NEUROENDOCRINE CHALLENGE STUDIES OF SEROTONIN AND GABA FUNCTION IN MANIA AND IN THE MECHANISM OF ACTION OF THE MOOD STABILIZER DIVALPROEX SODIUM

by

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ABSTRACT

In this thesis, we report on studies that investigated the roles of serotonin (5-hydroxytryptamine; 5-HT) and γ-aminobutyric acid (GABA) in the pathophysiology of mania and in the mechanism of action of the mood stabilizer, divalproex sodium (DVP) using neuroendocrine challenge paradigm.

Pharmacological challenges with the $5-HT_{1A}$ receptor agonist, ipsapirone and the $GABA_B$ receptor agonist, baclofen were administered to patients with mania and normal healthy volunteers. Following the administration of challenge agents, hormonal, hypothermic, and/or behavioral responses were measured at regular intervals. We found that 1) manic patients had significantly enhanced ACTH and cortisol responses to ipsapirone when compared to normal controls, but the hypothermic response to ipsapirone did not differ between the two groups; 2) one-week treatment with DVP significantly attenuated hypothermic response to ipsapirone in healthy controls but this did not modify the ACTH or cortisol response to ipsapirone; 3) GH response to baclofen was significantly increased in manic patients compared to normal controls; 4) one-week treatment with DVP significantly attenuated the GH response to baclofen in healthy controls.

The results of 5-HT neuroendocinre challenge studies suggest that manic patients have an increase in postsynaptic $5-HT_{1A}$ receptor sensitivity, which may be secondary to diminished 5-HT availability in central 5-HT synapses, and that DVP enhances 5-HT neurotransmission via a subsensitization of $5-HT_{1A}$ autoreceptors. These appear to support hypotheses that a 5-

HT deficit is involved in mania and that enhancement of 5-HT neurotransmission exerts a mood stabilizing effect. The findings of GABA neuroendocrine challenge studies suggest that manic patients also have an up-regulated hypothalamic $GABA_B$ receptor function, presumably resulting from a GABA deficit in brain, and that DVP treatment restores this deficit by enhancing GABAergic neurotransmission, thus leading to a down-regulation in hypothalamic $GABA_B$ receptor function. These experiments have provided some evidence for the contribution of 5-HT and GABA in mania and in the mechanism of action of DVP. However, it is very likely that other neurotransmitters such as norepinephrine and dopamine and their interaction with 5-HT and GABA also play important roles. More neurobiological studies on manic patients are warranted to enhance our understanding of this illness and its treatment.

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LIST OF ABBREVIATIONS

ACTH adrenocorticotropin hormone

ADP adenosine diphosphate

AMPA α-amino-3-hydroxy-5-methyl-4 isoxazole proprionic acid

ANCOVA analysis of covariance

ANOVA analysis of variance

AUC area under curve

BMI body mass index

CNS central nervous system

CRH corticotropin-releasing hormone

CSF cerebrospinal fluid

DA dopamine

5,7-DHT 5,7-dihydroxytryptamine

DSM-III-R Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised

DSM-IV Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition

DVP divalproex sodium

ECS electroconvulsive shock

ECT electroconvulsive therapy

EDTA ethylenediamine tetraacetic acid

FPIA Fluorescence Polarization Immunoassay

GABA γ-aminobutyric acid

GABA-T γ-aminobutyric acid transaminase

GAD glutamate decarboxylase

GH growth hormone

G proteins guanine nucleotide-binding proteins

5-HIAA 5-hydroxyindoleacetic acid

HPA hypothalamic-pituitary-adrenal

5-HTP 5-hydroxytryptophan

5-HT 5-hydroxytryptamine

HVA homovanillic acid

L-5-HTP 5-hydroxytryptophan

LSD lysergic acid

MAOIs monoamine oxidase inhibitors

MHPG 3-methoxy-4-hydroxyphenylglycol

MRS magnetic resonance spectroscopy

NE norepinephrine

NMDA N-methyl-D-aspartate

OCD obsessive compulsive disorder

8-OH-DPAT 8-hydroxy-2-(di-n-propylamino)tetraline

pCPA parachlorphenylalanine

PET positron emission tomography

PVN paraventricular nucleus

SCID Structure Clinical Interview for DSM-III-R Diagnosis, Patient Version

SCID-NP Structure Clinical Interview for DSM-III-R Diagnosis, Non-Patient Version

SD standard deviation

SEM standard error mean

SPSS Statistical Package for Social Sciences

SSRIs selective serotonin reuptake inhibitors

TCAs tricyclic antidepressants

VAS visual analog scale

Vd volume of distribution

YMRS Young Mania Rating Scale

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DEDICATION

This thesis is dedicated to my wife, Jasmine, and my children, Charlene and Charles,

for their love and support.

FORWARD

Portions of this thesis have been previously published or accepted for publication as follows:

1. Shiah, I.S., L.N. Yatham, R.W. Lam, and A.P. Zis. 1997. Effects of divalproex sodium on 5-HT$_{1A}$ receptor function in healthy human males: hypothermic, hormonal, and behavioral responses to ipsapirone. *Neuropsychopharmacology* 17:382-390.

2. Shiah, I.S., L.N. Yatham, R.W. Lam, and A.P. Zis. 1998. Divalproex sodium attenuates growth hormone response to baclofen in healthy human males. *Neuropsychopharmacology* 18:370-376.

3. Shiah, I.S., and L.N. Yatham. 1998. GABA function in mood disorders: an update and critical review. *Life Sci.* 63:1289-1303.

4. Yatham, L.N., I.S. Shiah, R.W. Lam, E.M. Tam, and A.P. Zis. 1999. Hypothermic, ACTH and cortisol responses to ipsapirone in patients with mania and healthy controls. *J. Affect. Disord.* 54; 295-301.

5. Shiah, I.S., L.N. Yatham, R.W. Lam, E.M. Tam, and A.P. Zis. 1999. Growth hormone response to baclofen in patients with mania: a pilot study. *Psychopharmacology* In Press.

6. Shiah, I.S., and L.N. Yatham. 1999. Serotonin in mania and in the mechanism of action of mood stabilizers: a review of clinical studies. *Bipolar Disord.* In Press.

For all portions of these papers that are reported in this thesis, I-Shin Shiah was the major contributor involved in proposing and conducting the research, analyzing the data and writing the papers.

CHAPTER I   INTRODUCTION, LITERATURE REVIEW AND THESIS OBJECTIVES

Bipolar disorder (commonly known as manic depressive illness) is a serious psychiatric disorder that affects 1% of the population (Nyberg et al. 1996). Patients with this disorder have both manic as well as major depressive episodes. Major depressive episodes are characterized by depressed, irritable or anxious mood, poor appetite and weight loss (or the opposite), sleep disturbance, excessive fatigue or tiredness, decreased activity level, loss of interest or pleasure in usual activities, decreased sexual drive, diminished ability to think or concentrate, feelings of worthlessness, or excessive guilt, and recurrent thoughts of death or self-harm (American Psychiatric Association 1994). In contrast, manic episodes are characterized by an elevated or irritable mood, overactivity, pressured speech, a decreased need for sleep, racing thoughts, distractibility, flight of ideas, an inflated self esteem sometimes amounting to grandiose delusion, and extravagant, disinhibited behavior (American Psychiatric Association 1994). The pathophysiology of bipolar disorder is not known. Although efforts have been made to investigate an association between this disorder and central neurotransmitter abnormalities over the past forty years, the neurochemical disturbances that underlie this illness remain elusive (Manji and Potter 1997).

Alterations in several neurotransmitter systems such as dopamine (DA), norepinephrine (NE), serotonin (5-hydroxytryptamine, 5-HT) and γ-aminobutyric acid (GABA) have been implicated in the pathophysiology of this illness (Manji and Potter 1997). However, there is a dearth of research into the role of these neurotransmitters in mania, although considerable evidence has accumulated supporting their roles in depression. In this thesis, we focused on

the roles of 5-HT and GABA in the pathophysiology of mania and its treatment, primarily because medications such as lithium, carbamazepine, and divalproex sodium (DVP) have been suggested to enhance either serotonergic or GABAergic transmission (Waldmeier 1987).

A.   Serotonin Function in Mania

Serotonin is an important neurotransmitter within the central nervous system (CNS). To date, based on pharmacological or molecular properties, at least 14 types of 5-HT receptors have been identified in the mammalian brain (Hoyer and Martin 1996; Hoyer and Martin 1997). They are 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}; 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}; 5-HT_3, 5-HT_4; 5-HT_{5A}, 5-HT_{5B}, 5-HT_6, and 5-HT_7 receptors. Except for the 5-HT_3 receptors, which are ligand-gated ion channels, all 5-HT receptor subtypes belong to the superfamily of G-protein-coupled receptors (Cooper, Bloom, and Roth 1996; Kroeze and Roth 1998). This indoleamine transmitter is involved in a variety of physiological and behavioral responses (Jacobs and Fornal 1995; Smith and Cowen 1997). Therefore, it is not surprising that disturbance in 5-HT function has been implicated in the pathophysiology of many psychiatric disorders including mood disorders. 5-HT was implicated in the pathophysiology of manic depressive illness by Strom-Oslen and Weil-Malherbe in as early as 1958. However, Prange et al. (1974) were the first to formulate the permissive hypothesis of 5-HT function for bipolar disorder. They postulated that a deficit in central serotonergic neurotransmission permits the expression of bipolar disorder; and that both the manic and the depressive phases of bipolar disorder are characterized by low central 5-HT function but differ in high versus

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low central catecholaminergic (i.e. NE and DA) neurotransmission. This hypothesis was primarily based on 1) the clinical observation that L-tryptophan, the precursor of 5-HT, was efficacious in the treatment of mania (Prange et al. 1974); 2) some earlier studies revealing decreased levels of 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (CSF) in both mania and depression (Coppen et al. 1972; Dencker et al. 1966; Mendels et al. 1972); and 3) animal studies showing that lithium enhanced brain 5-HT function (Knapp and Mandell 1973; Sheard and Aghajanian 1970; Tagliamonte et al. 1971). Although extensive evidence has accumulated since then to support a role for 5-HT in depression (Maes and Meltzer 1995; Malone and Mann 1993; Meltzer and Lowy 1987; Price et al. 1990; van Praag 1984, for review), relatively fewer studies examined its role in mania. This undoubtedly relates to the difficulty recruiting drug free manic patients for research studies.

Several approaches have been used to study 5-HT activity in mania: 1) CSF studies, 2) Postmortem studies, 3) Platelet studies, and 4) Neuroendocrine challenge studies.

1.   CSF Studies

CSF studies measured the levels of 5-HIAA, the major metabolite of 5-HT, with the assumption that 5-HIAA levels provide an index of central 5-HT activity (Moir et al. 1970; Williams et al. 1999). Although half the concentration of lumbar 5-HIAA comes from the spinal cord (Banki and Molnar 1981; Young and Gauthier 1981), the 5-HIAA levels in lumbar CSF may still reflect brain functioning since many of 5-HT axons and nerve terminals in cord originate in functionally important brain stem nuclei. Indeed it has been

shown that lumbar CSF 5-HIAA concentrations do correlate with brain 5-HIAA in humans (Stanley, Traskman-Bendz, and Dorovini-Zis 1985).

Studies of CSF 5-HIAA in manic patients have produced variable and inconsistent results. For example, baseline CSF 5-HIAA levels in manic patients compared to "non-depressed" controls have been reported as decreased in four studies (Banki 1977; Coppen et al. 1972; Dencker et al. 1966; Mendels et al. 1972), unchanged in nine studies (Aschroft et al. 1976; Ashcroft et al. 1966; Ashcroft and Glen 1974; Bowers, Heninger, and Gerbode 1969; Gerner et al. 1984; Goodwin et al. 1973; Sjostrom and Roos 1972; Swann et al. 1983; Wilk et al. 1972), and increased in three studies (Koslow et al. 1983; Ross and Sjostrom 1969; Vestergaard et al. 1978). However, when baseline CSF 5-HIAA levels were compared between manic and depressed patients, the results of previous studies were much more consistent. With the exception of one study (Ashcroft et al. 1966), all other studies found that CSF 5-HIAA levels in manics were not different from depressed patients (Ashcroft and Glen 1974; Banki 1977; Bowers, Heninger, and Gerbode 1969; Coppen et al. 1972; Gerner et al. 1984; Goodwin et al. 1973; Koslow et al. 1983; Mendels et al. 1972; Ross and Sjostrom 1969; Sjostrom and Roos 1972; Swann et al. 1994; Wilk et al. 1972).

Administration of probenecid blocks the active transport of 5-HIAA out of the CSF, leading to accumulation of 5-HIAA levels in CSF (Guldberg, Ashcroft, and Crawford 1966; Olsson and Roos 1968). This provides a more dynamic measure of central serotonergic activity than basal level of CSF 5-HIAA (Mendels and Frazer 1975; Post and Goodwin 1973; van Praag, Korf, and Puite 1970). Of the four studies that examined CSF 5-HIAA accumulation

following administration of probenecid in manics, depressives, and controls (Bowers, Heninger, and Gerbode 1969; Goodwin et al. 1973; Ross and Sjostrom 1969; Sjostrom 1973), two reported that both manic and depressed patients have diminished CSF 5-HIAA formation compared to controls (Ross and Sjostrom 1969; Sjostrom 1973) and one reported that manic patients have significantly lower CSF 5-HIAA accumulation than depressives and controls (Bowers, Heninger, and Gerbode 1969). Furthermore, Goodwin et al. (1973) found that manic and depressed patients have similar CSF 5-HIAA levels 18 hours after administration of probenecid. Although there was no control group data available for their 18 hours study, comparison with values obtained in the controls over a 9 hour period suggested that the rate of accumulation of 5-HIAA in both the manic and depressed patients may have been reduced (Mendels and Frazer 1975). Taken together, the results of the probenecid-induced accumulation studies appeared to support that both mania and depression are associated with a reduction in central 5-HT function.

2.   Postmortem Studies

The only study that assessed 5-HT and 5-HIAA levels in postmortem brains of subjects with a well-documented history of bipolar disorder reported significantly lower 5-HIAA levels and lower 5-HT/5-HIAA ratios in frontal and parietal cortex compared to postmortem brains from controls (Young et al. 1994). The results of this study therefore provide a support for regional decreases in central 5-HT activity.

3.   Platelet Studies

Platelets share many properties with central 5-HT neurons such as uptake, storage and release of 5-HT, 5-HT receptors and imipramine binding sites (Stahl 1977; Stahl 1985). They are easily accessible for study, and therefore have been used as a peripheral model for 5-HT neurons to study 5-HT function in psychiatric patients.

a.   Platelet 5-HT uptake

Meltzer et al. (1981) examined 5-HT uptake in 14 manic patients in comparison to 20 healthy controls. They noted a tendency for a decrease in 5-HT uptake in 4 manic patients and an increase in 7 manic patients, but manic patients as a group did not differ significantly from healthy controls. Similarly, Scott et al. (1979) reported no difference in 5-HT uptake in 8 manic patients compared to 26 healthy controls. Meagher et al. (1990) reported increased 5-HT uptake in 15 manic patients compared to 19 healthy controls. However, manic patients as a group had a large variation in their 5-HT uptake compared to control group in this study that could very well be due to the effects of medication. Indeed, when 5 drug free manic patients in this study were compared with controls, the 5-HT uptake was not different between the two groups. Marazziti et al. (1991) on the other hand, reported decreased 5-HT uptake in 7 manics compared to 12 healthy controls. Of the 7 manics, only 3 were drug free which confounds the interpretation of results.

b.   Platelet imipramine binding site

The imipramine binding site that is present in both 5-HT neurons and platelets appears to be closely linked to the 5-HT active transport site. The binding affinity of this site to imipramine in platelets has been examined to gain information about 5-HT neuron function in manic patients. Of the four studies that examined imipramine binding in manic patients, two showed an increase (Ellis et al. 1991; Lewis and McChesney 1985) while the other two showed no difference (Marazziti, Lenzi, and Cassano 1991; Muscettola, Di Lauro, and Giannini 1986) in binding in manics compared to depressed patients. However all four studies (Ellis et al. 1991; Lewis and McChesney 1985; Marazziti, Lenzi, and Cassano 1991; Muscettola, Di Lauro, and Giannini 1986) reported that binding was not different in manics when compared to healthy controls.

Taken together, the results of platelet studies that assessed 5-HT uptake or tritiated imipramine binding did not show any consistent differences between manic patients and controls. The discrepancy in findings between studies could be due to methodological differences or problems such as patient heterogeneity, confounding effects of psychotropic medication, and sample sizes.

c.   Other platelet 5-HT measures

In addition to platelet 5-HT uptake and $[^3H]$-imipramine binding, 5-HT-induced platelet calcium mobilization, a putative measure of $5-HT_2$-receptor sensitivity (Kagaya et al. 1990; Kusumi, Koyama, and Yamashita 1991), has also been examined in patients with mania.

Okamoto et al. (1994) reported that there is an increase in the rapid peak and prolonged plateau phase in platelet intracellular Ca^{2+} mobilization in 10 untreated acute manic patients compared to 14 matched healthy subjects and 10 euthymic bipolar patients treated mainly with lithium. As for the rapid peak, a similar result was also obtained in platelets of patients with bipolar depression (Dubovsky et al. 1992). This may suggest an increase in sensitivity of 5-HT$_2$ receptors in both mania and bipolar depression. In support of this hypothesis, a recent ex vivo study (Berk et al. 1995) showed that administration of 5-HT led to a significant elevation in platelet intracellular calcium levels in patients in both the manic and depressive phases of bipolar disorder, compared to normal controls and bipolar euthymic patients. In contrast, a recent study, using [^{125}I]-ketanserin as the radioligand, reported that platelet 5-HT$_2$ receptor binding sites in 29 drug-free male manic patients were not different from those in 29 male healthy controls (Velayudhan et al. 1999). Furthermore, another probe for assessing 5-HT$_2$-receptor sensitivity, the adenosine diphosphate (ADP) augmented 5-HT induced platelet aggregation response, was reported to be significantly lower in both manic and depressed patients compared to normal controls and it was reversed following clinical recovery (McAdams and Leonard 1992). The discrepancy between the studies that examined platelet 5-HT$_2$ receptor-mediated activity could be due to the differences in patient populations and/or to the nature of the means of measuring platelet 5-HT$_2$-receptor activity.

