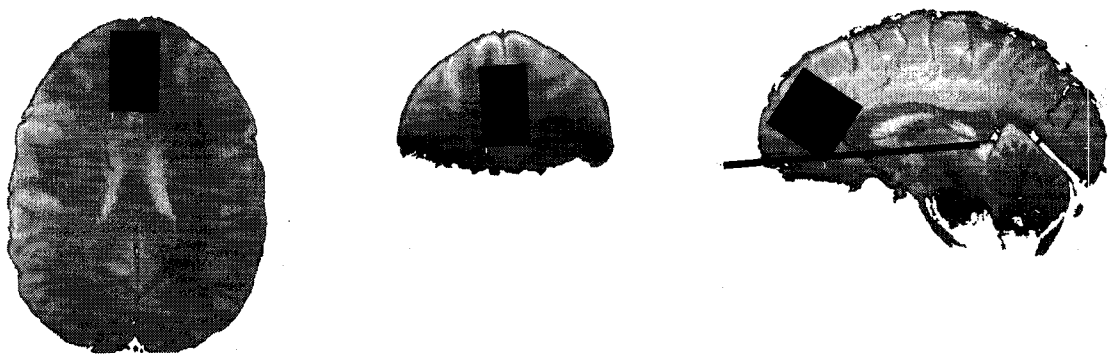


Figure 5-1. Voxel placement for ^{1}H MR spectroscopy and segmentation.

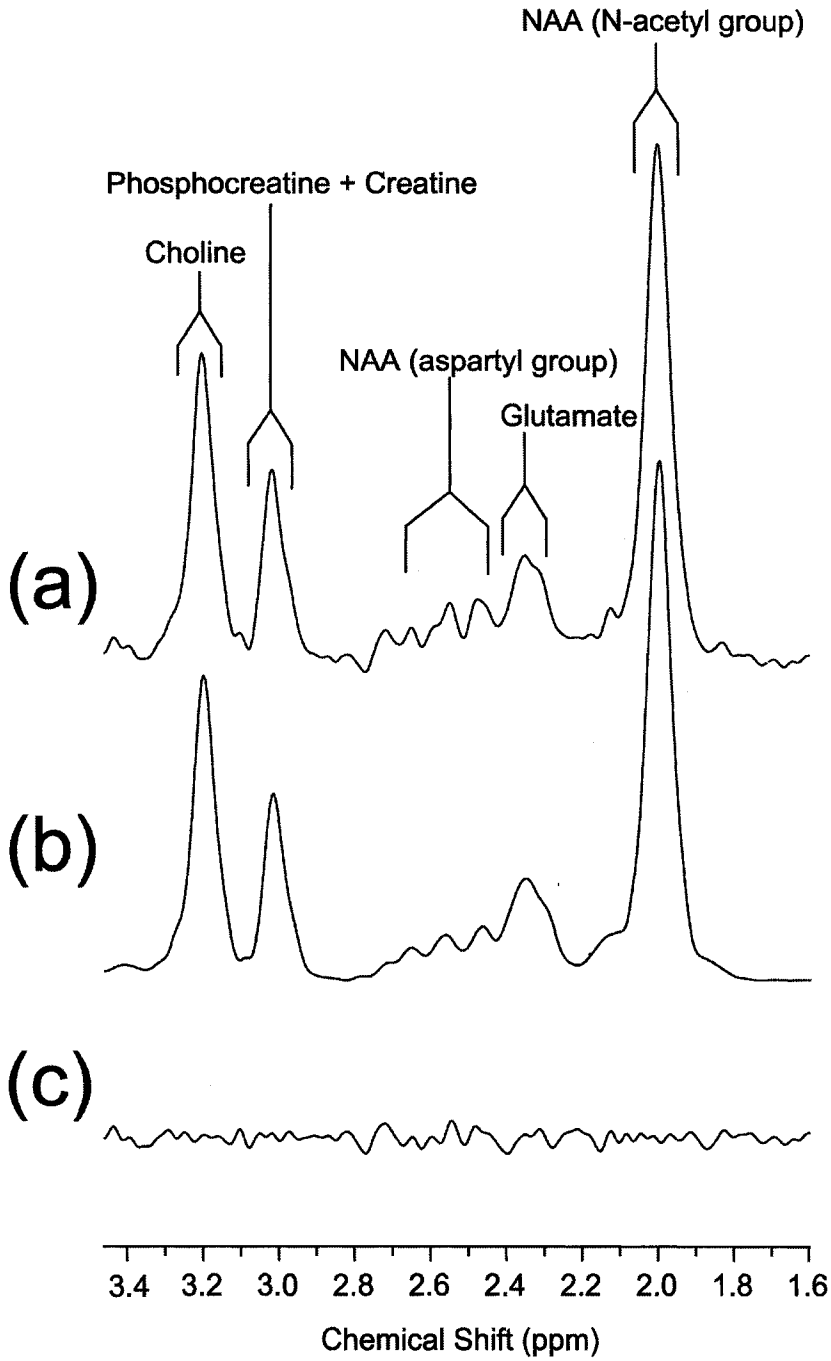


Figure 5-2. Typical ^{1}H MRS acquired from a 2 x 3 x 3 cm voxel located in the medial frontal cortex, with STEAM timings optimized for Glu (TE, TM = 240, 27 ms). (a) 2 Hz exponential filtered spectrum; (b) LCModel fit spectrum; (c) residual noise following subtraction of spectra in (a) and (b).

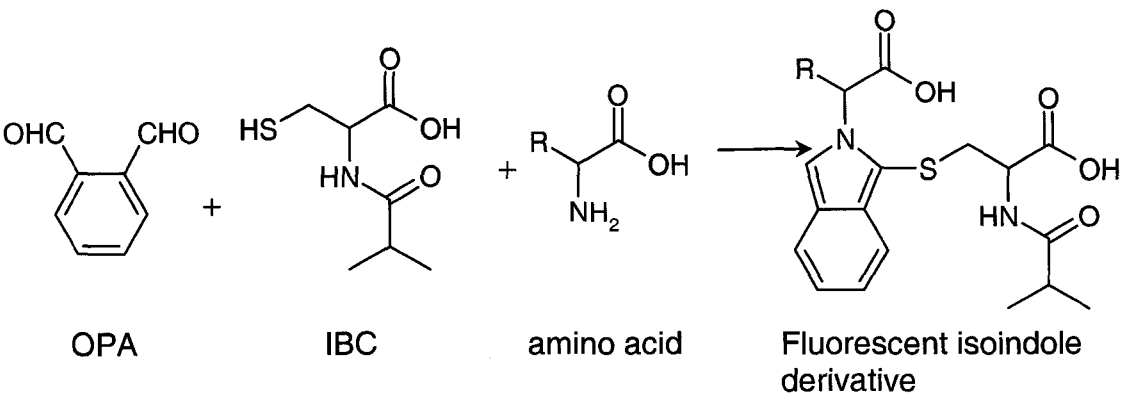
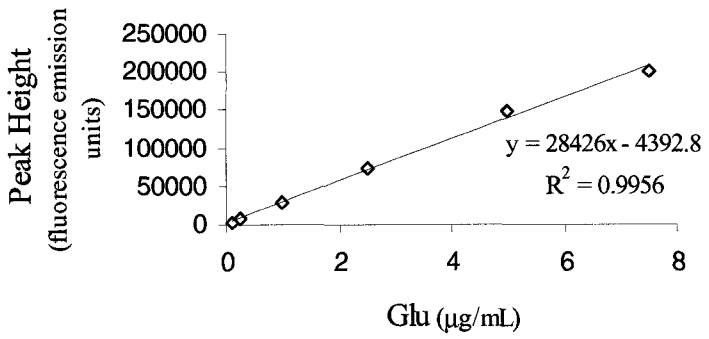
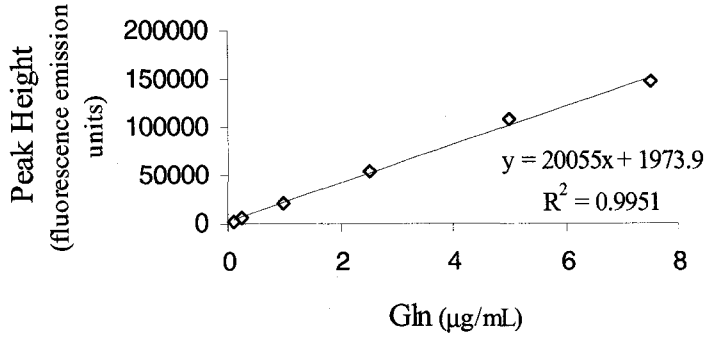


Figure 5-3. Amino acid derivatization with OPA and IBC.



A



B

Figure 5-4. Calibration curves used to quantify plasma Glu (A) and Gln (B).

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Chapter 6

The Relationship Between mPFC Glu Levels and Peripheral Markers of Glu Neurotransmission in First Episode Psychosis

6.1 INTRODUCTION

There is increasing evidence supporting Glu dysfunction in schizophrenia, with NMDA Glu receptor hypofunction potentially being a major contributor to the pathophysiology of the illness (Krystal et al 1994). Results from some ^{1}H MRS studies of *in vivo* brain Glu and Gln levels support the presence of glutamatergic dysfunction in some areas of the brain that are thought to be implicated in schizophrenia, including the prefrontal and anterior cingulate cortices, the hippocampus and the thalamus (Bartha et al 1997; Choe et al 1994; Kegeles et al 2000; Ohrmann et al 2006; Ohrmann et al 2005; Rowland et al 2005; Stanley et al 1996; Theberge et al 2002; Williamson et al 1999; Williamson et al 2003). Other ^{1}H MRS studies, however, have found no difference in brain Glu neurotransmission markers between patients and HCs (Bartha et al 1999; Block et al 2000; Stanley et al 1995; Wood et al 2007).

Findings of abnormal circulating (plasma/serum) Glu and Gln levels, including higher than normal Glu and lower than normal Gln levels in unmedicated chronically ill patients (Rao et al 1990; Tortorella et al 2001; van der Heijden et al 2005), and lower than normal Glu in drug-naïve FEPs (Palomino et al 2007), also suggest that Glu neurotransmission may be dysfunctional in schizophrenia. Furthermore, there is evidence to suggest that antipsychotics may exert some of their therapeutic effects by altering peripheral Glu levels. In two studies, serum Glu concentrations increased after a switch from conventional antipsychotics to olanzapine in schizophrenic patients (Evins et al 1997; Goff et al 2002). Tortorella et al also found that plasma Glu levels decreased in neuroleptic-resistant SZs after 12 weeks of clozapine treatment (Tortorella et al 2001), but other atypical antipsychotics had no effects on these levels (van der Heijden et al

2005). In a recent longitudinal investigation, Palomino et al found that plasma Glu levels, which were lower than normal in drug-naïve FEPs, progressively increased, and reached levels that were comparable to normal after six and 12 months of treatment with antipsychotics (Palomino et al 2007). Also, Maeshima et al found that SZs who were taking atypical antipsychotics had higher plasma Glu levels during the remission stage, compared to the active stage of a psychotic episode (Maeshima et al 2007).

Currently, high field strength ^{1}H MRS is one of the best methods for investigating *in vivo* Glu and Gln abnormalities in the brain. However, the availability of high field strength ^{1}H MRS is limited, and the methodologies between groups who have access can vary greatly. Given the relative ease and greater feasibility of using peripheral circulating measures clinically, it is important to determine if peripheral measures reflect those found centrally with MRS. In a previous investigation we found no relationship between plasma Glu and either mPFC Glu or Glx, and no relationship between plasma Gln and either mPFC Glu or Glx in young healthy male volunteers (Shulman et al 2006). Only one other study examined *in vivo* ^{1}H MRS-derived Glu brain measures and Glu serum levels simultaneously (Goff et al 2002). In this study the researchers found a significant increase in serum Glu concentrations, but no change in anterior cingulate Glx following a switch from conventional antipsychotics to olanzapine in SZs (Goff et al 2002). While the authors did not directly examine the correlation between peripheral Glu and central Glx, the discordance between peripheral and central changes are consistent with our results (Shulman et al 2006), and suggest a lack of a relationship between the two compartments.

To expand on our previous findings, in this study we examined the relationship between mPFC Glu and plasma Glu and Gln levels in FEPs. Furthermore, we examined

the relationship between mPFC Glu levels and levels of other peripheral compounds that are thought to modulate Glu neurotransmission, including the NASs DHEA, DHEAS, PREG and PREGS and the neuroactive KYN pathway metabolite KYNA. A longitudinal investigation of mPFC Glu and plasma Glu and Gln was also conducted in order to determine if Glu and Gln levels are altered in unmedicated FEPs, when compared to HCs, and examine if treatment with atypical antipsychotics affects these levels. In this investigation, we also determined if plasma Glu and Gln levels correlate with symptom severity.

6.2 METHODS

6.2.1 Study Participants

Ethical approval for this study was obtained from the University of Alberta Health Research Ethics Board, and written informed consent was obtained from all participants after all procedures had been fully explained. Twenty-one male FEPs, who were recruited from EEPIC and met the current EEPIC inclusion/exclusion criteria (Table 6-1), participated in the study. Thirty age-matched, male HCs with no psychopathology [as determined by the SCID-I (First et al 1994)] also participated in the study, and were recruited from the community via poster advertisement. Exclusion criteria for HCs included major neurological/medical illness, history of substance/alcohol abuse, history of significant head injury (loss of consciousness > 29 minutes), and a positive family history of Axis-I disorders in first or second degree relatives. Fifteen of the FEPs and 17 of the HCs underwent ^{1}H MRS analysis.

6.2.2 Clinical Assessment

Demographic and diagnosis data for all study participants are shown in Table 6-2, clinical rating scores are shown in Table 6-3, and medications taken by study participants are shown in Table 6-4. Of the 21 EEPIC patients included in the study, nine were antipsychotic-naïve, and the remaining 12 patients had a mean lifetime exposure to antipsychotics prior to entering EEPIC of 10.25 days. Demographic and diagnosis data for those individuals who underwent ^{1}H MRS analysis are shown in Table 6-5, clinical rating scores are shown in Table 6-6, and medications taken by these study participants are shown in Table 6-7. Of these 15 EEPIC patients, six were antipsychotic-naïve, and the remaining nine patients had a mean lifetime exposure to antipsychotics prior to entering EEPIC of 5.40 days. If a patient was taking any psychotropic medication it was discontinued for a minimum of two to seven days, as determined by the half-life of elimination of the specific drug. All FEPs completed a clinical assessment in an unmedicated state (baseline), as required by the EEPIC protocol. During this assessment the SCID-I (First et al 1994) was administered to all FEPs to determine diagnosis, the PANSS, subdivided into positive, negative and general symptom clusters (Kay et al 1987) was used to quantify psychopathology, the STAI (Spielberger et al 1970) and the BAI (Beck and Steer 1990) were used to assess symptoms of anxiety, the MIS (Eckblad and Chapman 1983) was used to assess magical thinking, and the PGWB (McDowell and Newell 1996) was used to assess subjective well-being. As per EEPIC protocol, following baseline assessment, all FEPs began taking one of three atypical antipsychotics (olanzapine, risperidone, or quetiapine), and were followed longitudinally. The treating EEPIC psychiatrists prescribe concomitant medication as necessary for standard patient

care. Symptom rating scales described above were administered to FEPs two months and six months post baseline assessment in order to track symptom changes throughout the course of treatment. All symptom rating scales, with the exception of the PANSS, were administered to HCs at baseline and two months after initial assessment. The HCs were unmedicated throughout the study.