4.   Neuroendocrine Challenge Studies

Neuroendocrine challenge paradigm offers a useful means of assessing central (hypothalamic) 5-HT function. This approach is based on the observation that 5-HT exhibits an excitatory

influence on the release of cortisol, adrenocorticotropin hormone (ACTH), prolactin and possibly growth hormone (GH) (Tuomisto and Mannisto 1985; Yatham and Steiner 1994, for review) and the assumption that the extent of release of hormones following a challenge with 5-HT agonist provides an index of the responsivity of 5-HT system (Yatham and Steiner 1993). Depending upon the probe employed, it is possible to assess the "net" central 5-HT activity or the sensitivity of various 5-HT receptor subtypes (Yatham and Steiner 1993).

This paradigm has been used extensively to assess 5-HT function in depression (Cowen 1998; Delgado and Charney 1991; Stahl 1992, for review) and other psychiatric disorders (Coccaro and Murphy 1990; Newman, Shapira, and Lerer 1998; Sandler, Coppen, and Harnett 1991, for review). There are a total of five neuroendocrine challenge studies to date that examined the role of 5-HT in mania by using different serotonergic probes. Studies that examined prolactin responses to serotonergic probes in manic patients have yielded conflicting results. Yatham (1994; 1996) reported no significant differences in prolactin responses to buspirone or d,l-fenfluramine between manic patients and matched healthy controls. In contrast, Thakore et al. (1996) demonstrated blunted prolactin response to d-fenfluramine, a more selective 5-HT probe, in manic patients in comparison to healthy subjects. The discrepancies in the prolactin responses between studies are likely to be due to difference in drug selectivity. It has been suggested that buspirone and d,l-fenfluramine possess additional DA effects whereas d-fenfluramine has more specific 5-HT effects with stimulation of 5-HT release and inhibition of its reuptake (van Praag, Lemus, and Kahn 1986). Since d-fenfluramine induced prolactin release is considered to reflect "net" 5-HT activity (Quattrone et al. 1983), the finding of Thakore et al. (1996) would suggest that "net"

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5-HT activity is decreased in mania, presumably due to low 5-HT availability in central 5-HT synapses. This would be expected to lead to enhanced postsynaptic 5-HT receptor sensitivity (Meltzer et al. 1984b).

In keeping with this hypothesis, two studies showed that cortisol response to 5-hydroxytryptophan (5-HTP) (Meltzer et al. 1984b) or d,l-fenfluramine (Yatham 1996), was enhanced in manic patients in comparison to healthy controls, although the difference in cortisol response between the two groups did not reach significance in the latter study.

In addition to prolactin and cortisol responses, GH release to sumatriptan, a putative 5-HT$_{1D}$ receptor agonist, has also been investigated in manic patients recently (Yatham et al. 1997b). This study did not find any significant difference in the GH response to sumatriptan between manic patients and normal controls. This might suggest that 5-HT$_{1D}$ receptor sensitivity be not altered in mania. However, since sumatriptan penetrates the CNS to only a limited extent (Dallas 1989), future studies using selective 5-HT$_{1D}$ probes with better blood-brain barrier permeability are warranted to explore further the role of 5-HT$_{1D}$ receptors in mania.

B.　5-HT in the Mechanism of Action of Mood Stabilizers

Effects of Lithium on 5-HT Function

There have been several excellent review articles indicating that lithium's antidepressant effects are related to its enhancement of 5-HT function in the CNS (Bunney and Garland-

Bunney 1987; Cowen et al. 1990; Lenox and Manji 1995; Meltzer and Lowy 1987; Price et al. 1990; Wood and Goodwin 1987). Therefore, we did not include those studies involving depressed patients, and only focused on the studies that examined lithium's effect on 5-HT activity in manics, healthy controls or euthymic bipolar patients. Four major paradigms have been utilized: 1) CSF studies, 2) Platelet studies, 3) Neuroendocrine challenge studies, and 4) Tryptophan depletion studies.

1.   CSF Studies

Mendels (1971) first reported that lithium significantly increased CSF 5-HIAA levels in two manic patients, and suggested that lithium's antimanic effect may be due to its enhancing effect on 5-HT neurotransmission. In a subsequent study, Wilk et al. (1972) also showed that successful treatment with lithium in two manic patients led to a sharp increase in CSF 5-HIAA levels. The two initial studies are limited by a small sample size. Likewise, other three earlier studies (Bowers, Heninger, and Gerbode 1969; Mendels et al. 1972; Sjostrom and Roos 1972) with negative findings are also difficult to interpret because of small sample sizes or their methodology and study design. The study of Bowers et al. (1969) included only 4 manic patients. Mendels et al. (1972) examined changes in CSF 5-HIAA levels from the perspective of clinical state rather than drug effects. Sjostrom and Ross (1972) failed to utilize a within-subject design and many of their patients received neuroleptics.

However, larger studies have generally shown that lithium increases CSF 5-HIAA levels in manic patients (Price et al. 1990). Fyro et al. (1975) reported a significant increase in CSF 5-

HIAA levels in 13 manic or hypomanic patients after 12 days treatment with lithium compared to before treatment. Bowers and Heninger (1977) reported a similar effect after a mean 27 days of lithium in 23 mixed affective disorder patients. Berrettini et al. (1985b) showed that 8 lithium-treated euthymic bipolar patients had significantly higher CSF 5-HIAA levels than they did drug-free after at least 2 weeks of lithium discontinuation. Swann et al. (1987) found that ten manic patients had higher CSF 5-HIAA levels after 18 days of lithium than before treatment, but the difference did not reach significance.

2.   Platelet/Blood Studies

a.   Platelet 5-HT uptake

There is conflicting evidence with regard to the effects of short-term lithium therapy on platelet 5-HT uptake. For example, Meltzer et al. (1983) reported that lithium treatment for 2-3 weeks led to a significant decrease in platelet 5-HT uptake in 14 drug-free manic patients. Poirier et al. (1988) also showed a significant decrease in platelet 5-HT uptake in 7 healthy subjects after 20 days of lithium administration. However, Murphy et al. (1969) reported that 1 week of lithium treatment led to a significant increase in platelet 5-HT uptake in 9 mixed affective disorder patients after. Scott et al. (1979) reported no significant change in platelet 5-HT uptake after lithium treatment for 5 days or 3 weeks in 7 healthy subjects, or for 5 days, 3 weeks, or 3 months in a small group of mixed affective patients. In contrast, studies of the effect of long-term lithium treatment on platelet 5-HT uptake have shown more consistent results. Meltzer et al. (1983) measured platelet 5-HT uptake in 7 manic patients

before and after at least 12 months of lithium treatment, and found that lithium treatment of at least 1-year duration was associated with a significant increase in the number of platelet uptake sites. Likewise, Born et al. (1980) reported that bipolar patients treated with lithium for at least 3 months had significantly greater platelet 5-HT uptake compared to those not on lithium or matched healthy controls. Coppen et al. (1980) showed that euthymic bipolar patients receiving lithium for 6 weeks or for 1 year had greater platelet 5-HT uptake compared to remitted drug free depressed patients. In keeping with these findings, Goodnick et al. (1984) observed that discontinuation of chronic lithium treatment for a mean of 4.4 years in 11 euthymic bipolar patients led to a decrease in platelet 5-HT uptake compared to on-lithium baseline.

b.   Platelet imipramine binding site

With regard to the effect of lithium on platelet $[^3H]$-imipramine binding, Baron et al. (1986) found a decrease in Bmax in platelet $[^3H]$-imipramine binding in 33 euthymic bipolar patients chronically treated with lithium for periods ranging from 3 to 15 years, compared to normal controls. They suggested that the density of $[^3H]$-imipramine binding sites in platelets is a trait marker for bipolar illness. In keeping with this, Wood et al. (1983) reported a decrease in platelet $[^3H]$-imipramine binding in 7 drug-free euthymic bipolar patients compared to 17 healthy controls; however, this was not the case in 16 lithium-treated euthymic bipolar patients. Moreover, two other studies also did not find any significant difference in Bmax $[^3H]$-imipramine binding between drug-free euthymic bipolar patients and normal controls (Berrettini et al. 1982; Mellerup, Plenge, and Rosenberg 1982).

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Similarly, Goodnick et al. (1984) reported that platelet [^{3}H]-imipramine binding in 7 euthymic lithium-treated bipolar patients was not altered by lithium discontinuation. Glue et al. (1986) reported that 20 days of lithium treatment had no significant effect on platelet imipramine binding in 8 normal healthy subjects. Poirier et al. (1988) also showed no significant change in platelet [^{3}H]-imipramine binding in 7 healthy subjects after 20 days of lithium administration. In summary, lithium treatment does not appear to alter the platelet imipramine binding site. To our knowledge, there is no study that measured platelet imipramine binding in drug-free acute manic patients before and after monotherapy with lithium.

c.   Other platelet/blood 5-HT measures

Several other platelet/blood 5-HT activities have also been used to examine the role of 5-HT in the mechanism of action of lithium. Wood et al. (1985) reported a significant increase in 5-HT-induced platelet aggregation response in 24 patients who had been treated with long-term lithium prophylaxis for a mean of 5.5 years compared to 22 drug-free depressed patients and 23 normal controls. Based on their finding, the authors suggest a supersensitivity in 5-HT$_2$ receptor function following lithium treatment. However, another two putative 5-HT$_2$ receptor measures, platelet binding sites for lysergic acid (LSD) and 5-HT-induced calcium mobilization in platelets, were not altered following lithium treatment (Glue et al. 1986; Kusumi, Koyama, and Yamashita 1994). Glue et al. (1986) reported that 20 days of lithium treatment had no significant effect on platelet LSD binding sites in 8 healthy subjects. Kusumi et al. (1994) reported no significant effect of 4 weeks of lithium administration (600

mg / day) on in 5-HT-induced calcium mobilization in the platelets of 7 healthy subjects. Additionally, two studies reported no significant effect of lithium treatment on platelet 5-HT content in healthy subjects (Glue et al. 1986; Poirier et al. 1988). Likewise, Artigas et al. (1989) reported no significant difference in whole blood 5-HT or plasma total tryptophan level between 22 patients treated chronically with lithium, 14 healthy controls and 11 patients treated chronically with antipsychotic drugs. However, they found that plasma 5-HIAA and plasma free 5-HT were significantly increased in lithium-treated patients compared to the other two groups (1989). Their findings therefore provide some support to the enhancing effect of lithium on 5-HT in the periphery.

3.   Neuroendocrine Challenge Studies

Many neuroendocrine challenge studies have examined the effects of lithium on 5-HT function in depressed patients (Cowen et al. 1989; Cowen et al. 1991; McCance-Katz et al. 1992; Meltzer et al. 1984a; Price et al. 1989; Shapira et al. 1992) or healthy controls (Cowen et al. 1990; Glue et al. 1986; Manji et al. 1991; McCance, Cohen, and Cowen 1989; Power, Dorkins, and Cowen 1993; Walsh, Ware, and Cowen 1991). Some (Cowen et al. 1990; Cowen et al. 1989; Cowen et al. 1991; Glue et al. 1986; McCance, Cohen, and Cowen 1989; Meltzer et al. 1984a; Price et al. 1990) but not all (Manji et al. 1991; Power, Dorkins, and Cowen 1993; Shapira et al. 1992; Walsh, Ware, and Cowen 1991) studies showed enhancement of 5-HT-mediated hormonal response following lithium treatment. However, there are only two studies that examined 5-HT function in manic patients before and after lithium treatment. Meltzer et al. (1984a) reported that a mean of 23.8 days of lithium

treatment alone led to an increase in cortisol response to 5-HTP in 7 manic patients, supporting the hypothesis that increasing serotonergic activity may have an antimanic effect. Yatham (1994) measured prolactin response to buspirone in 6 drug-free manic patients before and after 3 weeks of lithium treatment alone and found no treatment effect of lithium on the 5-HT$_{1A}$ receptor-mediated prolactin response. Similarly, Walsh et al. (1991) also showed no significant effect of lithium administration for 2 weeks on the hormonal responses to gepirone, a more selective 5-HT$_{1A}$ receptor agonist, in 10 healthy subjects. In the two studies that measured prolactin (Muhlbauer 1984) or cortisol response (Muhlbauer and Muller-Oerlinghausen 1985) to fenfluramine in euthymic bipolar patients receiving lithium, cortisol response was enhanced while prolactin release was unaltered compared to controls.

In summary, lithium appears to enhance the cortisol response to 5-HT precursor or 5-HT releaser, but does not seem to affect the prolactin response to 5-HT releaser or 5-HT$_{1A}$ receptor agonists in bipolar patients.

4.   Tryptophan Depletion Studies

Administration of an oral tryptophan free mixture containing large amounts of other amino acids induces hepatic protein synthesis and leads to rapid depletion of plasma tryptophan by nearly 80% within 5 hours (Gessa et al. 1974; Young et al. 1989; Young and Gauthier 1981; Young et al. 1985). This degree of tryptophan depletion reduces brain 5-HT to approximately 20% of normal levels in animals (Moja et al. 1989). In humans, the tryptophan depletion technique leads to lower CSF tryptophan levels (Carpenter et al. 1998) and a significant

reduction in 5-HT synthesis (Nishizawa et al. 1998). This paradigm has been used widely to study behavioral effects of depletion of 5-HT synthesis in various psychiatric disorders including major depression (Delgado et al. 1990; Delgado et al. 1998; Delgado et al. 1991; Reilly, McTavish, and Young 1997, for review).

This paradigm has also been used to study the effects of lithium on 5-HT function in euthymic bipolar patients. Benkelfat et al. (1995) reported that a total of 10 lithium-treated manic patients that had been stable and euthymic for more than a year did not relapse following the ingestion of tryptophan depletion mixture. In contrast, Cappiello et al. (1997) have shown that 2 out of 7 manic patients recently treated with lithium and had been in remission for less than a month had a symptomatic relapse following amino acid drink. This may suggest that maintenance of clinical improvement of manic symptoms in some recently lithium remitted but not in long term stable bipolar patients depends on the short-term availability of the tryptophan in brain. However, Cassidy et al. (1998) were unable to replicate such a finding in 4 recently manic patients successfully treated with lithium. Further tryptophan depletion studies with a larger number of patients are warranted to understand the role of 5-HT function in the antimanic effects of lithium in bipolar patients.

Effects of Anticonvulsants on 5-HT Function

In addition to lithium, anticonvulsants such as DVP (Calabrese, Woyshville, and Rapport 1994; McElroy et al. 1992, for review) and carbamazepine (Martin, Joffe, and Bebchuk 1994, for review) have been shown to be effective in the treatment and prophylaxis of mania. More

recently, several new anticonvulsants such as lamotrigine, gabapentin, and topiramate have also been reported to have some efficacy in manic patients (Post et al. 1998, for review). Since a 5-HT deficit is implicated in mania, and since lithium's antimanic effect may be related to its enhancing effect on 5-HT neurotransmission, one may expect that the efficacy of these anticonvulsants in manic illness may also be associated with their effects on 5-HT neurotransmission. Indeed, there has been some evidence from clinical studies to support this.

1.   Divalproex Sodium

Maes et al. (1997) measured plasma cortisol response to L-5-hydroxytryptophan (L-5-HTP), a 5-HT precursor, in 10 drug-free manic patients before and after valproate treatment for 3 weeks. They found that administration of L-5-HTP produced an increase in cortisol responses both before and after valproate treatment; however, the L-5-HTP induced cortisol response was significantly higher after treatment with valproate than before treatment. Their findings suggest that chronic treatment with valproate may enhance central 5-HT function in manic patients and appear to be consistent with the hypothesis that increasing 5-HT function plays a role in the antimanic effects of valproate. In addition, another study has also shown that valproate treatment leads to an increase in central 5-HT activity in humans. Fahn (1978) reported that treatment with valproate increased CSF level of 5-HIAA in a patient with post-anoxic intentional myoclonus. In contrast to the positive results of the above studies, Kusumi et al. (1994) reported no in vitro effect of DVP (100 μM) on basal calcium or 5-HT-induced intracellular calcium mobilization in the platelets of 7 healthy subjects.

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2.   Carbamazepine

To our knowledge, there is no study to date that examined the effects of carbamazepine on 5-HT activity in manic patients. However, some human studies have shown evidence for an increase in 5-HT function during carbamazepine treatment. For example, Elphick et al. (1990) studied plasma prolactin response to intravenous administration of tryptophan in 7 healthy human males before and after 10-day course of carbamazepine. They found that after the carbamazepine treatment, the prolactin response to tryptophan was significantly enhanced compared to before treatment. Moreover, carbamazepine treatment was reported to increase plasma total and free tryptophan in epileptic patients (Pratt et al. 1984), which could lead to an increase in brain 5-HT function (Fernstrom 1983). In contrast, Post et al. (1984) found no significant effect of carbamazepine on CSF levels of 5-HIAA in affectively ill patients. Kusumi et al. (1994) reported no in vitro effect of carbamazepine (10 μM) for 1 hour or 4 hours on basal calcium or 5-HT-induced intracellular calcium mobilization in the platelets of 7 healthy subjects. More recently, Mannel et al. (1997) administered d,l-fenfluramine challenge tests to 30 mixed affective disorder patients after a mean period of 9.2 months of prophylactic treatment with either lithium or carbamazepine. Of the 30 patients, 15 were treated with lithium and the other 15 received carbamazepine. The authors found that the cortisol response to d,l-fenfluarmine was significantly increased in lithium-treated patients compared to those receiving carbamazepine, whereas there was no significant difference in the prolactin response to d,l-fenfluramine between the two groups. Their findings are in keeping with the enhancing effect of lithium but not carbamazepine on 5-HT function.

However, the interpretation of their data was limited by a lack of placebo control, heterogeneity of diagnostic groups, and some patients taking neuroleptics within 72 hours prior to d,l-fenfluramine challenge testing.