6.2.3 Plasma Collection and Analysis

All study participants followed an overnight fast prior to blood collection, done between 8 and 10 am using vacutainers containing the anticoagulant ethylenediamine tetraacetic acid. Blood was collected from FEPs using the same regimen as that used for PANSS administration [i.e. at baseline (unmedicated state), and following two months and six months of treatment with antipsychotic medication]. Blood was also collected twice from unmedicated HCs: at baseline and two months after initial collection. Following blood collection the plasma was immediately separated by centrifugation, transferred into plastic tubes, and stored at the Neurochemical Research Unit, University of Alberta, in a -80°C freezer until analysis using various chromatographic techniques described previously (see chapters 2, 3 and 5).

6.2.4 mPFC Glu Analysis with ^{1}H MRS

The ^{1}H MRS technique used in this study is described in Chapter 5 and published elsewhere (Shulman et al 2006). In the previous method all metabolites were expressed as a ratio to Cr. To improve on this methodology all measures derived by MRS in this study are expressed as a ratio to water. The water reference data result from non-water-

suppressed STEAM acquisitions for a series of TE values. The water data were fitted to a multi-exponential using a non-negative-least-squares algorithm, yielding both the T_2 relaxation times present in the decay together with their relative proportions. An estimation of the water peak area at a theoretical TE of 0 ms was used to provide the denominator for metabolite concentration estimation.

6.2.5 Statistical Analysis

SPSS 12.0 software was used for all statistical analysis. All results are expressed as mean ± SD. For all statistical analyses, $\alpha = 0.05$ was set as the nominal level of significance.

MANOVA, with time (baseline and two months) as a within-subject factors and group (FEP and HC) as a between-subject factor, was used to analyze changes in plasma Glu and Gln concentrations, and changes in mPFC Glu levels.

Independent samples t-tests were done to determine if there were any significant between group differences in plasma Glu or Gln concentrations at baseline and at two months, separately, and paired samples t-tests were done to determine if there were significant differences in plasma concentrations between baseline and two month within each group separately. To determine if there were any significant differences in mPFC Glu levels between groups at baseline, an independent samples t-test was used. Due to the small two month FEP sample size, the Mann-Whitney U test was used to determine if these differences were present at two months.

In order to determine if there were significant differences in mPFC Glu levels between baseline and two months within the HC group, a paired samples t-test was used.

Due to the small two month FEP sample size, the Wilcoxon Signed Ranks test was used to determine if these differences were present in FEPs.

MANOVA, with time (baseline, two months, six months) as a within-subject factor, was also used to determine if there were any significant changes in plasma Glu and Gln concentrations between baseline, two months and six months in FEPs.

In order to determine if a linear relationship exists between plasma of Glu and Gln or mPFC Glu levels and clinical rating scores, correlation analyses between each plasma/mPFC amino acid level and each clinical rating score were done for baseline and two month measures, separately, in each group separately. The clinical rating scales included in correlation analyses in FEPs were PANSS-P, PANSS-N, PANSS-G, MIS, STAI and BAI. As the PANSS was not administered to HCs, only MIS, STAI and BAI were included in correlation analyses in HCs.

In order to determine if a linear relationship exists between central and peripheral markers of glutamatergic neurotransmission, correlation analyses between baseline mPFC Glu levels, as determined by MRS, and baseline concentrations of each plasma metabolite that is involved in, or that modulates glutamatergic neurotransmission, including Glu, Gln, DHEA, DHEAS, PREG, PREGS and KYNA, as determined by various chromatographic techniques described previously (see chapters 2, 3 and 5), was done in each group separately.

6.3 RESULTS

6.3.1 Plasma Glu Concentration Changes

MANOVA did not show a significant time effect (Wilks' multivariate tests of significance: $F_{1, 36} = 0.700$, $p = 0.408$) or a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 36} = 0.004$, $p = 0.951$) on plasma Glu concentration (Figure 6-1).

Independent samples t-tests did not reveal significant between-group differences in plasma Glu concentrations at baseline ($t_{48} = 0.097$, $p = 0.923$), or at two months ($t_{36} = 0.134$, $p = 0.894$) (Figure 6-1).

Paired samples t-tests did not show a significant differences in plasma Glu concentrations between baseline and two months within each group separately (FEPs: $t_8 = 0.271$, $p = 0.794$; HCs: $t_{28} = 1.283$, $p = 0.210$) (Figure 6-1).

MANOVA, with time (baseline, two months, six months) as a within-subject factor, did not reveal significant changes in plasma Glu concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2, 3} = 3.594$, $p = 0.160$) (Figure 6-2).

6.3.2 Plasma Gln Concentration Changes

MANOVA did not show a significant time effect (Wilks' multivariate tests of significance: $F_{1, 36} = 0.087$, $p = 0.770$) or a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 36} = 0.695$, $p = 0.410$) on plasma Gln concentration (Figure 6-3).

Independent samples t-tests did not reveal significant between-group differences in plasma Gln concentrations at baseline ($t_{48} = 1.847$, $p = 0.071$), or at two months ($t_{36} = 1.868$, $p = 0.070$) (Figure 6-3).

Paired samples t-tests did not show a significant difference in plasma Gln concentrations between baseline and two months within each group separately (FEPs: t_8 = 0.219, p = 0.832; HCs: t_{28} = 1.369, p = 0.182) (Figure 6-3).

MANOVA, with time (baseline, two months, six months) as a within-subject factor, did not reveal significant changes in plasma Gln concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2,\ 3}$ = 2.871, p = 0.201) (Figure 6-4).

6.3.3 mPFC Glu Level Changes

mPFC Glu, NAA, Cr and Cho levels are shown in Table 6-8. As the purpose of this investigation was to investigate abnormalities in glutamatergic neurotransmission, only mPFC Glu levels were used for statistical analysis. MANOVA revealed a significant time effect (Wilks' multivariate tests of significance: $F_{1,\ 16}$ = 6.566, p = 0.021), and a significant time x group effect (Wilks' multivariate tests of significance: $F_{1,\ 16}$ = 7.927, p = 0.015) on mPFC Glu levels (Figure 6-5).

Independent samples t-test did not reveal a significant between-group difference in mPFC Glu levels at baseline (t_{30} = 0.291, p = 0.773). The Mann-Whitney U test did not reveal a significant between-group difference in mPFC Glu levels at two months (U = 15, p= 0.167) (Figure 6-5).

The Paired samples t-test did not show a significant difference in mPFC Glu levels between baseline and two months in HCs (t_{13} = 0.292, p = 0.775), and the Wilcoxon Signed Ranks test did not show a significant difference in mPFC Glu levels between baseline and two months in FEPs (T = 0, p = 0.068) (Figure 6-5).

6.3.4 Relationship Between Plasma Glu and Gln, and mPFC Glu Levels and Clinical Rating Scores

Table 6-9 shows results from correlation analyses investigating the linear relationship between Glu and Gln plasma and mPFC Glu levels and PANSS scores in FEPs at baseline and at two months. There were no statistically significant linear relationships between plasma Glu or Gln concentrations or mPFC Glu levels and PANSS scores.

Results from correlation analyses investigating the linear relationship between Glu and Gln plasma and mPFC Glu levels and STAI, BAI and MIS scores in FEPs and HCs at baseline and at two months are presented in Table 6-10. There was a statistically significant positive correlation between plasma Glu concentrations and BAI and MIS scores in FEPs at baseline ($R_{1,17}$ = 0.522, p = 0.022 and $R_{1,18}$ = 0.487, p = 0.029, respectively). No other statistically significant linear relationships between plasma or mPFC amino acid concentrations and STAI, BAI and MIS scores were found.