3.   New Anticonvulsants: Lamotrigine, Gabapentin, Topiramate

Lamotrigine is a new anticonvulsant, which has been approved for use as an adjunct drug in treatment of refractory partial seizure with or without generalized tonic/clonic seizures (Messenheimer 1995). As reviewed by Calabrese et al. (1998), a series of clinical reports involving bipolar patients suggest that this medication is perhaps a mood stabilizer like lithium with antimanic and antidepressant properties. Although evidence suggests that the anti-epileptic action of lamotrigine may be related to its inhibition of voltage-sensitive sodium channel and suppression of subsequent release of glutamate (Gilman 1995), the mechanism of action underlying its efficacy in mood disorders remains unknown. To date, two studies examined the effects of lamotrigine on 5-HT function in humans. Southam et al. (1998) reported that lamotrigine treatment led to a concentration-dependent inhibition of 5-HT uptake in both human platelets and rat brain synaptosomes, raising the possibility that an alteration in 5-HT function may contribute to the efficacy of lamotrigine in bipolar illness. In contrast, another neuroendocrine challenge study did not find any significant effect of 1-week lamotrigine administration (100 mg / day) on 5-HT_{1A} receptor-mediated hypothermic and cortisol responses in ten healthy human males (Shiah et al. 1998c). More studies are clearly warranted to further explore the role of 5-HT function in the mechanism of action of lamotrigine.

Another new anticonvulsant that showed efficacy in the treatment of mood disorders is gabapentin (Erfurth et al. 1998; Knoll, Stegman, and Suppes 1998; McElroy et al. 1997; Ryback, Brodsky, and Munasifi 1997; Schaffer and Schaffer 1997; Young et al. 1997). Several mechanisms have been proposed to explain its pharmacology such as 1) competing with the amino acids leucine, isoleucine, valine and phenylalanine for transport; 2) increasing the concentration and the rate of synthesis of GABA in brain; 3) interacting with an auxiliary subunit of voltage-gated Ca^{2+} channels; 4) reducing the release of several monoamine neurotransmitters; and 5) inhibiting voltage-activated Na^+ channels (Taylor et al. 1998). Nevertheless, two clinical studies demonstrated that gabapentin significantly increased whole blood 5-HT level and CSF 5-HIAA level in healthy human subjects and epileptic patients respectively, suggesting that gabapentin may alter 5-HT activity in humans (Ben-Menachem, Persson, and Hedner 1992; Rao et al. 1988). To our knowledge, there is no other study that examined the effects of gabapentin on 5-HT function in patients with mood disorders or healthy humans.

The third novel anticonvulsant that has been suggested to have some benefit in the treatment of bipolar illness is topiramate (Marcotte 1998). Evidence from preclinical and clinical studies suggests that the antiepileptic mechanisms of topiramate may involve 1) blockade of voltage-sensitive sodium channels, 2) an enhancement of GABA activity, and 3) antagonism of kainate to activate the kainate/AMPA subtype of glutamate receptors (Perucca 1997; Rosenfeld 1997). Whether the above pharmacological properties contribute to the possible effect of topiramate in the treatment of mood disorders and whether topiramate also affects

central 5-HT neurotransmission remain unknown. More studies are needed to clarify these issues.

C. GABA Function in Mania

GABA is the most important inhibitory neurotransmitter of the brain. Up to 30-50% of all central synapses are GABAergic (Paredes and Agmo 1992). Noradrenergic, dopaminergic and serotonergic neurons are all under an inhibitory control of GABA (Haefely 1992). This neurotransmitter acts on two main receptors in the brain, $GABA_A$ and $GABA_B$ (Hill and Bowery 1981; Matsumoto 1989). Alterations in GABAergic activity and $GABA_A$ or $GABA_B$ receptor function may modify a wide variety of behavioral responses such as eating, sleep, sexual behavior, learning and memory (Paredes and Agmo 1992). It is plausible, therefore, that alteration in central GABAergic function may be involved in the pathophysiology of mood disorders. A role of GABA in bipolar disorder was first postulated by Emrich et al. (1980), based on the clinical observation that valproate, a GABA agonist, was effective in treatment for patients with bipolar disorder. They postulated that patients with this disorder have a GABA deficit. Since then, there is considerable evidence in the literature to support the hypothesis of low GABA function in depression (Shiah and Yatham 1998, for review). However, very few studies examined GABA function in mania.

To date, there are only four studies that examined GABA activity in manic patients by measuring CSF or plasma GABA concentrations. Gerner and colleagues (Gerner et al. 1984; Gerner and Hare 1981) reported that CSF GABA levels in manic patients were lower, but not

significantly, compared to those in normal controls. Similarly, Post et al. (1980) also showed lower (not significantly) CSF GABA levels in 8 manic patients compared to 41 normal controls. In contrast, Petty et al. (1993) showed that plasma GABA levels were significantly lower in both manic and depressed phases of bipolar patients when compared to healthy controls. Since plasma GABA levels have been suggested to reflect brain GABA activity (Petty 1994), their results suggest that low plasma GABA levels may be a shared biological marker in both depression and mania, and also implicate a low GABA function in mania.

D.   GABA in the Mechanism of Action of Mood Stabilizers

Mood stabilizers such as lithium, carbamazepine, and DVP have been shown to be effective in treatment of depression and mania (Kusumakar et al. 1997; Yatham et al. 1997a). However, the exact mechanisms of action underlying their therapeutic effect in mood disorders remain unclear. Given that low GABA function is involved in depression and possibly associated with mania, it is possible that the efficacy of these agents in mood disorders may be related to their capacity to restore low GABA function. There is some evidence from clinical studies to support this.

1.   CSF and Plasma Studies

Berrettini et al. (1983; 1986) reported that previously low levels of CSF GABA were normalized in bipolar patients being treated with lithium. In a recent paper, Petty et al. (1996), however, did not find any significant changes in plasma GABA levels in 13 bipolar

manic patients between before and after 3-week treatment with lithium. Similarly, Post et al. (1984) reported that carbamazepine did not significantly alter CSF GABA concentrations in affectively ill patients.

Studies of the effect of valproate on CSF and plasma GABA levels in humans are controversial. Zimmer et al. (1980) found that continued valproate treatment induced a significant increase by 70% in CSF GABA levels of six patients suffering from schizophrenia. In contrast, Lautin et al. (1980) found no differences in CSF GABA levels of six schizophrenic patients before and after treatment with valproate. Loscher and Siemes (1985) determined the CSF GABA levels in 31 children with epilepsy and 41 age-matched controls. They found that epileptic children treated with valproate had significantly higher CSF GABA levels than those patients without treatment and normal controls. Similarly, plasma GABA levels were significantly increased after treatment with valproate both for 7-42 days in 19 epileptic patients (Loscher and Schmidt 1981) and for 4 days in healthy subjects (Loscher and Schmidt 1980). It is of interest, however, to note that in a recent report (Petty et al. 1996), plasma GABA levels were significantly decreased rather than increased after 3 weeks of treatment with DVP in manic patients, and that higher pretreatment plasma GABA levels were significantly related to a better treatment response to DVP. The discrepant findings in treatment effect of valproate on CSF and plasma GABA levels between studies may be due to the differences in patient selection, assay methodology, or a combination.

2.   Positron Emission Tomography Studies

Recently, a positron emission tomography (PET) study, using $[^{11}C]$-flumazenil as a radioligand, assessed the role of GABA$_A$ receptors in the mechanism of action of valproate in patients with childhood and juvenile absence epilepsy (Prevett et al. 1995). The authors found that the regional cerebral volume of distribution (Vd) of $[^{11}C]$-flumazenil in patients not treated with valproate was not different from that in normal controls, whereas Vd was significantly lower in patients treated with valproate as compared with normal controls and patients not receiving valproate. Since Vd has been suggested to reflect benzodiazepine-GABA$_A$ receptor binding (Koeppe et al. 1991), their results may suggest that treatment with valproate down-regulated cerebral GABA$_A$ receptor binding. Such a down-regulation of GABA$_A$ receptor after valproate treatment presumably results from the persistent activating effect of valproate on GABA neurotransmission (Prevett et al. 1995). To our knowledge, there is no other neuroimaging study to date that examined the treatment effect of lithium, carbamazepine and valproate on GABA$_A$ receptors.

3.   Magnetic Resonance Spectroscopy (MRS) Studies

The technique of MRS has been used to measure GABA levels in brains of living humans (Mattson et al. 1994; Petroff et al. 1995). One recent study (Petroff et al. 1996) showed an increase in brain GABA levels in epileptic patients receiving gabapentin in a dose-dependent fashion. Although the mechanism underlying the efficacy of gabapentin in bipolar disorder remains unknown, the enhancing effect of gabapentin on GABA function may be related to its efficacy in treatment of bipolar disorder and this would be consistent with the GABA

hypothesis of bipolar disorder. This non-invasive technique should be employed to further investigate the role of GABA in mania and in the mechanism of action of mood stabilizers such as lithium, carbamazepine and DVP in future studies.

E.   Thesis Objectives

As reviewed above, there is some evidence in the literature suggesting that manic patients have low 5-HT and GABA function and that enhancement of 5-HT and GABA neurotransmission may underlie the mechanism of action of mood stabilizers. It would be of interest to determine if manic patients also have an abnormality in functioning of 5-HT and/or GABA receptor subtypes and functioning of these receptor subtypes is modified by effective mood stabilizers such as lithium and DVP. As mentioned earlier, 5-HT has at least 14 receptor subtypes ($5\text{-}HT_1$ to $5\text{-}HT_7$). Of these receptors, $5\text{-}HT_{1A}$ receptor subtype received the most attention in the study of neurobiology of mood disorders. Many neuroendocrine challenge studies have examined its role in depression. (Cowen et al. 1994; Lesch et al. 1990b; Meltzer and Maes 1994; Meltzer and Maes 1995; Moeller et al. 1994; Pitchot et al. 1995; Price et al. 1997; Rausch, Stahl, and Hauger 1990; Shiah et al. 1998a). In contrast, there is only one study to date that examined $5\text{-}HT_{1A}$ function in mania (Yatham 1994). In that study, prolactin response to buspirone was used to measure $5\text{-}HT_{1A}$ receptor function. Since buspirone has moderate affinity for DA-2 (D_2) receptors in addition to its $5\text{-}HT_{1A}$ receptor affinity (Peroutka 1985), it has been suggested that the prolactin response to buspirone might be due to D_2 receptor blockade (Meltzer, Lee, and Nash 1992). Hence, there is a clear need to examine $5\text{-}HT_{1A}$ receptor function in manic patients using more selective 5-

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HT$_{1A}$ receptor agonists such as ipsapirone. With regard to GABA receptors, there have been four neuroendocrine challenge studies that examined the role of GABA$_B$ receptors in depression (Davis et al. 1997; Marchesi et al. 1991; Monteleone et al. 1990a; O'Flynn and Dinan 1993). However, there is no study to date that examined GABA$_B$ function in manic patients.

DVP is an effective antimanic agent. It has a better side effect profile than lithium and carbamazepine, and is well tolerated by patients (Kusumakar et al. 1997). This medication exerts many biochemical and physiological effects (Loscher 1993, for review). For example, it has been shown to increase brain GABA concentration (Iadarola, Raines, and Gale 1979; Loscher 1981; 1982; Loscher and Vetter 1985; Simler et al. 1968), increase brain GABA release (Ekwuru and Cunningham 1990; Gram et al. 1988), inhibit the GABA degrading enzyme GABA transaminase (GABA-T) (Godin et al. 1969; Larsson et al. 1986; Loscher 1980; Maitre et al. 1978; Whittle and Turner 1978), increase the activity of the GABA synthesizing enzyme glutamate decarboxylase (GAD) (Loscher 1981; Phillips and Fowler 1982), increase brain GABA synthesis (Taberner, Charington, and Unwin 1980) and turnover (Loscher 1989), and enhance neuronal responses to GABA (Gent and Phillips 1980). It also increases brain 5-HT (Whitton and Fowler 1991) and 5-HIAA (Horton et al. 1977; Hwang and van Woert 1979) concentrations. It has been reported to increase brain concentrations of glycine (Martin-Gallard et al. 1985) and taurine (Patsalos and Lascelles 1981), and reduce brain aspartate level (Chapman et al. 1982) as well as inhibit its release (Crowder and Bradford 1987). The other effects of this medication include decreased dopamine turnover, decreased N-methyl-D-aspartate (NMDA)-mediated currents, direct neuronal effects (i.e.

reducing sodium influx and increasing potassium efflux) (Keck and McElroy 1998, for review). More recently, molecular biological studies showed that DVP, like lithium, has an effect on the intracellular signal transduction pathways such as guanine nucleotide-binding proteins (G proteins), protein kinase C isoenzymes, phosphoinositide system, and intracellular calcium (Dubovsky et al. 1992; Li et al. 1993; Manji et al. 1996; 1999; Post, Weiss, and Chuang 1992; Stoll and Severus 1996, for review). However, most of these findings were obtained from animal studies and it is unknown if DVP exerts similar effects on various neurotransmitter systems in humans. Therefore, the purposes of this thesis were to ascertain the roles of 5-HT$_{1A}$ and GABA$_B$ receptors in the pathophysiology of mania and in the mechanism of action of DVP. We used neuroendocrine challenge paradigm to accomplish this.

There are a total of four experiments included in this thesis. In the first experiment, we compared the hypothermic, hormonal and behavioral responses to the 5-HT$_{1A}$ receptor agonist ipsapirone between manic patients and matched normal controls. Then we examined the treatment effects of DVP on 5-HT$_{1A}$ receptor function by measuring the hypothermic, hormonal and behavioral responses in ten healthy subjects before and after one-week treatment with DVP. In the third study, we compared the GH responses to baclofen, a GABA$_B$ receptor agonist between patients with mania and matched healthy controls. Finally, we determined the treatment effect of DVP on the GABA$_B$ receptor function by measuring the GH response to baclofen in ten healthy subjects before and after one-week of DVP treatment. These four studies were performed separately over a period of three years. Manic

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patients and normal controls for each study were recruited independently, and each subject participated in only one experiment.

CHAPTER II   SEROTONIN-1A RECEPTOR FUNCTION IN MANIA

A.   Introduction

Serotonin (5-HT) neuroendocrine challenge tests are a valid and useful means of measuring 5-HT neurotransmission in human. This approach is based on the observation that 5-HT exhibits an excitatory influence on the release of hypothalamic-pituitary-adrenal (HPA) axis hormones, prolactin, and possibly GH, and the assumption that the extent of release of hormones to challenge with various 5-HT agonists provides an index of the responsivity of 5-HT system (Yatham and Steiner 1993). Depending on the challenge agents used, it is possible to assess the responsivity of central 5-HT_{1A}, 5-HT_2 receptors, or the overall functioning of 5-HT system (Yatham and Steiner 1993).

When given orally to humans, ipsapirone, a 5-HT_{1A} partial agonist, causes dose-dependent decrease in body temperature, and increase in ACTH and cortisol release, which are blocked by 5-HT_{1A} antagonists (Lesch et al. 1990d). This suggests that the hypothermia and ACTH and cortisol release induced by ipsapirone are mediated by 5-HT_{1A} receptors and that these responses provide a valid index of 5-HT_{1A} responsivity in humans (Yatham and Steiner 1993). Previous studies have used the hypothermic and hormonal responses to examine the 5-HT_{1A} receptor sensitivity in patients with unipolar (Lesch et al. 1990a; Meltzer and Maes 1995) and bipolar depressions (Shiah et al. 1998a), obsessive-compulsive disorder (Lesch et al. 1991), panic disorder (Lesch et al. 1992), and chronic fatigue syndrome (Dinan et al. 1997). To our knowledge, there is no study to date that examined the ipsapirone-induced

responses in patients with mania. We report here the results of an investigation on the effects of ipsapirone on the body temperature and ACTH and cortisol release in patients with mania compared to healthy volunteers.

B.   Methods

We studied a total of six manic patients (four males and two females, mean age 45.0 years, S.D.=14.1) and six matched healthy subjects (four males and two females, mean age 42.5 years, S.D.= 13.2). All study subjects were physically healthy and gave written informed consent for participation in the study. The DSM-IV diagnosis of bipolar disorder – current episode mania was made by the consensus of the research team using the information generated from a clinical interview and a structured clinical interview for DSM-III-R diagnosis (SCID) (Spitzer et al. 1990). Those that met criteria for other Axis I diagnosis, substance or alcohol abuse were excluded. The healthy subjects had no lifetime history of psychiatric illness as determined by a SCID-non-patient version (SCID-NP) (Spitzer et al. 1992) and were free of a family history of an Axis I psychiatric disorder in their first-degree relatives, as determined by a clinical history. The severity of manic symptoms was assessed by Young Mania Rating Scale (YMRS) (Young et al. 1978). Manic patients had a mean score of 28.5 (S.D.=6.5) on YMRS. All patients that participated in the study had been drug free (went off their mood stabilizers) for more than 2 weeks, with the exception of one manic patient who was on lithium (lithium level: 0.6 mEq / l), at the time they were admitted to hospital. Upon admission, they were started on lorazepam on a prn basis (mean dose received was less than 0.5 mg per day) for control of behavioral symptoms. Ipsapirone challenge test

was usually done the following day after their admission to hospital. None of the patients was allowed to receive lorazepam during 12 hours preceding the test session.

The ipsapirone challenge test was conducted on the Mood Disorders Clinical Research Unit, Vancouver Hospital & Health Sciences Center, UBC site. Study subjects reported to the unit between 11:00 a.m. and 11:30 a.m.. An indwelling intravenous catheter was inserted in a forearm vein at 11:30 a.m.. All the subjects were not allowed to eat or sleep until the procedure was completed. After obtaining a blood sample for baseline hormone levels, and measuring body temperature, a single dose of 0.3 mg / kg of ipsapirone was given orally to all subjects at 2:00 p.m. (time "0"). Further blood samples and temperature readings were obtained at 30-min intervals for 3 hours. Behavioral responses to ipsapirone were rated with 100 mm visual analog scales (VAS, 0 = not at all, 100 = most ever) on four subjective states (nausea, drowsiness, anxiety, and headache) by the subjects at 0, 60, 120, and 180 minutes. Room temperature ranged between 20°C and 22°C. Sublingual temperature was recorded by using a high-resolution digital thermistor probe (IVAC co., San Diego, California). Venous samples were collected into tubes containing EDTA, stored on ice, and centrifuged within 60 minutes. Serum was separated and stored at -80°C for assay at a later time. All samples were assayed in a random order by a lab technician blind to the study conditions. ACTH and cortisol were measured by radioimmunoassay (Ciba Corning Diagnostic Corp. , USA). Inter- and intra-assay coefficients of variation were 7.8% and 3.0% for ACTH and 4.0% and 2.0% for cortisol.

C. Statistical Analysis

Fisher's exact test and Mann-Whitney U test were performed to compute difference in sex, age, baseline body temperature, ACTH and cortisol levels between groups. Hypothermic and hormonal and behavioral responses to ipsapirone were calculated as the net maximal response (labeled as Δ max BT, Δ max ACTH, Δ max Cortisol, and Δ max VAS scores respectively), that is, the peak response minus baseline. Mann-Whitney U test (for Δ max) and repeated measures analyses of variance (ANOVA) were used to determine if there was a significant difference in hypothermic, hormonal, and behavioral responses between patients with mania and healthy controls. Relationships between variables were assessed by means of Spearman's rank order correlation coefficient. All tests were two-tailed, with significance set at $P < 0.05$.

D. Results

Demographic, hormonal and body temperature data for manic patients and healthy controls are presented in Table 1. As the hypothermic, ACTH and cortisol responses to ipsapirone of the single manic patient receiving lithium were similar to other manic patients that were drug free, we included this subject in the analysis. There was no significant difference in sex (Fisher's exact test, $P=1.0$), age ($U=0.57$, $P=0.57$), baseline body temperature ($U=13.5$, $P=0.47$), or baseline ACTH ($U=15.5$, $P=0.69$) and cortisol levels ($U=10.0$, $P=0.20$) between manic patients and healthy subjects. Similarly, the hypothermic response to ipsapirone measured as for Δ max BT did not differ between manic patients and healthy subjects ($U=16.5$, $P=0.81$). However, the ACTH response to ipsapirone measured as Δ max ACTH in

manic patients was significantly enhanced when compared with healthy subjects (U=3.0, P<0.02). Likewise, the cortisol response to ipsapirone measured as Δ max Cortisol was also significantly increased in manic patients compared to healthy controls (U=4.0, P<0.03) (see Table 1 for details).

The body temperature, ACTH and cortisol levels at various time points, following ipsapirone administration, are plotted in Figures 1-3. Repeated measures ANOVA on body temperature data, showed a significant time effect (F=4.29, d.f.=6,60, P<0.002), but no significant group effect (F=0.33, d.f.=1,10, P=0.58), nor a significant interaction between time and group (F=0.70, d.f.=6,60, P=0.65), indicating no difference in hypothermic response to ipsapirone between manic patients and healthy controls (see Figure 1). In contrast, repeated measures ANOVA on plasma ACTH data, showed a significant time effect (F=6.33, d.f.=6,60, P<0.00001), a significant group effect (F=5.71, d.f.=1,10, P<0.04), and a significant time by group interaction effect (F=4.52, d.f.=6,60, P<0.002) (see Figure 2). As expected, repeated measures ANOVA on plasma cortisol data also revealed a significant time effect (F=13.12, d.f.=6,60, P<0.00001), a significant time by group interaction effect (F=3.22, d.f.=6,60, P<0.009), and a trend toward a group effect (F=3.76, d.f.=1,10, P=0.08) (see Figure 3). This indicates that the administration of ipsapirone at time "0" led to a significant increase in ACTH and cortisol levels in both manic patients and healthy controls, but the hormonal responses were significantly enhanced in manic patients as compared to healthy controls.

Table 1. Demographic, hormonal and body temperature data for patients with mania and healthy controls

	Age (years)	Sex	Baseline BT (°C)	Δmax BT (°C)	Baseline ACTH (ng/l)	Δmax ACTH (ng/l)	Baseline Cortisol (μg/dl)	Δmax Cortisol (μg/dl)
Mania								
1	29	Female	37.3	-0.8	9.5	72.1	4.6	10.3
2	32	Male	36.5	-0.5	8.1	95.6	9.5	16.0
3	37	Male	36.6	-0.8	11.7	132.5	4.7	12.2
4	52	Male	36.1	0.0	23.9	120.3	4.4	16.0
5	60	Female	36.8	-0.8	16.2	33.8	8.1	13.0
6	60	Male	35.4	-0.5	27.0	166.8	7.1	14.0
Mean ± SD	45.0 ± 14.1^a	—	36.4 ± 0.7^c	-0.55 ± 0.31^e	16.1 ± 7.8^g	103.5 ± 47.0^i	6.4 ± 2.2^k	13.6 ± 2.2^m
Health controls								
1	32	Female	36.9	-0.1	13.1	4.5	8.1	5.7
2	30	Male	36.9	-0.4	5.0	33.3	4.7	11.1
3	31	Male	36.6	-0.6	21.6	73.0	4.9	11.1
4	47	Male	36.2	-0.9	15.8	21.2	9.1	6.7
5	57	Female	36.3	-0.5	9.5	61.8	8.5	12.7
6	58	Male	36.9	-0.6	16.7	21.6	10.1	8.8
Mean ± SD	42.5 ± 13.2^b	—	36.6 ± 0.3^d	-0.50 ± 0.26^f	13.6 ± 5.8^h	35.9 ± 26.3^j	7.6 ± 2.3^l	9.4 ± 2.8^n

Patient No. 5 was the single manic patient receiving lithium (lithium level: 0.6 mEq / l) during the study
Δmax: Peak minus baseline responses to ipsapirone challenge, ACTH: adrenocorticotropin hormone, BT: body temperature
Mann-Whitney U test (2-tailed): [a] vs. [b], $P=0.57$; [c] vs. [d], $P=0.47$; [e] vs. [f], $P=0.81$; [g] vs. [h], $P=0.69$; [k] vs. [l], $P=0.20$; [i] vs. [j], $P<0.02$; [m] vs. [n], $P<0.03$

Figure 1.   Mean (± S.E.M.) hypothermic responses to ipsapirone in manic patients and healthy controls
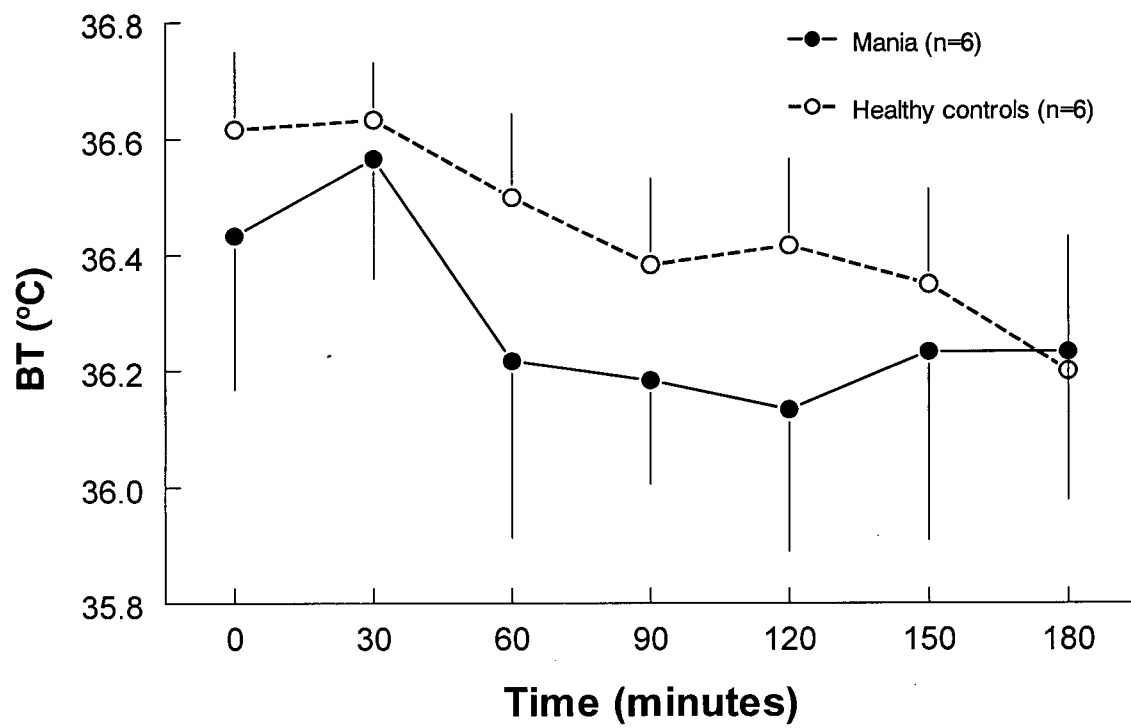
Figure 2.   Mean (± S.E.M.) ACTH responses to ipsapirone in manic patients
             and healthy controls
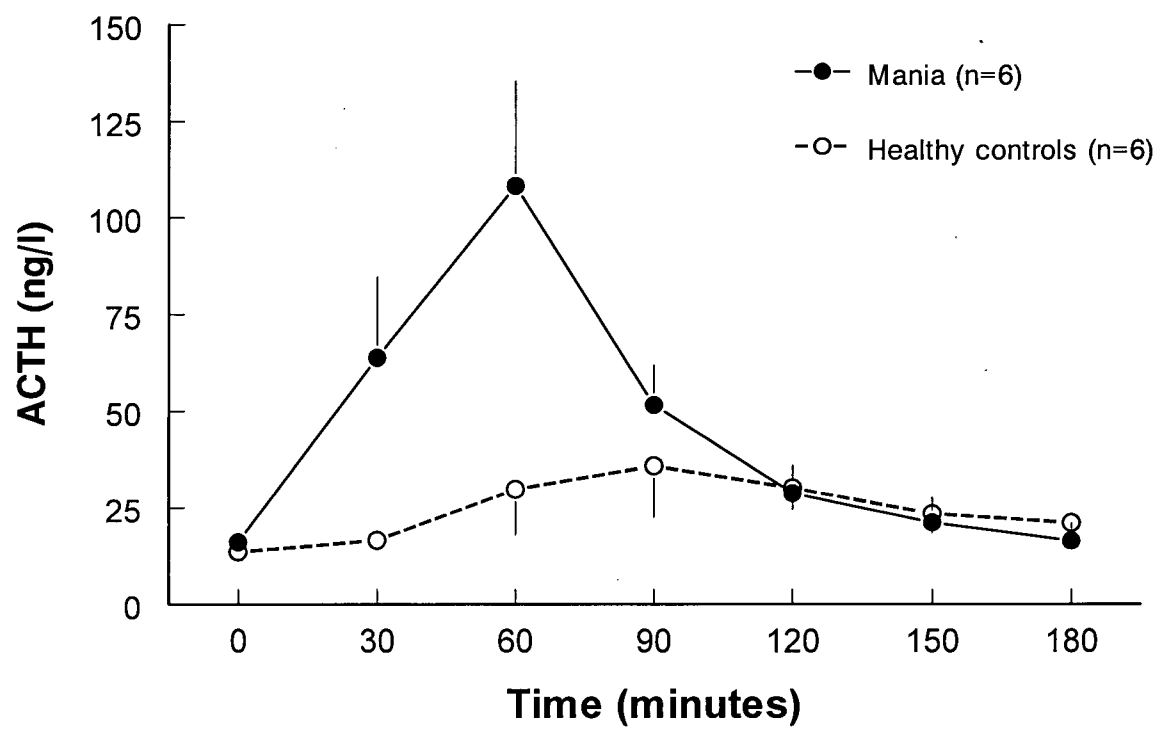
Figure 3.   Mean (± S.E.M.) cortisol responses to ipsapirone in manic patients
            and healthy controls
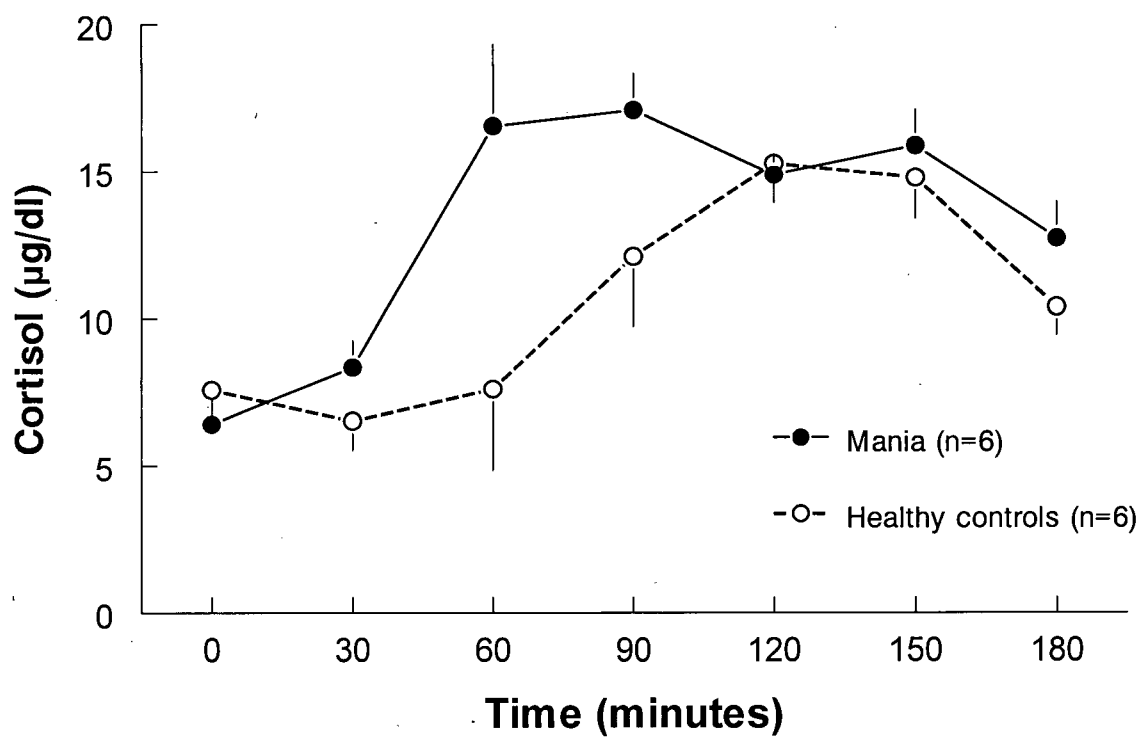
For behavioral measure, Δ max VAS scores for nausea, drowsiness, anxiety, and headache were calculated by subtracting baseline values from peak values, and analyzed by Mann-Whitney U test. There were no differences in Δ max VAS scores of nausea (U=15.0, P=0.61), drowsiness (U=16.5, P=0.81), anxiety (U=13.5, P=0.39), or headache (U=11.0, P=0.21) between manic patients and healthy controls. Similarly, repeated measures ANOVA on VAS score data showed no significant time, group, or time x group effects on each of the above subjective states (data not shown).

As expected, there was a significant positive correlation between Δ max ACTH and Δ max Cortisol when manic patients and healthy controls were pooled together (r=0.77, P<0.004, n=12). In contrast, there was no significant correlation between Δ max BT and Δ max ACTH (r=0.11, P=0.74, n=12) or Δ max Cortisol (r=0.28, P=0.37, n=12). No significant correlation was found between YMRS scores and Δ max BT (r=-0.32, P=0.54, n=6), Δ max ACTH (r=-0.03, P=0.96, n=6) or Δ max cortisol (r=-0.43, P=0.40, n=6) in manic patients.

E.   Discussion

The major findings of the present study were 1) patients with mania exhibited enhanced ACTH and cortisol responses to ipsapirone when compared to healthy controls; 2) there was no significant difference in hypothermic response to ipsapirone between manic patients and healthy controls; and 3) there was a significant positive correlation between the ACTH and cortisol responses to ipsapirone when manic patients and healthy subjects were pooled.

Since ACTH and cortisol responses elicited by 5-HT$_{1A}$ receptor agonists have been shown to be mediated by postsynaptic 5-HT$_{1A}$ receptors (Gilber et al. 1988; Koenig, Gudelsky, and Meltzer 1987) , our findings would suggest an increased postsynaptic 5-HT$_{1A}$ receptor sensitivity in manic patients. Cortisol responses to other 5-HT challenges, such as 5-hydroxytryptophan (5-HTP) (Meltzer et al. 1984b) and d,l-fenfluramine (Yatham 1996), have also been reported to be enhanced in manic patients in comparison to healthy controls, although the difference in cortisol responses between two groups did not reach significance in the latter study. Our results, thus are in agreement with the previous studies, suggesting that serotonergic receptor sensitivity is enhanced in mania.

Studies that examined prolactin responses to serotonergic probes in manic patients have yielded conflicting results. Yatham (1996; 1994) reported no significant differences in prolactin responses to buspirone or d,l-fenfluramine between manic patients and matched healthy controls. In contrast, Thakore et al. (1996) demonstrated blunted prolactin response to d-fenfluramine, a more selective 5-HT probe, in manic patients in comparison to healthy subjects. The discrepancies in the prolactin responses between studies are likely to be due to difference in drug selectivity. It has been suggested that buspirone and d,l-fenfluramine possess additional DA effects whereas d-fenfluramine has more specific 5-HT effects with stimulation of 5-HT release and inhibition of its reuptake (van Praag, Lemus, and Kahn 1986). Since d-fenfluramine induced prolactin release is considered to reflect "net" 5-HT activity (Coccaro and Murphy 1990), the finding of Thakore et al. (1996) would suggest that "net" 5-HT activity is decreased in mania, presumably due to low 5-HT availability in central 5-HT synapses. This would be expected to lead to an increase in postsynaptic 5-HT receptor

sensitivity, and the findings of our study and that of Meltzer et al. (1984b) are in keeping with this hypothesis.