6.3.5 Relationship Between mPFC Glu Levels and Peripheral Markers of Glutamatergic Neurotransmission

In HCs there were no statistically significant linear relationships between baseline mPFC Glu levels and each of the following baseline plasma concentrations: Glu ($R_{1, 15}$ = 0.034, p = 0.896), Gln ($R_{1, 15}$ = 0.231, p = 0.327), DHEA ($R_{1, 15}$ = 1.144, p = 0.582), DHEAS ($R_{1, 15}$ = 0.201, p = 0.439), PREG ($R_{1, 15}$ = 0.184, p = 0.480), PREGS ($R_{1, 15}$ = 0.161, p = 0.536), or KYNA ($R_{1, 15}$ = 0.271, p = 0.311). Similarly, in FEPs there were no

statistically significant linear relationships between baseline mPFC Glu levels and each of the following baseline plasma concentrations: Glu ($R_{1, 13} = 0.102$, $p = 0.716$), Gln ($R_{1, 13} = 0.110$, $p = 0.696$), DHEA ($R_{1, 13} = 0.051$, $p = 0.857$), DHEAS ($R_{1, 13} = 0.299$, $p = 0.279$), PREG ($R_{1, 13} = 0.093$, $p = 0.741$), PREGS ($R_{1, 13} = 0.024$, $p = 0.932$), or KYNA ($R_{1, 13} = 0.505$, $p = 0.065$).

6.4 DISCUSSION

This study was, to our knowledge, the first investigation examining the relationship between high field strength ^{1}H MRS-derived central Glu and circulating Glu and Gln in FEPs. This was also the first investigation of the relationship between brain Glu levels and other peripheral markers of Glu neurotransmission. In this study, we also examined plasma Glu and Gln, and mPFC Glu levels in FEPs in an unmedicated state, examined how these levels changed following treatment with atypical antipsychotics, compared these concentrations to those found in HCs, and established if a relationship exists between these levels and symptom severity. We found that mPFC Glu levels did not correlate with any of the peripheral markers of Glu neurotransmission examined in this study. Plasma Glu and Gln concentrations in FEPs were comparable to those found in HCs throughout the study; however, there was a significant time x group effect on mPFC Glu levels. Plasma Glu concentrations were also directly correlated with BAI and MIS sores in FEPs at baseline, but not at two months in our study.

In our study, mPFC Glu levels did not correlate with plasma Glu or Gln in HCs or in FEPs. Similarly, mPFC Glu levels did not correlate with other Glu modulating compounds found in plasma, including the NASs DHEA, PREG, DHEAS, PREGS, and

the KYN pathway metabolite KYNA. Our results are in agreement with those from our previous study, where we found no relationship between mPFC Glu and Glx levels and plasma Glu and Gln levels in HCs (Shulman et al 2006). Our results are also consistent with a study conducted by Goff et al, who found a discordant change in serum Glu and anterior cingulate Glx levels following pharmacological intervention (Goff et al 2002). Together, these studies suggest that peripheral markers of Glu neurotransmission are not useful predictors of Glu levels in the prefrontal and anterior cingulate cortices. However, it remains unknown if these peripheral markers correlate with Glu levels found in other brain areas. Also, it is uncertain if plasma NAS and KYNA levels correlate with levels in the brain, because a method for quantifying *in vivo* brain NAS and KYNA levels is currently not available.

In their study, Goff et al found that following a switch from typical antipsychotics to olanzapine, brain Glx levels increased in those patients with improvement in negative symptoms, compared to patients who experienced no change in, or worsening of negative symptoms (Goff et al 2002). These findings provide preliminary evidence that MRS may be a clinically useful tool for assessing neurochemical abnormalities and medication effects in psychiatric disorders. Our results support this idea, as we found a significant time x group effect on mPFC Glu levels. However, in our study, mPFC Glu levels did not change significantly in FEPs after two months of treatments with atypical antipsychotics, and at two months, mPFC Glu levels in FEPs were comparable to those found in HCs. A possible explanation for these seemingly discrepant results, is that due to the small two month FEP sample size in our study (n = 4), we had to use non-paramateric statistical analysis for multiple comparison purposes. Non-parameric statistical tests are less

powerful (i.e. there is a higher probability of committing a type II statistical error) than their equivalent parametric tests, which may account for these statistically non-significant results. In the future, as our FEP sample size increases, we will re-examine the effects of atypical antipsychotics on mPFC Glu levels using paramateric statistical analysis.

Consistent with one previous report (Alfredsson and Wiesel 1989), but inconsistent with another (Palomino et al 2007), we found that plasma Glu and Gln levels in unmedicated FEPs were comparable to those in HCs. We also found that the atypical antipsychotics had no effect on plasma Glu or Gln levels. These results are inconsistent with those found in a similar longitudinal investigation where plasma Glu level changes were measured in drug-naïve FEPs, and after one, six and 12 months, during which time patients were treated with typical or atypical antipsychotics, with lithium or other mood stabilizers together with atypical antipsychotics, or received no treatment (Palomino et al 2007). The researchers found that plasma Glu levels were lower than normal at the onset of psychosis and after one month of treatment, and that these levels progressively increased, and reached levels that were comparable to normal after six and 12 months of treatment. Plasma Glu levels have also been reported to increase in SZs following a switch from typical to atypical antipsychotics (Evins et al 1997; Goff et al 2002), and after remission of an acute psychotic episode in SZs taking atypical antipsychotics (Maeshima et al 2007). Although our results are not in agreement with results in these previous studies, Figure 6-2 shows a non-statistically significant increase in plasma Glu levels following six months of treatments with atypical antipsychotics. Perhaps, as the six month sample size increases, this difference will reach statistical significance, and be consistent with results reported in the literature.

We found that plasma Glu concentrations were positively correlated with BAI and MIS scores in FEPs at baseline, but not at two months. No other statistically significant correlations between plasma or mPFC Glu or Gln levels and symptom severity were found. The MIS is a measure of schizotypy and delusional thinking (Eckblad and Chapman 1983), and a positive correlation between MIS scores and plasma Glu levels suggest that Glu may contribute to delusional symptoms of schizophrenia. BAI scores reflect anxiety levels experienced by an individual throughout the previous week, and the presence of a correlation between Glu levels and anxiety levels is not surprising since excessive Glu is thought to be implicated in anxiety (Bergink et al 2004). However, Glu plasma levels were not correlated with BAI or MIS scores in FEPs at two months, or in HCs throughout the study, indicating that the relationship between plasma Glu and the severity of these symptoms needs to be re-examined in the future.

A potential limitation in our study is that clinical assessment and phlebotomy were not done simultaneously, but rather hours-days apart. This makes it difficult to examine the relationship between some symptoms and Glu levels because some of the symptoms rating scores, particularly STAI scores, are time-dependent. However, scores from other clinical rating scales used in our study, including the MIS, PANSS, and BAI, remain stable for at least a week. Therefore, we were effectively able to examine the relationship between Glu levels and scores derived using these symptom rating scales.

Another concern is that phlebotomy and MRS were conducted on different days and under different conditions (fasting and non-fasting, respectively), which may account for the non-significant correlations between peripheral and central measures of Glu neurotransmission. We addressed this issue in our previous investigation by examining

the stability of plasma Glu and Gln levels over time, and by examining the effects of fasting on mPFC Glu levels (Shulman et al 2006). In that study we found that fasting had no effect on mPFC Glu levels in HCs (Shulman et al 2006). Also, similar to the results in this study, we previously found no statistically significant difference in Glu or Gln plasma levels between baseline and two months in HCs (Shulman et al 2006). Another limitation, which is a concern in most studies investigating the pathophysiology of schizophrenia during the early phase of the illness, is that the FEP group in our study was a heterogeneous group of patients that had different diagnoses and were treated with different antipsychotics. As our FEP sample increases, we will divide the FEP sample into separate diagnostic and treatment groups and re-examine the variables tested in this study.