With regard to hypothermic response, we found that the administration of ipsapirone led to similar magnitude of decrease in body temperature in both manic patients and healthy controls. Given that presynaptic 5-HT$_{1A}$ autoreceptors have been suggested to mediate the hypothermia induced by 5-HT$_{1A}$ receptor agonists in animal studies (Glaser and De Vry 1992), our finding of no significant difference in hypothermic response to ipsapirone between manic patients and healthy controls might suggest that presynaptic 5-HT$_{1A}$ autoreceptors are not altered in mania.

Although the current findings are consistent with the results of previous studies, several potential limitations to this study still need to be considered. First, since we did not include a placebo control condition to minimize the effects of confounding variables such as nonspecific stress effects, we can not exclude the possibility of a type I error of inference. Second, we studied only six manic patients, one of which was on lithium. Third, blood levels of ipsapirone were not measured. We, therefore, cannot exclude the possibility that the differences in ACTH/cortisol responses to ipsapirone between manic patients and healthy controls may have been due to differences in the absorption and/or bioavailability of ipsapirone between the two groups.

In conclusion, we found enhanced ACTH and cortisol responses to ipsapirone in manic patients in comparison to healthy controls but the hypothermic response was not different

between the two groups. This might suggest that manic patients have an increase in postsynaptic 5-HT_{1A} receptor sensitivity, which may be secondary to diminished 5-HT availability in central 5-HT synapses, and that the sensitivity of presynaptic 5-HT_{1A} autoreceptors is not altered in manic patients. However, the results of this study should be viewed as preliminary, in light of the above limitations. Further studies with a larger number of manic patients and a placebo control design are required to replicate our findings before any firm conclusion can be drawn.

CHAPTER III EFFECTS OF DIVALPROEX SODIUM ON SEROTONIN- 1A RECEPTOR FUNCTION

A. Introduction

Valproate has been shown to be effective in treatment and prevention of acute mania and somewhat beneficial in treatment and prevention of depression (McElroy et al. 1997). However, the mechanisms of action underlying its efficacy in mood disorders are still unknown. Since disturbances in serotonergic neurotransmission are implicated in the pathophysiology of mood disorders, it is possible that the efficacy of valproate may be related to its putative actions on 5-HT function.

Several reports have shown that treatment with valproate may enhance central 5-HT function in animals and humans. In rodents for example, valproate increases the synthesis of 5-HT and elevates brain concentration of its metabolite 5-HIAA (Kempf, Mack, and Mandel 1982; Shukla 1985; Whitton et al. 1985). More recently, experiments with in vivo microdialysis have shown that valproate increased extracellular concentrations of 5-HT in several rat brain regions (Biggs et al. 1992; Whitton and Fowler 1991). In humans, treatment with valproate increased CSF concentrations of 5-HIAA in a patient with post-anoxic intentional myoclonus (Fahn 1978). Maes and colleagues (1997) examined cortisol responses to L-5-HTP, a 5-HT precursor, in 10 manic patients before and after 4-6 weeks of treatment with valproate. They found that the L-5-HTP-induced cortisol responses in manic patients were significantly higher after treatment with valproate than before treatment. Since the cortisol responses to L-5-HTP are probably mediated by central 5-HT mechanisms (Maes and Meltzer 1995), their

finding suggests that chronic treatment with valproate may enhance central 5-HT function in manic patients.

Ipsapirone hydrochloride, a pyrimidinylpiperzaine 5-HT$_{1A}$ partial agoinst (Peroutka 1988), has high affinity and selectivity for 5-HT$_{1A}$ recognition sites and negligible affinity for the 5-HT$_{1B}$, 5-HT$_{1C}$, 5-HT$_{1D}$, 5-HT$_2$ and 5-HT$_3$ subtypes (Lesch et al. 1992). When given orally to humans, it causes dose-dependent increase in ACTH and cortisol secretion and decrease in body temperature (Lesch et al. 1989a; Lesch et al. 1989b). Pretreatment with pindolol, a β-adrenergic receptor blocker with 5-HT$_{1A}$ receptor antagonistic properties, blocks ACTH and cortisol release as well as hypothermic response, whereas betaxolol, another β$_1$-adrenergic blocker without any affinity for 5-HT$_{1A}$ receptors, does not have any effect on hormone release or temperature (Lesch et al. 1990c; Lesch et al. 1990d). This would suggest that ACTH and cortisol release and hypothermia induced by ipsapirone, are indeed mediated by 5-HT$_{1A}$ receptors and that these responses would provide a valid index of 5-HT$_{1A}$ receptor sensitivity in humans (Yatham and Steiner 1993).

In the present study, we examined the effects of valproate on 5-HT$_{1A}$ receptor function in humans by measuring hypothermic, ACTH/cortisol and behavioral responses to ipsapirone in male healthy volunteers before and after one-week treatment with DVP.

B. Methods

Ten male healthy volunteers were recruited to participate in the study (mean age ± S.D.: 29.4 ± 10.5 years). All subjects were screened by a research psychiatrist with a SCID-NP (Spitzer et al. 1992), a medical history and a physical examination. All subjects were free of physical and psychiatric illness, and were free of a family history of an Axis I psychiatric disorder in first-degree relatives. All subjects were medication free for a minimum of 2 weeks prior to testing, smoked less than 10 cigarettes per day, and ingested no more than five beers per week and three cups of coffee per day. This study was approved by the Clinical Research Ethics Committee of University of British Columbia. Written informed consent was obtained from all subjects before enrollment in the study.

Subjects arrived at the Mood Disorders Clinical Research Unit of Vancouver Hospital & Health Science Center between 11:00 a.m. and 11:30 a.m.. They wore normal indoor attire, and after being weighed (mean weight ± S.D.: 71.6 ± 7.3 kgs), reclined on a bed in a comfortable position with the head elevated. An indwelling intravenous catheter was inserted in a forearm vein at 11:30 a.m. and kept patent by a normal saline infusion. Room temperature ranged between 20 and 22 °C. After having had a standard light lunch between 12:00 noon and 12:30 p.m., all subjects were not allowed to eat, sleep or watch television until the procedure was completed, though visits to the bathroom were allowed.

Each subject received 0.3 mg / kg of ipsapirone hydrochloride tablets orally at 2:00 p.m. (time "0"). The average dose ± S.D. was 22.2 ± 2.6 mg. Sublingual body temperature was recorded at -30, 0, 30, 60, 90, 120, 150, 180 minutes using a high resolution thermistor probe (IVAC co., San Diego, California) and digital readings were obtained at the end of a 1-

minute recording period. For measurement of plasma hormone levels, blood samples were obtained at -30, 0, 15, 30, 45, 60, 75, 90, 105, 120, 150, and 180 minutes, collected into prechilled tubes containing EDTA and placed on ice. After collecting all the samples, they were immediately centrifuged using a refrigerated centrifuge, and serum separated and kept frozen at -80 °C for assay at a later time. Behavioral responses to ipsapirone were rated with 100 mm VAS (0=not at all, 100= most ever) on seven subjective states (nausea, drowsiness, anxiety, headache, depression, concentration, energy) by the subjects at 0, 60, 120, 180 minutes. Pulse rates and blood pressure were clinically monitored throughout.

After the pretreatment study, the subjects took 1000 mg / day of DVP (500 mg in the morning and 500 mg in the evening) for one week on an outpatient basis. During the week, they were requested to record any adverse events, which were related to the administration of DVP. The adverse events were classified as mild (not affecting usual activity), moderate (mild disruption in usual activity) and severe (major disruption in usual activity). Subjects took the last dose of DVP at 9:00 a.m. on the 8th day, and ipsapirone challenge was repeated on the same day commencing at 11:00 a.m.. An additional blood sample was taken at the end of ipsapirone challenge test for measuring valproic acid levels to check for compliance (mean valproic acid level ± S.D.: 629.0 ± 130.7 μmol / l). Dose and drug administration as well as thermoregulatory, endocrine, and behavioral monitoring were identical to the pretreatment studies.

The assays were performed in the Clinical Chemistry Laboratory, Vancouver General Hospital. Valproic acid plasma concentrations were determined using Fluorescence

Polarization Immunoassay (FPIA) technique. ACTH and cortisol were measured by Radioimmunoassay (Ciba Corning Diagnostic Corp, USA). Inter- and intra-assay coefficients of variation were 7.8% and 3.0% for ACTH and 4.0 % and 2.0 % for cortisol. All plasma samples from a given subject were assayed in the same batch, and all assays were performed by a lab technician blind to the study conditions.

C. Statistical Analysis

Both temperature and hormonal data at time "-30" and " 0" points before and after DVP treatment did not differ and were averaged to obtain a single baseline value for each variable. Baseline body temperature and hormone differences were assessed by paired t-tests. The hypothermic and hormonal responses to ipsapirone were calculated as: 1) the net change from baseline at each timepoint (labeled as Δ), and 2) the net maximal response (labeled as Δ_{max}), that is, the peak response minus baseline. Repeated measures ANOVA and ANCOVA were used to examine the treatment effect of DVP on the hypothermic and hormonal responses measured by the net change from the baseline. Post-hoc tests were carried out using paired t-tests. The Δ_{max} data were not normally distributed and were analyzed with Wilcoxon's sign rank tests. Changes in VAS ratings (peak response minus baseline) were also assessed by Wilcoxon's sign rank tests. Relationships between variables were assessed by means of Pearson's product moment correlation. Because of the large number of correlations performed, the significance level was set at $P < 0.01$. All significant levels reported were two-tailed. Data were reported as mean plus or minus standard deviation. The data were analyzed using the SPSS software.

D.   Results

There was a trend toward lower baseline body temperature (36.7 $\pm$ 0.4 vs 36.6 $\pm$ 0.2 ^{0} C; t=2.17, d.f.=9, P=0.06) after DVP treatment compared with pre-treatment. Before the DVP treatment, ipsapirone (0.3 mg / kg) produced a significant hypothermic response. However, following one week of DVP, the hypothermic response was significantly attenuated (Figure 4). Repeated measures ANCOVA using pre- and post-treatment baseline body temperature as covariates showed a trend for treatment effect (F=4.56, d.f.=9,1, P=0.06), a significant time effect (F=10.11, d.f.=54,6, P<0.001) and a significant interaction effect (F=3.96, d.f.=54,6, P<0.003). Post hoc analyses using paired t tests showed a significantly attenuated hypothermia at 120, 150, 180 minutes after DVP treatment compared to pre-treatment (t=-2.35, d.f.=9, P<0.05; t=-4.33, d.f.=9, P<0.003; t=-2.28, d.f.=9, P<0.05, respectively).

As shown in Figure 5, the ipsapirone-induced hypothermic response as measured by Δ_{max} body temperature was also significantly attenuated after the DVP treatment when compared with pre-treatment (pre- vs post-treatment, -0.63 $\pm$ 0.3 vs -0.40 $\pm$ 0.2 ^{0}C; Z=-2.07, P<0.05).

There was no significant difference in baseline plasma ACTH levels in ten subjects before and after DVP treatment (16.2 $\pm$ 7.2 vs 12.6 $\pm$ 12.2 ng / l; t=-0.91, d.f.=9, P=0.38). Repeated measures ANOVA on Δ ACTH data showed a trend for time effect (F=2.50, d.f.=4,36, P=0.06) but no treatment effect (F=0.04, d.f.=1,9, P=0.85) or interaction between time and treatment (F=1.20, d.f.=4,36, P=0.33) (Figure 6). The plasma ACTH response to ipsapirone

measured by Δ_{max} was not altered by the DVP treatment, either (50.0 ± 78.9 vs 48.2 ± 91.5 ng / l; Z=-0.26, P=0.80) (Figure 7).

There was no significant difference in baseline plasma cortisol levels in ten subjects between pre-treatment and post-treatment conditions (78.4 ± 23.0 vs 110.4 ± 57.5 ug / dl; t=-2.02, d.f.=9, P=0.08). Repeated measures ANOVA on Δ cortisol data showed a significant time effect (F=5.32, d.f.=10,90, P<0.001) but no treatment effect (F=0.16, d.f.=1,9, P=0.70) or an effect for treatment x time interaction (F=1.72, d.f.=10,90, P=0.09) (Figure 8). In addition, the plasma cortisol response to ipsapirone measured by Δ_{max} was not altered by the DVP treatment, either (82.2 ± 48.2 vs 73.2 ± 78.4 ug / dl; Z=-0.05, P=0.96) (Figure 9).

We compared the maximal behavioral responses independent of time with baseline responses using Wilcoxon's sign rank tests. We found that ipsapirone overall significantly increased drowsiness (Z=-2.82, P<0.005), nausea (Z=-2.50, P<0.02), headache (Z=-2.22, P<0.03) and anxiety (Z=-2.13, P<0.04), decreased energy (Z=-3.36, P<0.001) and concentration (Z=-2.90, P<0.004), but had no significant effect on depression (Z=-1.34, P=0.18). However, none of the behavioral responses measured as Δ_{max} VAS scores was significantly altered by DVP treatment (data not shown). The adverse events with DVP, which were reported with open questionnaires, included headache (10% with mild, moderate, and severe degree respectively), drowsiness (20% with mild degree and 10% with moderate degree), nausea (20% with moderate degree), diarrhea (10% with mild degree), and feeling tired (10% with mild degree).

Figure 4. Mean (± S.E.M.) hypothermic response to ipsapirone (measured as change from baseline, Δ BT) in healthy male humans before and after DVP treatment.
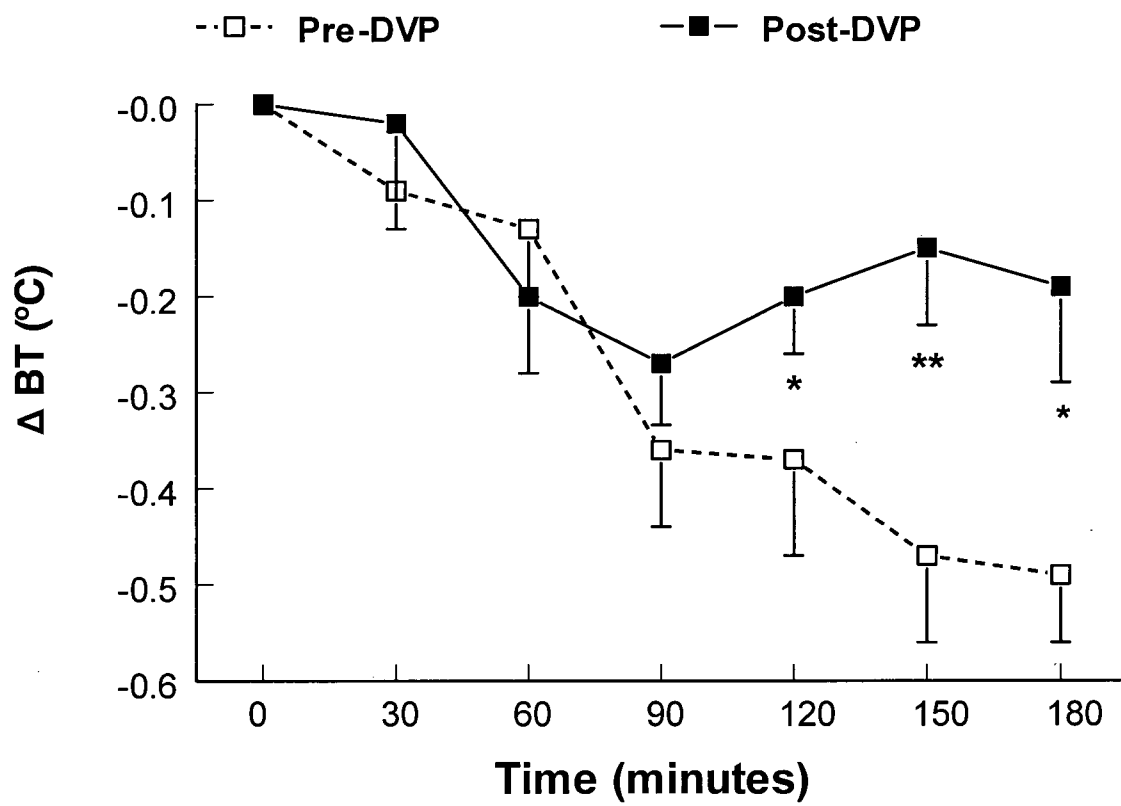
Figure 5. Mean (± S.E.M.) net maximal hypothermic response to ipsapirone (labeled as Δ max BT) in healthy male humans before and after DVP treatment.
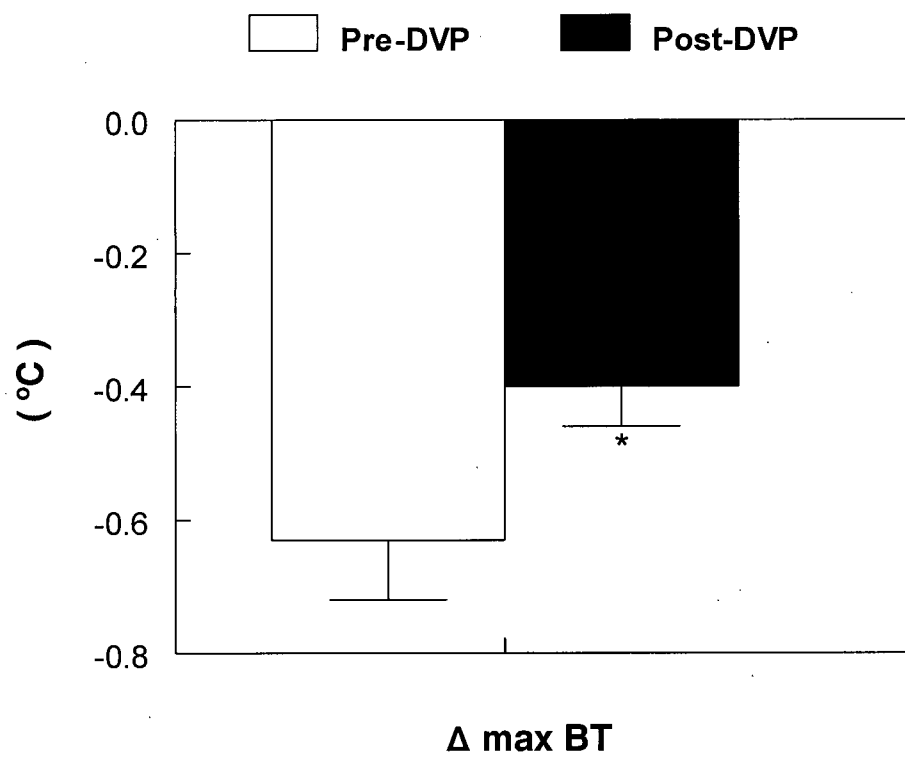
Figure 6. Mean (± S.E.M.) ACTH response to ipsapirone (measured as change from baseline, Δ ACTH) in healthy male humans before and after DVP treatment.
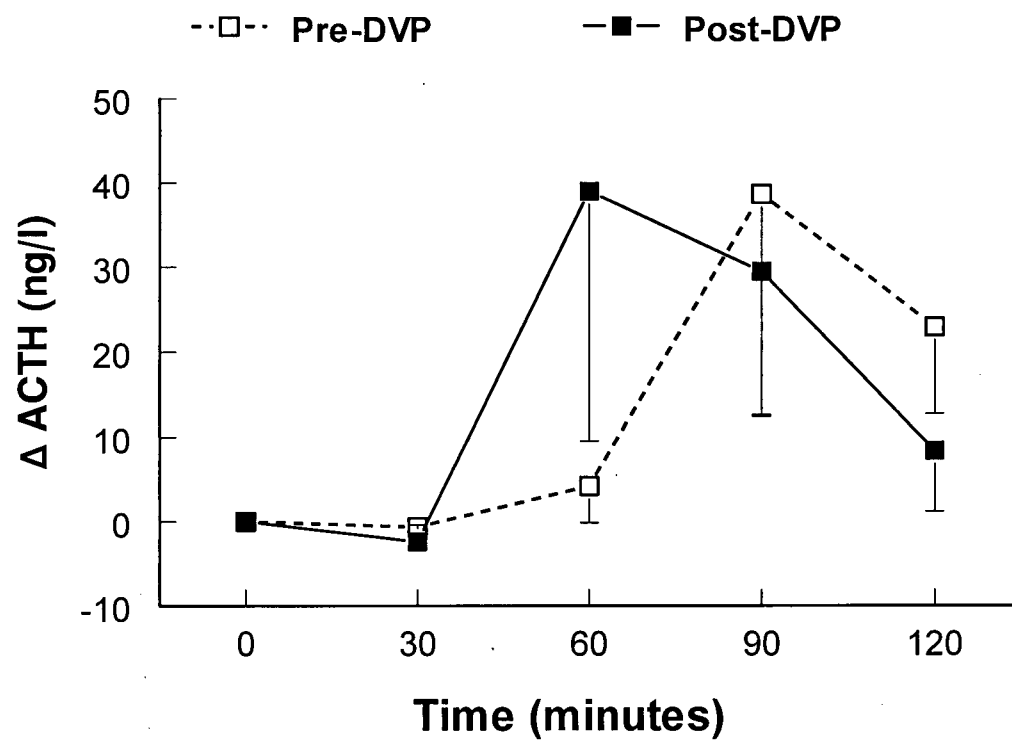
Figure 7. Mean (± S.E.M.) net maximal ACTH response to ipsapirone (labeled as Δ max ACTH) in healthy male humans before and after DVP treatment.

Δ max ACTH
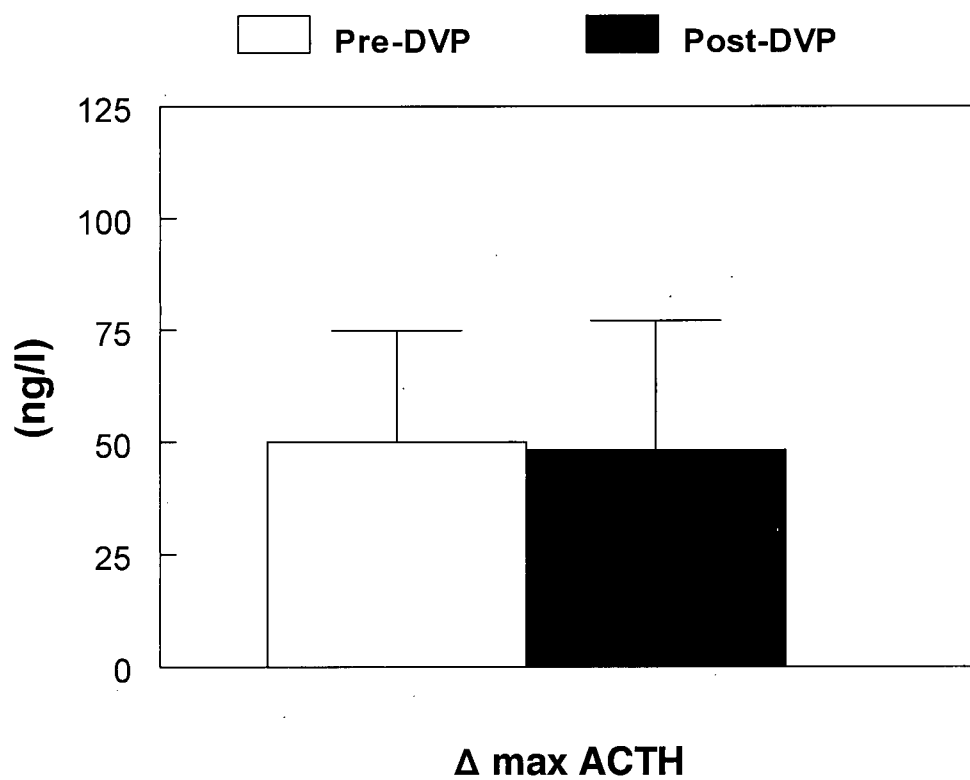
Figure 8.   Mean (± S.E.M.) cortisol response to ipsapirone (measured as change from baseline, Δ Cortisol) in healthy male humans before and after DVP treatment.

Time (minutes)
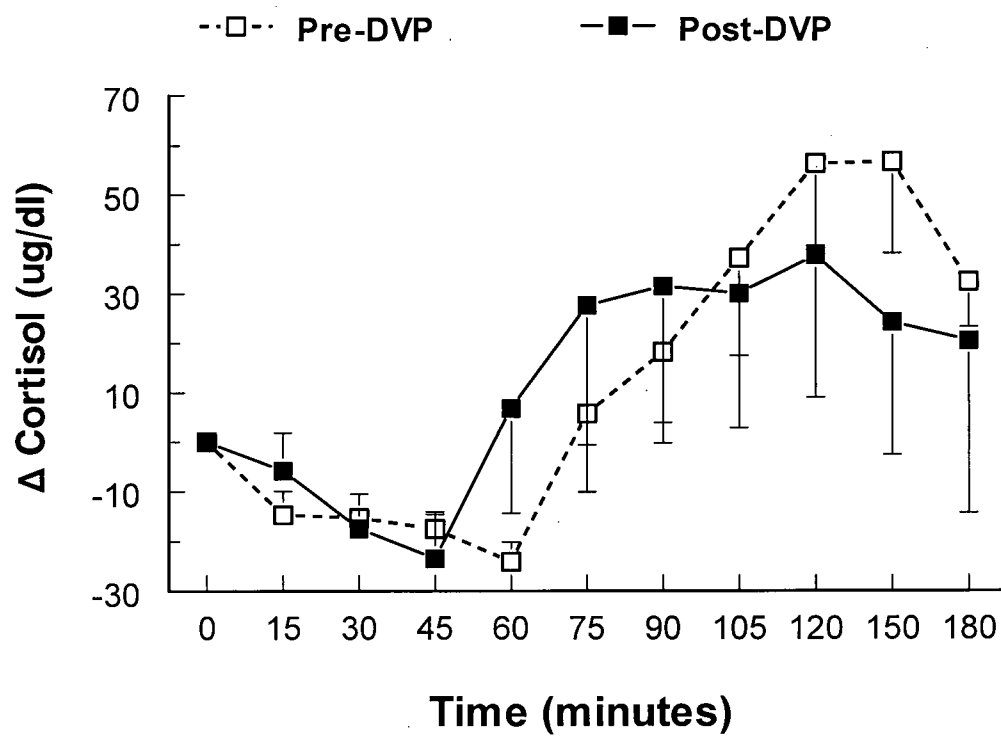
Figure 9.   Mean (± S.E.M.) net maximal cortisol response to ipsapirone (labeled as
            Δ max Cortisol) in healthy male humans before and after DVP treatment.

Δ max Cortisol
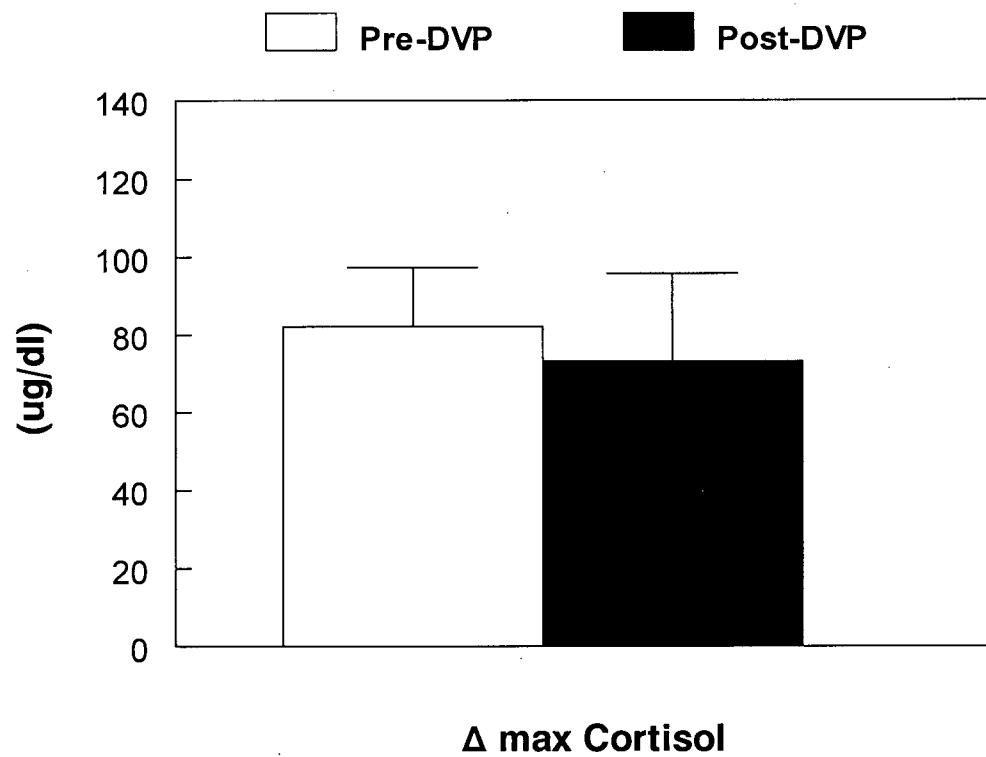
As well, one subject reported mild gum bleeding in the first two days of the drug administration but the symptom spontaneously remitted. The other one reported vomiting once on the second day of the drug administration. Three out of the ten subjects did not report any significant side effects.

There were no significant correlations between Δ_{max} hypothermic response and ACTH or cortisol responses either before or after DVP treatment. There were no significant correlations between plasma valproic acid levels and Δ_{max} hypothermic, ACTH or cortisol responses post-treatments. There was a significant correlation between the Δ_{max} VAS scores of anxiety and the Δ_{max} hypothermic responses (r=0.91, P<0.001) before DVP treatment but there were no significant correlations between any other behavioral responses to ipsapirone and the hypothermic or endocrine responses either before or after DVP treatment.

E.   Discussion

Our major findings were 1) ipsapirone significantly decreased body temperature and increased plasma cortisol levels in male healthy humans; there was a strong trend toward an increase in plasma ACTH levels to ipsapirone challenge; 2) one week treatment with DVP significantly attenuated the hypothermia induced by ipsapirone but it had no effect on the ACTH and cortisol responses to ipsapirone; 3) ipsapirone significantly decreased energy, concentration, and increased anxiety, headache, drowsiness and nausea, but there were no significant treatment effects of DVP on these behavioral responses.

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Our study demonstrated a significant hypothermic effect of ipsapirone in healthy men. This is consistent with previous findings that 5-HT$_{1A}$ receptor agonists such as buspirone (Anderson and Cowen 1990), ipsapirone (Lesch et al. 1990c), gepirone (Anderson, Cowen, and Grahame-Smith 1990), and flesinoxan (Seletti et al. 1995) induced hypothermia in humans. The hypothermic response induced by ipsapirone in humans is antagonized by pindolol pretreatment, suggesting that the response is 5-HT$_{1A}$ receptor mediated (Lesch et al. 1990c).

There has been some controversy in the animal literature about whether hypothermia induced by 5-HT$_{1A}$ agonists is mediated by somatodendritic autoreceptors or post-synaptic 5-HT$_{1A}$ receptors. In the mouse, there is clear evidence that the hypothermic response to the prototypic 5-HT$_{1A}$ agonist 8-hydroxy-2-(di-n-propylamino)tetraline (8-OH-DPAT) is mediated by presynaptic 5-HT$_{1A}$ receptors because the hypothermia induced by 8-OH-DPAT is abolished by 1) destruction of presynaptic 5-HT neurons with the neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT) (Goodwin, DeSouza, and Green 1985a; Martin et al. 1992) and, 2) depletion of 5-HT stores by a pretreatment with parachlorphenylalanine (pCPA), an inhibitor of 5-HT synthesis (Goodwin, DeSouza, and Green 1985a). This was replicated by Bill et al. (1991) who also showed that both 5,7-DHT lesions and pCPA treatment led to a significant decrease in brain 5-HT as well as 5-HIAA levels in mice. Furthermore, they also demonstrated that pretreatment with agents which facilitate 5-HT release acutely such as selective serotonin reuptake inhibitors (SSRIs), the 5-HT precursor, 5-HTP, or a 5-HT releasing agent fenfluramine, markedly attenuated or abolished the 8-OH-DPAT induced hypothermia in this species. Taken together, these findings would suggest that an autoreceptor mediated inhibition of 5-HT release, on to an (as yet) unidentified postsynaptic

5-HT receptor, is primarily responsible for the hypothermic response in mice (Bill et al. 1991).

However, the findings for rats are much less consistent. Goodwin et al. (1987) reported that pCPA administration for two weeks led to attenuation of 8-OH-DPAT-induced hypothermia. Hillegaart (1991) found that a direct injection of 8-OH-DPAT into 5-HT cell bodies in the dorsal raphe nucleus of rats led to clear hypothermia. These results suggest that the 5-HT$_{1A}$ receptors mediating hypothermia in rats are located presynaptically. In contrast, Hjorth (1985) and Hutson et al. (1987) reported that 5,7-DHT lesions or pCPA treatment enhanced 8-OH-DPAT-induced hypothermia suggesting that the relevant 5-HT$_{1A}$ receptors are located postsynaptically. Further evidence in support of the hypothesis that hypothermia in rats is mediated by postsynaptic 5-HT$_{1A}$ receptors has been provided by O'Connell et al. (1992) who showed that pretreatment with pCPA did not affect the 8-OH-DPAT or BMY 7378 (BMY 7378 is a buspirone analogue with 5-HT$_{1A}$ receptor agonist properties) induced hypothermia in rats (O'Connell, Sarna, and Curzon 1992). In addition, Bill et al. (1991) found no effect of 5-HTP or fenfluramine on 8-OH-DPAT-induced hypothermia in rats; since these drugs increase 5-HT secretion, they would be expected to antagonize hypothermia induced via activation of 5-HT$_{1A}$ presynaptic receptors. Taken together, the findings of the studies in rats suggest that the 8-OH-DPAT induced hypothermia in rats is probably mediated by a mixed pre- and post-synaptic 5-HT$_{1A}$ receptor activation.

Given the discrepancy in the mechanism of 8-OH-DPAT-induced hypothermia between the mouse and rat, it is hard to generalize the findings of rodent studies to humans. To our

knowledge, there is only one human study to date that addressed this issue. Blier et al. (1994), using the hypothermic response to buspirone as an index of 5-HT_{1A} receptor function in healthy volunteers, reported that tryptophan depletion paradigm had no effect on the buspirone induced hypothermia. Based on this, they concluded that hypothermia induced by 5-HT_{1A} receptor agonists in humans was mediated by an activation of postsynaptic 5-HT_{1A} receptors (Blier et al. 1994). However, buspirone does not appear to induce hypothermia consistently in humans (Lee and Meltzer 1991), suggesting that buspirone-induced hypothermia is not likely to be a good measure of 5-HT_{1A} receptor function in humans. Furthermore, if the hypothermic responses to 5-HT_{1A} agonists in humans like 5-HT_{1A}-mediated ACTH/cortisol responses are mediated by an activation of postsynaptic 5-HT_{1A} receptors, an unaltered hypothermic response to ipsapirone would be expected after DVP treatment in the present study given that the endocrine responses were not affected by DVP. We, however, found that DVP treatment attenuated the hypothermic response to ipsapirone but did not affect endocrine responses. Therefore, our results are consistent with the hypothesis that hypothermic response to ipsapirone in humans is mediated through presynaptic receptors, and that DVP alters 5-HT neurotransmission by affecting presynaptic 5-HT_{1A} autoreceptors but not postsynaptic 5-HT_{1A} receptors.