This study was the first investigation that examined the relationship between high field strength ^{1}H MRS-derived central Glu and circulating Glu and Gln, and the relationship between brain Glu levels and other peripheral markers of Glu neurotransmission in FEPs. In this study, we also established if mPFC or plasma Glu levels are altered in unmedicated FEPs and if treatment with antipsychotics affects these levels. Our results suggest that the measurement of plasma Glu is not an appropriate tool for investigating the abnormalities in, and the effects of medication on Glu neurotransmission in the mPFC, and provides strong support for using a more direct approach, such as high field strength MRS, for such investigations.

6.5 TABLES AND FIGURES

Table 6-1. EEPIC inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
Age 16-35 years	Subjects requiring mood stabilizers at study entry
Diagnosis of schizophrenia or schizophreniform disorder	Head injury with > 29 minutes loss of consciousness
Less than 1 year of active-phase symptoms	Active substance abuse or dependence disorder (i.e. within past 30 days)
Less than 3 months of lifetime prior exposure to antipsychotic medication	Exposure to long acting depot neuroleptic medication
Current psychotic symptoms requiring long term antipsychotic treatment	Known sensitivity to olanzapine, risperidone, or quetiapine
	Serious past or current CNS/medical illness

Table 6-2. Diagnostic and demographic data for all FEPs and HCs.

		FEP n=21	HC n=30
Age	Mean ± SD (years)	22.97±3.28	21.18±3.36
DSM-IV diagnosis	Schizophrenia	n=6	n=0
	Schizophreniform disorder	n=3	n=0
	Substance-induced psychotic disorder	n=5	n=0
	Brief psychotic disorder	n=1	n=0
	Psychotic disorder not otherwise specified	n=5	n=0
	Psychotic disorder due to a general medical condition	n=1	n=0
First degree family history	No history	n=14	n=30
	Schizophrenia	n=4	n=0
	Bipolar disorder	n=2	n=0
	Major depressive disorder	n=1	n=0
Handedness	Right handed	n=17	n=25
	Left handed	n=4	n=5
Marital status	Married or common law	n=0	n=6
	Single	n=21	n=24
Number of children	No children	n=21	n=29
	Two children	n=0	n=1
Occupation	Administrative/minor professional	n=0	n=4
	Clerical/sales/technician/farmer	n=0	n=1
	Skilled manual employee	n=2	n=0
	Unskilled employee	n=6	n=0
	Student	n=5	n=25
	Unemployed	n=8	n=1
Highest level of education achieved	Graduated professional/graduate school	n=0	n=1
	Part graduate/professional school	n=0	n=2
	Graduated four year college school	n=1	n=7
	Graduated two year college/ technical school	n=1	n=0
	Part college	n=7	n=19
	Graduated high school	n=5	n=1
	Attended grade 7-12 without graduating	n=7	n=0

Table 6-3. Clinical rating scores for all FEPs and HCs.

Clinical rating scales	FEP *baseline* n=21	*2 months* n=11	*6 months* n=5	HC *baseline* n=30	*2 months* n=29
PANSS-P	19.76±5.88	13.40±5.06	10.60±1.52	N/A	N/A
PANSS-N	20.38±6.00	17.90±7.46	16.40±4.67	N/A	N/A
PANSS-G	40.62±10.60	34.70±10.89	31.20±4.21	N/A	N/A
PANSS-T	80.76±20.23	66.00±22.36	58.20±9.01	N/A	N/A
MIS	11.05±5.99	9.70±6.83	8.00±7.46	2.53±2.16	2.07±2.09
STAI	44.70±11.07	42.10±6.90	40.40±10.43	26.80±5.68	25.55±5.08
BAI	15.80±10.54	6.20±5.73	3.83±2.99	2.93±2.94	4.17±5.70
PGWB	57.33±18.45	68.30±7.10	66.00±8.88	90.93±9.55	90.28±10.11

All results are expressed as mean ± SD. PANSS-P, PANSS-N, PANSS-G, and PANSS-T refer to the positive, negative, general, and total PANSS scores, respectively.

Table 6-4. Medications taken by all FEPs and HCs throughout the study.

Time	Anti-psychotics	Group	n	Other psychotropic medication	Group	n	Other medication	Group	n
Baseline							minocycline	FEP	1
							fluticasone	HC	1
							salbutamol	HC	2
							celecoxib	HC	1
							budesonide	HC	1
							formoterol	HC	1
							isotretinoid	HC	1
2 months	olanzapine	FEP	4	mirtazapine	FEP	1	minocycline	FEP	1
	quetiapine	FEP	1	divalproax	FEP	1	fluticasone	HC	1
	risperidone	FEP	3	lorazepam	FEP	1	salbutamol	HC	2
	olanzapine+ quetiapine	FEP	1	venlafaxine	FEP	1	celecoxib	HC	1
	risperidone+ quetiapine	FEP	1				budesonide	HC	1
							formoterol	HC	1
							isotretinoid	HC	1
6 months	olanzapine	FEP	1	miratazapine	FEP	1	minocycline	FEP	1
	quetiapine	FEP	1	venlafaxine	FEP	1			
	risperidone	FEP	3						

All FEPs were unmedicated (not taking psychotropic medication) at baseline, and all HCs were unmedicated (not taking psychotropic medication) throughout the duration of the study. The daily doses for antipsychotic medication ranged between 5-20 mg, 150-500 mg, and 1.5-4 mg for olanzapine, quetiapine, and risperidone, respectively. The daily doses for other psychotropic medications were 15 mg, 750 mg, 1 mg, and 150 mg for mirtazapine, divalproax, lorazepam, and venlafaxine, respectively.

Table 6-5. Diagnostic and demographic data for FEPs and HCs that underwent ^{1}H MRS analysis.

		FEP n=15	HC n=17
Age	Mean ± SD (years)	22.16±3.36	21.69±2.35
DSM-IV diagnosis	Schizophrenia	n=4	n=0
	Schizophreniform disorder	n=3	n=0
	Substance-induced psychotic disorder	n=3	n=0
	Brief psychotic disorder	n=1	n=0
	Psychotic disorder not otherwise specified	n=3	n=0
	Psychotic disorder due to a general medical condition	n=1	n=0
First degree family history	No history	n=11	n=17
	Schizophrenia	n=2	n=0
	Bipolar disorder	n=1	n=0
	Major depressive disorder	n=1	n=0
Handedness	Right handed	n=12	n=14
	Left handed	n=3	n=3
Marital status	Married or common law	n=0	n=3
	Single	n=15	n=14
Number of children	No children	n=15	n=16
	Two children	n=0	n=1
Occupation	Administrative/minor professional	n=0	n=0
	Clerical/sales/technician/farmer	n=0	n=0
	Skilled manual employee	n=2	n=0
	Unskilled employee	n=6	n=0
	Student	n=2	n=16
	Unemployed	n=5	n=1
Highest level of education achieved	Graduated professional/graduate school	n=0	n=1
	Part graduate/professional school	n=0	n=0
	Graduated four year college school	n=1	n=2
	Graduated two year college/ technical school	n=1	n=0
	Part college	n=6	n=14
	Graduated high school	n=3	n=1
	Attended grade 7-12 without graduating	n=4	n=0

Table 6-6. Clinical rating scores for FEPs and HCs that underwent ^{1}H MRS analysis.

Clinical rating scales	FEP		HC	
	baseline	*2 months*	*baseline*	*2 months*
	n=15	n=4	n=17	n=14
PANSS-P	18.27±5.68	12.00±6.06	N/A	N/A
PANSS-N	19.40±5.29	15.00±7.39	N/A	N/A
PANSS-G	38.27±8.79	29.25±10.05	N/A	N/A
PANSS-T	75.93±16.77	56.25±21.09	N/A	N/A
MIS	10.53±6.26	8.50±4.44	2.88±2.34	2.14±2.11
STAI	44.79±12.80	47.00±6.78	26.35±5.97	24.64±4.16
BAI	15.21±8.44	5.25±4.99	3.41±3.14	6.36±7.11
PGWB	54.27±20.84	61.25±4.11	90.94±10.54	86.79±12.05

All results are expressed as mean ± SD. PANSS-P, PANSS-N, PANSS-G, and PANSS-T refer to the positive, negative, general, and total PANSS scores, respectively.