Previous studies have demonstrated that chronic treatment with amitriptyline in depressed patients (Lesch, Disselkamp-Tietze, and Schmidtke 1990), fluoxetine in patients with obsessive compulsive disorder (OCD) (Lesch et al. 1991), paroxetine in healthy subjects (Wing et al. 1996), and various antidepressant treatments including tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), SSRIs, electroconvulsive shock (ECS) and

lithium in mice (Goodwin, DeSouza, and Green 1985b; Goodwin et al. 1986; Maj and Moryl 1992; Martin et al. 1992), attenuated hypothermic responses to 5-HT$_{1A}$ receptor agonists. Our finding of an attenuation of ipsapirone-induced hypothermia by DVP in healthy males is consistent with these earlier reports, suggesting that subsensitivity of the 5-HT$_{1A}$ autoreceptors may be a change induced by most antidepressant treatments and could serve as an important factor in their therapeutic action. However, it is of some interest to note that a recent study in rats did not find any effect of valproate on hypothermia induced by 8-OH-DPAT (Khaitan, Calabrese, and Stockmeier 1994). The discrepancy in findings between our study and the rat study is likely to be due to species differences in the responsivity of 5-HT$_{1A}$ receptors to valproate treatment.

Given in sufficient doses, ipsapirone and other 5-HT$_{1A}$ receptor agonists increase plasma ACTH and cortisol in rodents and humans (Cowen et al. 1990; Gilber et al. 1988; Koenig, Gudelsky, and Meltzer 1987; Urban et al. 1986). The endocrine responses elicited by the 5-HT$_{1A}$ receptor agonists have been shown to be mediated by postsynaptic 5-HT$_{1A}$ receptors which stimulate corticotropin-releasing hormone (CRH) release in the paraventricular nucleus (PVN) of the hypothalamus (Bagdy and Makara 1994; Gilber et al. 1988; Koenig, Gudelsky, and Meltzer 1987). Our results showed that ipsapirone significantly increased plasma ACTH/cortisol levels in humans, supporting a role for 5-HT$_{1A}$ receptor in regulating HPA axis activation.

As regards to the effects of psychotropic drugs on the postsynaptic 5-HT$_{1A}$ receptor-mediated endocrine responses, Aulakh et al. (1988) reported that repeated treatment of rats with

imipramine, clomipramine or clorgyline had no effect on the rise of ACTH caused by 8-OH-DPAT in rats. Gartside et al. (1992) reported that the ACTH response to 8-OH-DPAT in rats was unaffected by amitriptyline, ECS or lithium. Li and colleagues (1994; 1993) demonstrated that chronic treatment with fluoxetine but not desipramine attenuated the endocrine responses to 8-OH-DPAT and ipsapirone in male rats. More recently, Akiyoshi et al. (1995) investigated 8-OH-DPAT- induced cortisol in male rats after treatment with mianserin (2, 10 mg / kg), imipramine (5 mg / kg), desipramine (5 mg / kg), and doxepine (5 mg / kg) for 1 day or 3 weeks. They found that chronic mianserin (10 mg / kg) and doxepine significantly increased the 8-OH-DPAT-induced cortisol response whereas acute antidepressants and chronic imipramine, desipramine, mianserin (2 mg / kg) treatment did not change it.

In human studies, Lesch et al. (1991) reported that a significant reduction in postsynaptic 5-HT_{1A}-mediated ACTH/cortisol responses was obtained during a course of fluoxetine treatment in patients with OCD, and Sargent et al. (1997) found that 17 days treatment with paroxetine (30 mg / day) produced an attenuation of endocrine responses to gepirone in healthy humans. In contrast, Walsh et al. (1991) reported that ACTH/cortisol responses induced by gepirone in healthy males were not altered by 7 days of lithium treatment (800 mg / day), and Lesch (1991) reported that the ACTH and cortisol responses to ipsapirone were not altered during chronic treatment with amitriptyline in depressed patients. We have in a recent study found that treatment with lamotrigine, a newer anticonvulsant with antimanic and antidepressant properties, had no effect on the cortisol response to ipsapirone in healthy humans (Shiah et al. 1998c). In addition, in the present study, one week treatment

with DVP (1000 mg / day) did not alter the ACTH/cortisol responses to ipsapirone in healthy males. This may suggest that DVP does not affect postsynaptic $5-HT_{1A}$ receptors. Taken together, the results of the present study and the previous animal and human endocrine studies suggest that the ACTH/cortisol responses to $5-HT_{1A}$ receptor agonists appear to be attenuated by SSRIs such as fluoxetine and paroxetine, but not by other antidepressant treatments including TCAs, MAOIs, electroconvulsive therapy (ECT), and mood stabilizers such as lithium, DVP and lamotrigine. However, the mechanism by which SSRIs attenuate $5-HT_{1A}$ receptor-mediated hormone responses is still unclear.

In conclusion, we found that DVP attenuated the hypothermia induced by ipsapirone but did not alter the hormonal and behavioral responses, suggesting that DVP may enhance 5-HT neurotransmission via a subsensitization of $5-HT_{1A}$ autoreceptors but does not appear to have an effect on postsynaptic $5-HT_{1A}$ receptors. Studies of $5-HT_{1A}$-mediated function after DVP treatment in patients with mood disorders may further establish the role of $5-HT_{1A}$ receptors in the mechanisms of action of DVP in mood disorders.

CHAPTER IV   GABA-B RECEPTOR FUNCTION IN MANIA

A.   Introduction

A role for GABA in mood disorders was first postulated by Emrich et al. (1980), based on the clinical observation that valproate, a GABA agonist, was effective in treatment of bipolar disorder. They hypothesized that patients with this disorder have a GABA deficit. Since then, some evidence from preclinical and clinical studies has accumulated supporting a role for GABA in depression but very few studies to date examined GABA function in mania (Shiah and Yatham 1998).

Three studies examined CSF GABA levels in acute manic patients (Gerner et al. 1984; Gerner and Hare 1981; Post et al. 1980). Although the CSF GABA levels were not significantly different compared to normal controls, the levels were in general found to be lower in manic patients compared to controls. Berrettini et al. (1985a; 1986) on the other hand, reported that CSF GABA levels were decreased in recently recovered manic-depressive patients, and that these values normalized in longer-recovered bipolar patients. In addition, Petty et al. (1993) reported that mean levels of plasma GABA were significantly lower in both manic and depressed phases of bipolar patients when compared to matched normal controls. Given that plasma GABA has been suggested to reflect brain GABA activity (Petty 1994), low plasma GABA may represent a shared biologic correlate in mania and depression, and appear to support the GABA deficit hypothesis of mood disorders.

In addition to low GABA levels, manic patients may also have an alteration in sensitivity of $GABA_A$ or $GABA_B$ receptors. To date, to the best of our knowledge, no study assessed the sensitivity of these receptors in acute manic patients. In humans, $GABA_B$ receptor function can be assessed using a neuroendocrine challenge paradigm with baclofen as a pharmacological probe. Baclofen is a $GABA_B$ receptor agonist which when given orally increases GH levels (Koulu, Lammintausta, and Dahlstrom 1979). The magnitude of GH release to baclofen challenge is considered to provide an index of sensitivity of $GABA_B$ receptors. This paradigm has been used in previous studies to assess $GABA_B$ receptor function in patients with major depression (Davis et al. 1997; Marchesi et al. 1991; Monteleone et al. 1990a; O'Flynn and Dinan 1993). In this study, we measured GH response to baclofen challenge in ten manic patients and 10 healthy controls to ascertain $GABA_B$ receptor function in acute mania.

B.   METHODS

A total of ten manic patients (8 women and 2 men; mean age $\pm$ S.D. = 34.1 $\pm$ 3.9) and ten healthy controls (6 women and 4 men; mean age $\pm$ S.D. = 38.4 $\pm$ 2.3) were recruited for the study. The mean body mass indices (BMI) $\pm$ S.D. for manic patients and healthy controls were 25.3 $\pm$ 5.1 and 24.1 $\pm$ 2.4 kg / m^2 respectively. The DSM-IV diagnosis of bipolar disorder - current episode mania (American Psychiatric Association 1994) was made by the consensus of the research team using the information generated from a clinical interview by a psychiatrist, and a SCID (Spitzer et al. 1990) administered by a research assistant. The mean $\pm$ S.D. numbers of previous manic and depressive episodes were 0.6 $\pm$ 1.2 (range: 0 - 4) and

1.1 $\pm$ 1.3 (range: 0 - 4) respectively. The mean $\pm$ S.D. years since diagnosis was 2.3 $\pm$ 4.8 years (range: 0 - 15). Previous medication treatments for these patients include mood stabilizers: lithium and DVP, antidepressants: fluoxetine and paroxetine, and antipsychotics: haloperidol, loxapine and thiothixene. Those that met criteria for other Axis I diagnoses, substance or alcohol abuse were excluded. The severity of manic symptoms was assessed by YMRS (Young et al. 1978) the day before the challenge procedure. Manic patients had a mean ($\pm$ S.D.) YMRS score of 24.3 $\pm$ 6.3. The healthy subjects had no lifetime history of psychiatric illness as determined by a SCID-NP (Spitzer et al. 1992) and were free of a family history of an Axis I psychiatric disorder in their first-degree relatives.

All study subjects were physically healthy and gave written informed consent for participation in the study, which had been approved by the Clinical Research Ethics Committee of the University of British Columbia. All patients that participated in the study had been neuroleptic, antidepressant, and mood stabilizer free (went off their medications) for more than 2 weeks by the time they were admitted to hospital. Upon admission, they were started on lorazepam on a prn basis for control of behavioral symptoms. The baclofen challenge test was usually done the following morning after their admission to hospital. None of the patients was allowed to receive lorazepam during 8 hours proceeding the test session. Of the seven manic women that were included in analysis, five were tested during the follicular phase (day 2 to day 9), one was postmenopausal and one received hysterectomy in 1987. Of the six female healthy volunteers, five were tested during the follicular phase and one was postmenopausal.

The baclofen challenge test was conducted on the Mood Disorders Clinical Research Unit, Vancouver Hospital and Health Sciences Center, UBC site. The subjects, having fasted from midnight, presented for testing between 8:00 and 8:30 a.m. An intravenous cannula was inserted in a forearm vein at 8:30 a.m. All study subjects including manic patients lied down on a bed and rested through out the neuroendocrine test procedure. Testing was carried out in all subjects in fasting condition. They were not allowed to sleep or leave the bed, though brief visits to the washroom were allowed. The first blood sample for baseline GH level was taken at 10:00 a.m. (time"0"). Baclofen 20 mg was given orally at this time, and further blood samples were obtained at 30-minute intervals for the following 3 hours. The blood was immediately centrifuged and serum stored at -80°C until analysis.

GH was assayed by Quantitope HGH Radioimmunoassay (Kallestad diagnostics). The samples were assayed blind to the diagnostic status of subjects. All samples from each subject were assayed in the same batch. The sensitivity of GH was 0.2 ng / ml. The interassay coefficients of variation were 10.8% for GH pool of 2.6 ng / ml, 6.6% for GH pool of 5.8 ng / ml, and 5.7% for GH pool of 11.3 ng / ml. The intraassay coefficients for GH were 6.8%, 5% and 9.1% for GH pools of 2.5 ng / ml, 5.4 ng / ml, and 35.2 ng / ml, respectively.

C.   Statistical Analysis

The Kolmogorov-Smirnov (K-S) test was used to ascertain the normality of GH data distribution (P values for K-S tests on GH data were all larger than 0.1). Fisher's exact test and Student's t test were used to compute differences in sex, age, baseline GH level between

patients with mania and healthy controls. GH response to baclofen was measured as 1) the net change from baseline at each time point after challenge (labeled as Δ GH), and 2) the net area under the time curve (labeled as AUC GH), using a trapezoidal method with subtraction of the baseline values. Analysis of variance (ANOVA) with repeated measures (for Δ GH data) and Student's t test (for AUC GH data) were used to determine if there was a significant difference in GH response to baclofen between manic patients and healthy controls. Relationships between variables were assessed by means of Pearson's correlation coefficient. Values reported are means ± S.D.s, unless otherwise specified. All tests were two-tailed, with significance set at $P<0.05$.

D.   Results

One out of the 10 manic patients had a baseline GH level greater than 5 ng / ml and was excluded from data analysis because, after a GH secretory episode, the pituitary is relatively refractory (Vance et al. 1985). There was no significant difference in sex (Fisher's exact test, $P=0.63$) age ($t=-5.6$, d.f.=17, $P=0.57$) or BMI ($t=0.23$, d.f.=17, $P=0.81$) between the remaining 9 manic patients and 10 healthy controls. Likewise, the baseline GH levels in patients with mania (1.1 ± 0.6 ng / ml, n=9) were not significantly different from those (2.0 ± 1.5 ng / ml, n=10) in healthy controls ($t=-1.79$, d.f.=17, $P=0.09$). The GH levels at various time points following baclofen challenge are plotted in Figure 10. Repeated measures ANOVA on Δ GH data showed a significant time effect ($F=6.67$, d.f.=6,102, $P<0.001$), a significant group effect ($F=4.54$, d.f.=1,17, $P<0.05$), but no significant time x group effect ($F=1.76$, d.f.=6,102, $P=0.11$). Furthermore, the GH response to baclofen measured as AUC

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GH was also significantly enhanced in manic patients (368.6 ± 404.7 ng x min / ml, n=9) when compared to healthy controls (23.5 ± 288.5 ng x min / ml, n=10) (t=2.16, P<0.05) (see Figure 11).

These results indicate that the administration of baclofen at time "0" led to a significant increase in GH release both in manic patients and healthy controls, but the GH response to baclofen was significantly enhanced in manic patients when compared to healthy controls. However, we did not find a significant correlation between the YMRS scores and the GH response to baclofen measured as AUC GH in manic patients (Pearson's r=0.07, P=0.86, n=9). Similarly, there was no significant correlation between the GH to baclofen measured as AUC GH and age (Pearson's r=-0.17, P=0.67, n=9), sex (Pearson's r=-0.33, P=0.39, n=9), or baseline GH level (Pearson's r=-0.31, P=0.42, n=9).

E.   Discussion

The major finding of the present study was that administration of baclofen, a $GABA_B$ receptor agonist, increased plasma GH levels both in patients with mania and healthy controls, but the GH response to baclofen was significantly higher in manic patients compared to healthy controls.

Since the extent of GH response to baclofen is considered to provide an in vivo index of hypothalamic $GABA_B$ receptor function in humans, our finding of enhanced baclofen induced GH response in mania suggests that hypothalamic $GABA_B$ receptors are upregulated

Figure 10.   Mean (± S.E.M.) growth hormone (GH) to baclofen in manic patients and healthy controls
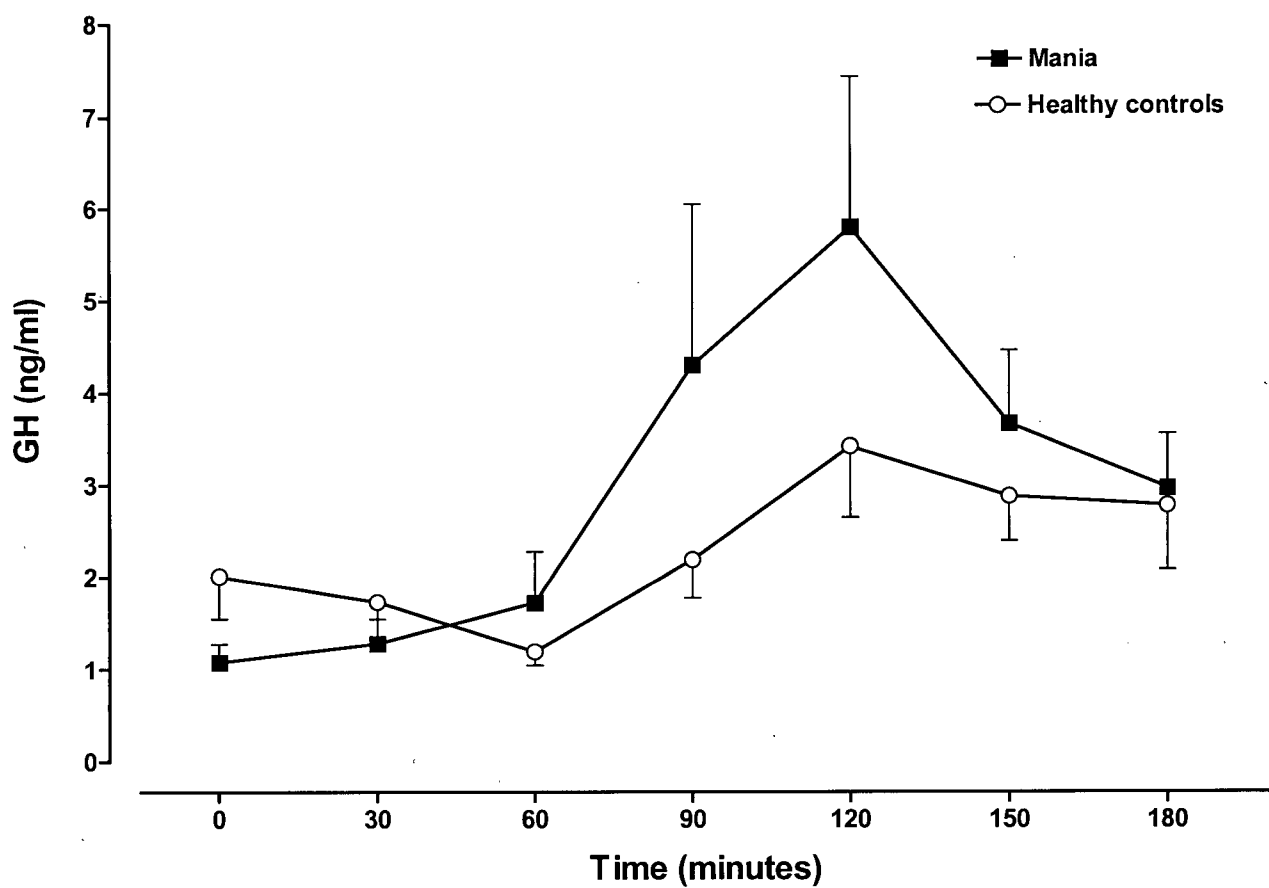
Figure 11.   GH response to baclofen measured as the net area under the time curve (labeled as AUC GH), using trapezoidal method with subtraction of baseline values in manic patients and healthy controls.
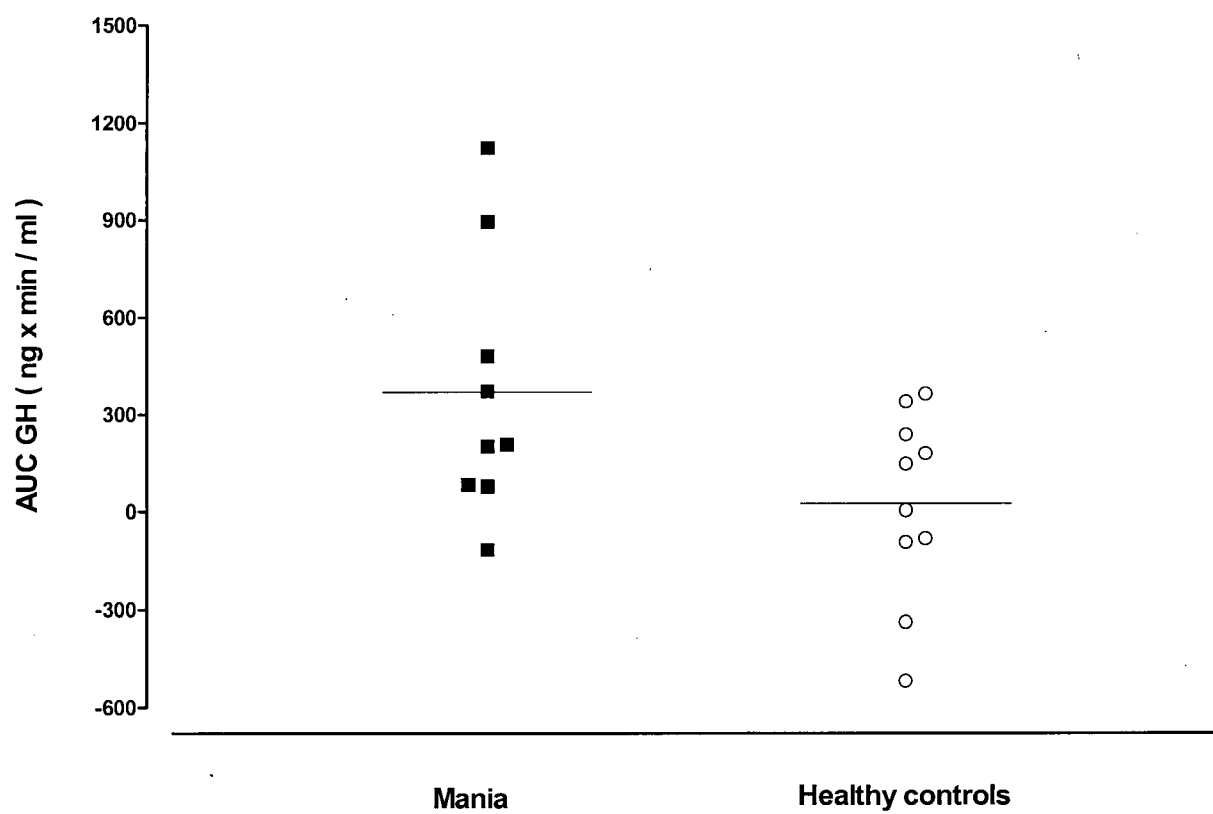
in mania. However, it is unknown if the up-regulation of $GABA_B$ receptors in hypothalamus is due to a primary abnormality or a secondary response to decreased brain GABA levels in this condition.