Table 6-7. Medications taken by FEPs and HCs that underwent ^{1}H MRS analysis.

Time	Anti-psychotics	Group	n	Other psychiatric medication	Group	n	Other medication	Group	n
Baseline							salbutamol	HC	1
							budesonide	HC	1
							formoterol	HC	1
							isotretinoid	HC	1
2 months	olanzapine	FEP	2	mirtazapine	FEP	1	salbutamol	HC	
	risperidone	FEP	2	venlafaxine	FEP	1	isotretinoid	HC	

All FEPs were unmedicated (not taking psychotropic medication) at baseline, and all HCs were unmedicated (not taking psychotropic medication) throughout the duration of the study. The daily doses for antipsychotic medication ranged between 5-20 mg, 150-500 mg, and 1.5-4 mg for olanzapine, quetiapine, and risperidone, respectively. The daily doses for other psychotropic medications were 15 mg and 150 mg for mirtazapine and venlafaxine, respectively.

Table 6-8. mPFC metabolite levels in FEPs and HCs at baseline and at two months.

	Baseline		**2 months**	
	FEPs (n=15)	**HCs (n=17)**	**FEPs (n=4)**	**HCs (n=14)**
Glu	7.84±1.78	8.74±2.16	11.10±2.59	8.85±1.91
NAA	9.79±1.31	10.11±1.34	10.28±0.89	10.19±0.89
Cho	1.84±0.27	1.88±0.40	1.86±0.19	1.93±0.32
Cre	8.74±2.49	9.91±2.28	7.30±3.50	9.34±1.51

All results are expressed as mean ± SD.

Table 6-9. Relationship between plasma Glu and Gln, and mPFC Glu levels and PANSS scores, subdivided into PANSS-P, PANSS-N and PANSS-G in FEPs at baseline and at two months. R and p values are displayed.

		PANSS-P		**PANSS-N**		**PANSS-G**	
Time	**Amino acid**	R	p	R	p	R	p
Baseline	Plasma Glu	0.298	0.202	0.116	0.625	0.175	0.460
	Plasma Gln	0.234	0.320	0.016	0.946	0.106	0.658
	mPFC Glu	0.087	0.757	0.373	0.170	0.472	0.076
2 months	Plasma Glu	0.425	0.254	0.053	0.891	0.098	0.802
	Plasma Gln	0.014	0.971	0.403	0.282	0.230	0.552
	mPFC Glu	0.907	0.093	0.064	0.936	0.730	0.270

Table 6-10. Relationship between plasma Glu and Gln, and mPFC Glu levels and STAI, BAI and MIS scores in FEPs and HCs at baseline and at two months. R and p values are displayed.

			STAI		**BAI**		**MIS**	
Time	**Group**	**Amino acid**	R	p	R	p	R	p
Baseline	FEP	Plasma Glu	0.408	0.083	***0.522***	***0.022****	***0.487***	***0.029****
		Plasma Gln	0.106	0.667	0.332	0.165	0.126	0.596
		mPFC Glu	0.346	0.225	0.341	0.233	0.034	0.905
	HC	Plasma Glu	0.060	0.752	0.178	0.346	0.102	0.593
		Plasma Gln	0.264	0.159	0.057	0.746	0.181	0.340
		mPFC Glu	0.251	0.330	0.057	0.829	0.271	0.292
2 months	FEP	Plasma Glu	0.258	0.502	0.289	0.451	0.369	0.328
		Plasma Gln	0.069	0.860	0.195	0.616	0.017	0.966
		mPFC Glu	0.443	0.557	0.902	0.098	0.817	0.183
	HC	Plasma Glu	0.061	0.751	0.052	0.790	0.131	0.497
		Plasma Gln	0.087	0.655	0.131	0.497	0.228	0.243
		mPFC Glu	0.471	0.089	0.438	0.117	0.087	0.767

* $p < 0.05$

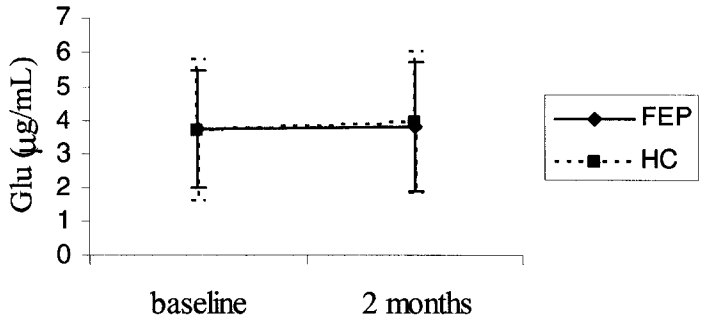

Figure 6-1. Mean (± SD) Glu concentrations at baseline and at two months in FEPs (unmedicated and medicated, respectively) and HCs (unmedicated at both time intervals). MANOVA did not show a significant time effect (Wilks' multivariate tests of significance: $F_{1, 36} = 0.700$, $p = 0.408$) or a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 36} = 0.004$, $p = 0.951$) on plasma Glu concentration. Plasma Glu concentrations were not significantly different between groups at baseline ($t_{48} = 0.097$, $p = 0.923$), or at two months ($t_{36} = 0.134$, $p = 0.894$). There were no significant differences between baseline and two month plasma Glu concentrations within each group separately (FEPs: $t_8 = 0.271$, $p = 0.794$; HCs: $t_{28} = 1.283$, $p = 0.210$).

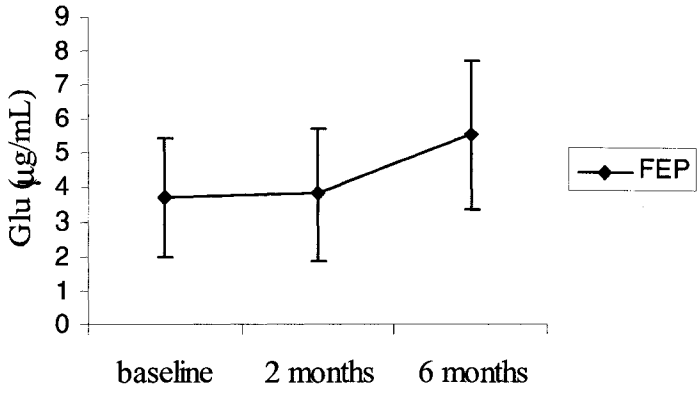

Figure 6-2. Mean (± SD) Glu concentrations at baseline, at two months and at six months. MANOVA did not reveal significant changes in plasma Glu concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2,3} = 3.594$, $p = 0.160$).

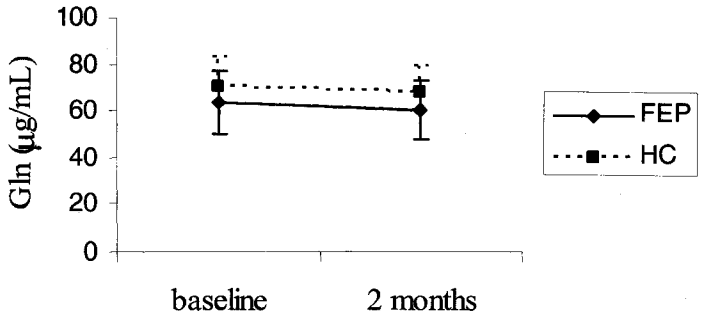

Figure 6-3. Mean (± SD) Gln concentrations at baseline and at two months in FEPs (unmedicated and medicated, respectively) and HCs (unmedicated at both time intervals). MANOVA did not show a significant time effect (Wilks' multivariate tests of significance: $F_{1, 36} = 0.087$, $p = 0.770$) or a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 36} = 0.695$, $p = 0.410$) on plasma Gln concentration. Plasma Gln concentrations were not significantly different between groups at baseline ($t_{48} = 1.847$, $p = 0.071$), or at two months ($t_{36} = 1.868$, $p = 0.070$). There were no significant differences between baseline and two month plasma Gln concentrations within each group separately (FEPs: $t_8 = 0.219$, $p = 0.832$; HCs: $t_{28} = 1.369$, $p = 0.182$).

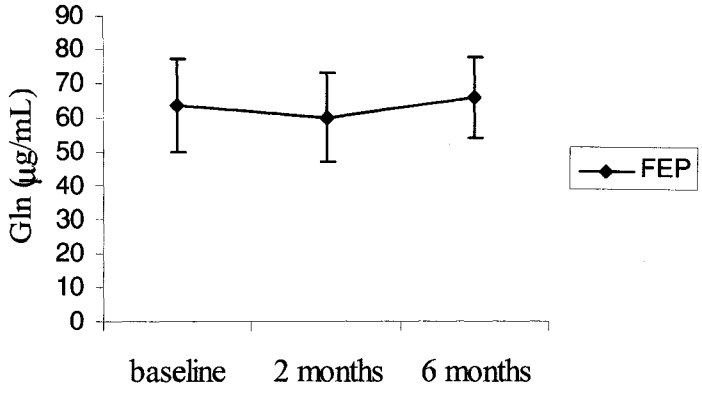

Figure 6-4. Mean (± SD) Gln concentrations at baseline, at two months and at six months. MANOVA did not reveal significant changes in plasma Gln concentrations between baseline, two months and six months in FEPs (Wilks' multivariate tests of significance: $F_{2,3} = 2.871$, $p = 0.201$).

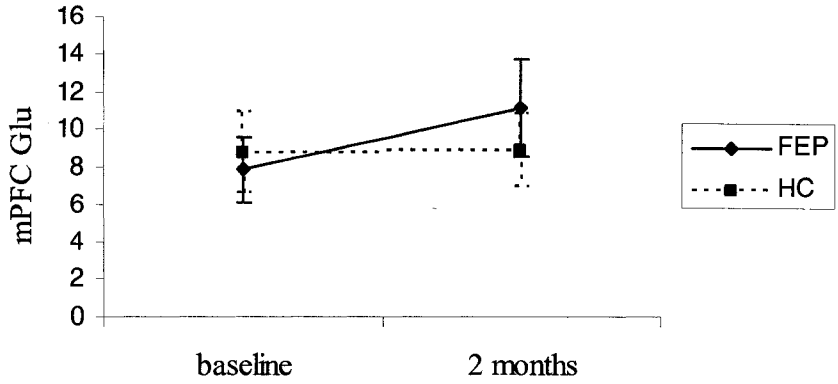

Figure 6-5. Mean (± SD) mPFC Glu levels at baseline and at two months in FEPs (unmedicated and medicated, respectively) and HCs (unmedicated at both time intervals). MANOVA showed a significant time effect (Wilks' multivariate tests of significance: $F_{1, 16} = 6.566$, $p = 0.021$), and a significant time x group effect (Wilks' multivariate tests of significance: $F_{1, 16} = 7.927$, $p = 0.015$) on mPFC Glu levels. mPFC Glu levels were not significantly different between groups at baseline ($t_{30} = 0.291$, $p = 0.773$), or at two months ($U = 15$, $p= 0.167$). There were no significant differences between baseline and two month mPFC Glu levels within each group separately (FEPs: $T = 0$, $p = 0.068$, $p = 0.794$; HCs: $t_{13} = 0.292$, $p = 0.775$).

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Chapter 7

General Discussion

7.1 SUMMARY OF RESULTS

In this thesis I investigated neuroactive compounds that modulate Glu and GABA neurotransmission in individuals with schizophrenia. I first used a crossover, randomized, PLA-controlled design to examine DHEA and PREG plasma concentration changes after a challenge with the panicogenic agent CCK-5 in chronically ill SZs, PDs and HCs. This was followed by a series of longitudinal studies examining Glu, NASs and KYN pathway metabolites in FEPs, where I quantified these compounds in the plasma of unmedicated FEPs and investigated how these levels changed throughout the initial course of treatment with atypical antipsychotics. I also determined if plasma levels of compounds that modulate glutamatergic neurotransmission correlate with *in vivo* brain Glu levels determined by ^{1}H MRS. The major findings from these studies are summarized below.

7.1.1 CCK-5-Induced NAS Changes in SZs and PDs

In the study examining CCK-5-induced changed in plasma NAS levels, I found that chronically ill medicated SZs and PDs have similar NAS plasma concentrations prior to panic induction, and a similar pattern in CCK-5-induced changes in plasma NAS concentrations.

I found that chronically ill SZs and PDs had lower DHEA and PREG levels than HCs prior to CCK-5 administrations. As these NASs are negative $GABA_A$ and positive NMDA receptor modulators (Baulieu and Robel 1998; Compagnone and Mellon 2000; Macdonald and Olsen 1994; Rupprecht and Holsboer 1999; Twyman and Macdonald 1992), low PREG and DHEA levels in SZs may contribute to NMDA receptor hypofunction thought to be present in the illness. Alternatively, lower PREG and DHEA

levels may be compensatory changes that serve to correct the hypoGABAergic state hypothesized in schizophrenia. In PDs, lower levels of these putatively anxiogenic NASs may serve as a counterregulatory mechanism against the occurrence of spontaneous panic attacks (Strohle et al 2002).

I also found that there was a greater increase in DHEA plasma concentrations immediately following CCK-5 injection in PDs, compared to HCs. Because DHEA is a putative anxiogenic agent in acute anxiety paradigms, the lower increase in HCs may be a protective mechanism against anxiety, and this mechanism may be either blunted or missing in PDs. There was also a smaller decrease in plasma DHEA and/or PREG concentrations 180 minutes following CCK-5 injection in SZs and PDs, compared to HCs. The ability to decrease these putatively anxiogenic NASs may be a mechanism that helps restore normal functioning after acute anxiety. Thus, healthy individuals may have a greater capacity to restore normal functioning than both SZs and PDs following acutely anxious situations.

The similar pattern of NAS plasma level fluctuations found in SZs and PDs in this study may partially explain why SZs have a high rate of comorbid anxiety disorders (Tibbo et al 2003).

7.1.2 Longitudinal Investigation of Neuroactive Compounds in First Episode Psychosis

The longitudinal investigation of plasma Glu, NAS and KYN metabolite levels in FEPs revealed that plasma 3α,5β-THP concentrations were higher in unmedicated FEPs than in HCs, and that two months of treatment with atypical antipsychotics normalized

these concentrations. These results indicate that 3α,5β-THP may be implicated in both the pathophysiology of psychosis and in the mechanism of action of antipsychotic drugs. Since 3α,5β-THP is a positive $GABA_A$ receptor modulator (Follesa et al 2001), higher than normal 3α,5β-THP concentrations in unmedicated FEPs may serve to compensate for the hypothesized hypoGABAergic state in schizophrenia (i.e. 3α,5β-THP may act as an endogenous antipsychotic agent). The finding that two months of treatment with antipsychotics "normalizes" 3α,5β-THP levels in FEPs can be interpreted in two ways: 1) atypical antipsychotics decrease 3α,5β-THP levels; or 2) levels decline because the endogenous antipsychotic mechanism of increasing 3α,5β-THP is no longer necessary after atypical antipsychotic administration.

I also found that while DHEAS, PREGS and KYNA plasma concentrations in unmedicated FEPs were comparable to those found in HCs, these levels declined after two months of treatment with atypical antipsychotics. These results are consistent with results from preclinical studies, where antipsychotics were found to decrease DHEAS (Nechmad et al 2003) and KYNA levels (Ceresoli-Borroni et al 2006) in rats. The similar pattern of KYNA and NAS level fluctuations seems to be contradictory since KYNA is a negative NMDA receptor modulator, while DHEAS and PREGS are positive NMDA receptor modulators (Birch et al 1988; Rupprecht and Holsboer 1999; Twyman and Macdonald 1992). However, DHEAS and PREGS are also negative $GABA_A$ receptor modulators (Rupprecht and Holsboer 1999; Twyman and Macdonald 1992). Perhaps antipsychotic-induced changes in NAS plasma levels reflect the effects of antipsychotics on the GABA system, while antipsychotic-induced changes in KYNA levels reflect the effects of antipsychotics on the Glu system.

7.1.3 Relationship Between Brain Glu Levels and Peripheral Markers of Glutamatergic Neurotransmission

I found that mPFC Glu levels did not correlate with any peripheral markers of Glu neurotransmission. These results suggest that peripheral markers of Glu neurotransmission are not useful predictors of Glu levels in the PFC. It remains unknown if these peripheral markers correlate with Glu levels in other brain areas or with synaptic Glu levels (i.e. levels of Glu that activate receptors).

7.2 LIMITATIONS AND FUTURE DIRECTIONS

A major limitation in the study examining CCK-5-induced changes in plasma NASs was that SZs were taking antipsychotic drugs throughout the duration of the study. Thus, it is possible that the lower DHEA and PREG levels found in SZs may have been caused by antipsychotics. Indeed, in a preclinical investigation it was found that the atypical antipsychotic clozapine, but not the typical agent haloperidol, decreased cortical DHEA and DHEAS following eight days of treatment in rats (Nechmad et al 2003). However, contrary to these preclinical results, sulpiride, a typical antipsychotic, increased DHEA levels in HCs (Oseko et al 1986). Also, in a recent investigation of DHEA and DHEAS serum level fluctuations in medicated SZs, there were no associations between NAS changes and the type or dose of antipsychotic medication that patients were on (Ritsner et al 2006). As it is unknown how antipsychotics alter NAS levels in humans, and as I did not include an unmedicated schizophrenic group in the study, I could not

exclude the possibility that the low DHEA and PREG levels found in the SZs sample were due to medication effects.

Aside from the possible confounding effect of antipsychotics on NAS levels, another issue with investigating NASs in chronically ill SZs, is that the patients had a long and heterogeneous psychiatric illness/medication history. These include previously diagnosed Axis I disorders, multiple hospitalizations and extensive exposure to different types of psychotropic agents. Furthermore, although all the patients were chronically ill (i.e. ill for longer than one year), the duration of illness varied from patients to patient.

In order to control for these possibly confounding variables and determine how antipsychotics affect NAS levels, as well as levels of other neuroactive compounds, I conducted a series of longitudinal investigations of NASs, KYN metabolites and Glu in FEPs. However, these studies had methodological issues as well. One major limitation was that the FEPs were a heterogeneous group of patients with different diagnoses. Due to the duration criteria when diagnosing schizophrenia, a person has to be assessed six months after they experience their first psychotic episode before a diagnosis of schizophrenia can be established, and only five patients completed their six month assessment (three with schizophrenia and two with substance-induced psychosis). Furthermore, as there are currently no laboratory diagnostic tests for schizophrenia, the diagnosis is subjective and dependent on the treating psychiatrist. Thus, the diagnosis may change throughout a patient's lifetime. This issue is a concern in all studies that investigate schizophrenia pathology, as well as the pathology of other psychiatric disorders. The only way to resolve this issue is to improve diagnostic procedures.

The FEPs included in our studies also took different atypical antipsychotics, which may have affected plasma metabolite levels differently. This has been demonstrated in both preclinical and clinical studies. For example, olanzapine (Barbaccia et al 2001; Marx et al 2000; Marx et al 2003), but not risperidone (Marx et al 2000) increased brain 3α,5α-THP levels in rats. Also, clozapine (Tortorella et al 2001), but not other atypical antipsychotics (van der Heijden et al 2005) decreased plasma Glu levels in neuroleptic-resistant SZs, and a significant increase in plasma Glu concentrations following a switch from typical to atypical antipsychotics was found in SZs (Evins et al 1997; Goff et al 2002). Unfortunately, the two month FEP sample size in my studies was too small to statistically examine the differential effects of different antipsychotics on plasma metabolite levels, and some of the patients took more than one type of antipsychotic, thus further complicating the relationship between antipsychotic drugs and plasma level changes. In the future, as the FEP sample size increases, it will be interesting to examine how different antipsychotics affect neuroactive compound plasma levels.

As with all studies that use peripheral metabolite levels to investigate the neurochemistry of brain disorders, I cannot be certain that peripheral levels reflect those found in the brain. With regard to NASs, which are lipophilic compounds, it is likely that they are able to cross the BBB. This was demonstrated by O'Dell et al who found that brain and plasma NASs increase in parallel after acute ethanol administration in rats, and adrenalectomy/gonadectomy prevents the increase in NASs (O'Dell et al 2004). These findings implicate peripheral endocrine glands, rather than the brain, as the source of some NASs following ethanol administration, indicating that peripheral NASs can

penetrate the BBB. This issue has only been addressed in humans in one postmortem study that yielded inconclusive results. In this study Marx et al found that brain PREG and 3α,5α-THP concentrations exceeded, while brain DHEA levels were comparable to those found in serum or plasma (Marx et al 2006). The researchers did not examine other NASs, and it is possible that postmortem changes affected the distribution of the NASs in various body compartments. Thus, further investigation into the distribution of brain and plasma NASs in humans is needed.

This issue is even more complex for KYNA, which is a polar molecule that has limited ability to penetrate the BBB. However, KYN, the precursor for KYNA, crosses the BBB via large neutral amino acid carriers (Fukui et al 1991; Klivenyi et al 2004). The CNS KYN pathway is dependent on KYN originating in the periphery (Jauch et al 1993), and it is estimated that 60% of brain KYN is derived from peripheral sources (Klivenyi et al 2004). After entering the brain, KYN is converted into KYNA in astrocytes and into other KYN metabolites in microglia (Schwarcz and Pellicciari 2002). Because KYNA brain levels are dependent on peripheral KYN, which can cross the BBB, peripheral KYNA levels may predict levels found in the brain. Indeed, plasma KYNA levels found in our study (nM range) are similar to those reported in the human brain (Moroni et al 1988; Turski et al 1988). However, until an *in vivo* technique that allows for quantification of brain NASs and KYN metabolites is available, we cannot determine with certainty whether plasma levels of these compounds reflect those found in the brain.

In this thesis I investigated the relationship between plasma and brain Glu levels. Although I found that there was no relationship between plasma and mPFC Glu levels, I did not investigate the relationship between plasma levels and levels found in other brain

areas. Furthermore, I cannot be certain that Glu levels in the mPFC voxel examined in my studies reflect Glu levels found in the synapses (i.e. levels of Glu that activate receptors) in this brain area. More sensitive *in vivo* techniques that can differentiate between synaptic and intracellular Glu levels are needed to conclusively establish how plasma and brain Glu levels are related.

Another relationship that needs to be explored is that between *in vivo* brain GABA levels and plasma levels of neuroactive compounds that affect GABAergic neurotransmission. This type of investigation would aid in understanding the GABAergic abnormalities present in schizophrenia. As the sensitivity and specificity of MRS technology is constantly improving, this investigation will be possible in the not-so-distant future. Our biomedical engineering team is also currently developing a sequence for quantifying *in vivo* brain glycine levels. This technology can be used for investigating glutamatergic neurotransmission abnormalities present in schizophrenia.

By integrating and simultaneously using different laboratory techniques for investigating the various neurochemical abnormalities at different stages of schizophrenia pathology, we can better piece together and solve the complex puzzle of schizophrenia pathophysiology. These types of longitudinal investigations will also improve our understanding of the mechanisms of action of currently available antipsychotics. Indeed, the work presented in this thesis helped decipher if NASs and KYN metabolites are implicated in the pathophysiology of psychosis, or in the mechanism of action of antipsychotic drugs. This knowledge will be useful for directing future research in this area.

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