In a recent study (Shiah et al. 1998b), we have demonstrated that DVP, a mood stabilizer, significantly attenuated GH response to baclofen in healthy humans, and the attenuation effect of DVP was significantly correlated with the blood level of valproic acid, suggesting that DVP down-regulated hypothalamus $GABA_B$ receptors in humans. Furthermore, the only animal study that examined the effects of another mood stabilizer, lithium on GABA receptor binding in hypothalamus, also showed a decrease in [^{3}H]-GABA binding sites in rat hypothalamus after chronic administration of lithium (Maggi and Enna 1980). The current study result thus is in agreement with the previous studies, raising the possibility that DVP or lithium treatment in mania normalizes the up-regulated hypothalamic $GABA_B$ receptor function in this condition. Since mood stabilizers such as lithium and DVP have been suggested to enhance GABAergic neurotransmission (Waldmeier 1987), one may speculate that the up-regulated hypothalamic $GABA_B$ receptor function in mania is secondary to a GABA deficit in brain, whereas chronic treatment with DVP or lithium restores this deficit by enhancing GABAergic neurotransmission, thus leading to a down-regulation of hypothalamic GABA receptors.

However, it needs to be pointed out that other three animal studies (Lloyd, Thuret, and Pilc 1985; Motohashi 1992; Motohashi, Ikawa, and Kariya 1989) have shown that chronic administration of mood stabilizers including lithium, carbamazepine, and valproate sodium up-regulated rather than down-regulated $GABA_B$ receptors in rat hippocampus or frontal

cortex. The possible explanations for the discrepancy between the studies could be due to either differential treatment effects of mood stabilizers on $GABA_B$ receptors in different brain regions, or species differences in $GABA_B$ receptor responsivity to mood stabilizers.

In addition to GABA, catecholamine neurotransmitters such as DA and NE have also been implicated in the pathophysiology of bipolar disorder and mania in particular (Buki and Goodnick 1998, for a review). In general, it is considered that there is excess NE or DA in mania and depleted NE or DA in depression. Given that GABA has an inhibitory effect on noradrenergic and dopaminergic neurons, one may expect that a low GABA function in mania will lead to an enhancement of NE and/or DA neurotransmission in this condition. Our finding of increased GH response to baclofen in mania, presumably resulting from a primary GABA deficit, appears to be consistent with this hypothesis. Further studies are needed to clarify the relationship between multiple neurotransmitter systems including GABA, NE, and DA, during the manic state.

This study has several methodological limitations: First, we did not use a placebo condition to control for nonspecific stress effects. Therefore, we can not exclude the possibility of a Type I error of inference (Thompson, Maes, and Meltzer 1994). Second, lorazepam on a prn basis was used to control the behavioral symptoms of manic patients up to 8 hours prior to neuroendocrine challenge. Although all patients were tested within 48 hours of initiating treatment with lorazepam on a prn basis and each patient had received a total of less than 8 mg prior to the testing, one could still argue that this may have altered the $GABA_B$ receptor sensitivity. Third, although we did not find a significant relationship between gender (coded

by dummy variables) and the GH response to baclofen (measured as AUC GH), the small number of male manic patients (only 2 male patients) did not allow us to perform subgroup analysis to determine whether there is a gender difference in the GH response to baclofen between male and female manics. Future studies with more male manic patients are needed to address this issue. Fourth, blood levels of baclofen were not measured. We, therefore, can not exclude the possibility that the differences in GH response to baclofen between manic patients and healthy controls may have been due to differences in the absorption and/or bioavailability of baclofen between the two groups. Finally, although baclofen is a potent agonist at $GABA_B$ receptors, it also has some affinity for α_2-adrenoceptors (Fung, Swarbrick, and Fillenz 1985). Since α_2-adrenoceptors also regulate GH release, one could argue that enhanced GH release observed in the present study in manic patients is due to increased α_2-receptor sensitivity. This possibility is not supported by the previous studies that reported blunted GH release to direct and indirect α_2-receptor agonists in mania (Ansseau et al. 1987; Dinan et al. 1991). However, studies with more selective $GABA_B$ receptor agonists are needed to clarified this issue.

In summary, we found that baclofen induced GH response was significantly enhanced in manic patients in comparison to healthy controls. This might suggest that manic patients have an up-regulated hypothalamic $GABA_B$ receptor function, which may be secondary to a primary deficit of GABA neurotransmission in this condition. However, our results should be viewed as preliminary in light of the above limitations. Further studies with a larger number of manic patients and a placebo control design are needed to replicate our finding before any firm conclusion can be drawn.

CHAPTER V   EFFECTS OF DIVALPROEX SODIUM ON GABA-B RECEPTOR FUNCTION

A.   Introduction

Divalproex sodium (DVP) is an anticonvulsant, which contains valproic acid and sodium valproate in a one-to-one molar ratio. This medication has recently been shown to be effective in treatment of acute mania (Bowden et al. 1994) and depression (Davis et al. 1996a), suggesting that, like lithium, it has antimanic and antidepressant properties. Although the mechanisms underlying the therapeutic effect of DVP in mood disorders have not been elucidated, the increase in GABA levels in brain is the most often cited mechanism of action for the anticonvulsant activity of DVP (Maes and Calabrese 1994, for a review).

GABA is the main inhibitory neurotransmitter in the mammalian brain, and GABAergic transmission modulates a variety of other neurotransmitter and neuropeptide systems (Lloyd et al. 1989; Miller and Ferrendelli 1990). This neurotransmitter acts on two main receptors in the brain, $GABA_A$ and $GABA_B$ (Hill and Bowery 1981; Matsumoto 1989). In the past decade, some evidence has accumulated implicating a role for $GABA_B$ receptors in the pathophysiology of mood disorders and in the mechanism of action of antidepressants and mood stabilizers. In animal studies, for instance, a decrease in cortical $GABA_B$ binding has been shown in the animal models of depression such as the helpless rats and the olfactory bulbectomized rats (Lloyd and Morselli 1987). Chronic administration of TCAs normalizes cortical $GABA_B$ receptor density in both helpless (Martin et al. 1989) and bulbectomized (Joly et al. 1987) rats. Furthermore, chronic administration of antidepressants including

TCAs, MAOIs, SSRIs, atypical antidepressants, mood stabilizers such as lithium, carbamazepine, valproate, and ECS, has been reported to increase $GABA_B$ receptors in rat cortex or hippocampus (Lloyd and Pichat 1987; Lloyd, Thuret, and Pilc 1985; Motohashi, Ikawa, and Kariya 1989; Pratt and Bowery 1993; Szekely, Barbaccia, and Costa 1987). Several other studies, however, were unable to confirm up-regulation of $GABA_B$ receptors in the rat frontal cortex following antidepressant treatments (Cross and Horton 1987; Cross and Horton 1988; Engelbrecht, Russell, and Taljaard 1994; McManus and Greenshaw 1991).

In humans, one way to assess $GABA_B$ receptor function is to measure GH release following administration of baclofen, a β-p-chlorophenyl derivative of GABA and a $GABA_B$ receptor agonist (Muller 1987). This endocrine challenge paradigm is based on two observations: 1) that hypothalamic $GABA_B$ receptor sites are involved in the modulation of GH secretion (Gamse et al. 1980; Muller 1987), and 2) that the administration of baclofen induces a significant increase in GH concentrations in normal healthy humans (Koulu, Lammintausta, and Dahlstrom 1979). Using this paradigm, two out of four studies (Davis et al. 1997; Marchesi et al. 1991; Monteleone et al. 1990a; O'Flynn and Dinan 1993) demonstrated a significant reduction of GH response to baclofen in patients with major depression compared with matched healthy controls, supporting the idea that down-regulated $GABA_B$ receptor function is associated with depression. To our knowledge, there is no study to date that examined the $GABA_B$ receptor function in manic patients. Similarly, no studies to date examine the effect of DVP on $GABA_B$ receptors in humans. The purpose of this study, therefore, was to examine the effect of DVP on the $GABA_B$ receptor function in humans by

measuring GH release to baclofen challenge in ten male healthy volunteers before and after one-week DVP treatment.

B.   Methods

Ten male healthy volunteers (mean age ± S.D.: 25.9 ± 5.8 years) gave written informed consent for participation in the study which had been approved by the Clinical Research Ethics Committee of the University of British Columbia. Subjects were evaluated with a SCID-NP (Spitzer et al. 1992), a medical history and a physical examination by a research psychiatrist. All subjects had no past or present psychiatric history and had no current medical conditions. They were free of a family history of psychiatric illness in first-degree relatives, as determined by a clinical history. They were also medication-free for a minimum of 4 weeks prior to study, smoked less than 5 cigarettes per day, and ingested no more than the equivalent of five beers per week and three cups of coffee per day.

Subjects, having fasted from midnight, reported to the research unit between 8:00 a.m. and 8:30 a.m. on the day of the baclofen test. After being weighed (mean weight ± S.D.: 79.0 ± 14.5 kgs), they reclined on a bed in a comfortable position with the head elevated. An intravenous cannula was inserted in a forearm vein at 8:30 a.m. and subjects were allowed to rest but not sleep, smoke or eat. The first blood sample for baseline GH level was taken at 10:00 a.m. (time "0"). Baclofen 20 mg was given orally at this time and further blood samples were collected every 30 minutes for 3 hours (6 samples). Subjects also rated at baseline, 60, 120, 180 minutes on 100 mm VAS (0=not at all, 100=most ever) on four

subjective states (nausea, drowsiness, headache, concentration). Pulse rate and blood pressure were recorded at regular intervals throughout the procedure.

After the pre-treatment study, subjects received DVP 1000 mg / day (500 mg in the morning and 500 mg in the evening) for 7 consecutive days on an outpatient basis. During the 7 days, subjects were asked to record the side effects of DVP with an open questionnaire. The side effects reported in the questionnaire were classified as mild, moderate, and severe, and were defined by "not affecting usual activity", "mild disruption in usual activity", and "major disruption in usual activity", respectively. Subjects took the last dose of DVP at 10:00 p.m. on the 7th day, and baclofen challenge was repeated at 8:30 a.m. of the 8th day. An additional blood sample was taken at 10:00 a.m. (12 hours after the final dose) on the day of testing for measuring the blood levels of valproic acid to check for compliance (mean valproic acid level $\pm$ S.D.: 513.8 $\pm$ 92.4 μmol / l). Dose and drug administration as well as hormonal and behavioral monitoring were identical to those in the pre-treatment study.

Venous samples were collected into tubes containing EDTA, stored on ice and centrifuged within 60 minutes. Serum was separated and then was stored at -80^0 C for assay at a later time. All samples from each subject were assayed in the same batch. The samples were assayed by a lab technician blind to the study conditions. Valproic acid concentrations were determined using FPIA technique. GH was assayed by Quantitope HGH Radioimmunoassay (Kallestad diagnostics). The sensitivity of GH assay was 0.2 ng / ml. The inter-assay coefficients of variation were 10.8 % for GH pool of 2.6 ng / ml, 6.6 % for GH pool of 5.8 ng

/ ml, and 5.7 % for GH pool of 11.3 ng / ml. The intra-assay coefficients for GH were 6.8%,

5%, and 9.1% for GH pools of 2.5 ng / ml, 5.4 ng / ml, 35.2 ng / ml respectively.

C.   Statistical Analysis

Wilcoxon's sign rank test was used to compare the baseline GH levels between pre- and

post-treatment conditions, because the GH data were not normally distributed. Repeated

measures analysis of variance (ANOVA) was used to examine the treatment effect of DVP

on the GH responses to baclofen, which were measured as the net change from baseline at

each time point following challenge (labeled as Δ GH). Post-hoc comparisons were carried

out using Wilcoxon's sign rank tests. The GH responses were also calculated as 1) the net

maximal response (labeled as Δ_{max} GH), that is, the peak response minus baseline, 2) the net

area under the time curve (labeled as AUC GH), using trapezoidal method with subtraction

of the baseline values. The change in Δ_{max} GH was defined by the difference between pre-

treatment Δ_{max} GH and post-treatment Δ_{max} GH. Behavioral responses to baclofen (labeled as

Δ_{max} VAS scores) were calculated as the difference between baseline VAS scores and

maximal peak scores. The Δ_{max} and AUC GH data as well as Δ_{max} VAS scores were analyzed

with Wilcoxon's sign rank tests. Relationships between variables were assessed by means of

Spearman's rank order correlation coefficients. All significance levels reported were two-

tailed. Data were reported as mean ± S.D.. The data were analyzed using the SPSS software.

D.   Results

The data for baseline, peak, Δ_{max}, and AUC GH of the ten subjects are presented in Table 2.

Two out of the ten subjects were excluded from data analysis. One subject did not have an

increase in GH response to baclofen challenge before DVP treatment (defined by Δ_{max} GH >

0.5 ng / ml) (Davis et al. 1996b), and the other one had baseline GH levels above 5 ng / ml

post-treatment. The exclusion of subjects with large baseline GH levels is standard practice

because after a GH secretory episode, the pituitary is relatively refractory (Vance et al.

1985). For the remaining 8 subjects, there was no significant difference in baseline plasma

GH levels between pre-treatment and post-treatment conditions (0.56 ± 0.32 vs 0.98 ± 0.70

ng / ml; $Z=-1.54$, $P=0.12$). The baclofen induced GH responses measured as Δ_{max} GH was

significantly attenuated after DVP treatment (pre- vs post-treatment, 4.34 ± 3.30 vs $2.78 \pm$

3.15 ng / ml, $Z=-1.96$, $P=0.05$). There was also a significant reduction in the AUC GH after

DVP treatment compared with pre-treatment (pre- vs post-treatment, 320.3 ± 219.8 vs 129.7

± 207.2 ng x min / ml; $Z=-2.52$, $P<0.02$).

Δ GH levels at various time points for the 8 subjects are plotted in Figure 12. Repeated

measures ANOVA on the Δ GH data showed a significant time effect ($F=3.72$, d.f.=6,42,

$P<0.006$), a significant treatment effect ($F=21.67$, d.f.=1,7, $P<0.002$), but no significant

interaction between time and treatment ($F=1.38$, d.f.=6,42, $P=0.24$). Post hoc analyses using

Wilcoxon's signed ranks tests showed a significantly attenuated GH response at 90 minutes

after DVP treatment compared with pre-treatment (pre- vs post-treatment, 3.84 ± 3.59 vs

1.09 ± 1.61 ng / ml, $Z=-2.36$, $P<0.02$).

As shown in Figure 13, there was a significant positive correlation between the changes in Δ_{max} GH response (that is, the pre-treatment Δ_{max} GH minus post-treatment Δ_{max} GH) and the blood levels of valproic acid (Spearman's r=0.79, P<0.03), suggesting that the higher the blood levels of valproic acid, the more attenuated the GH responses to baclofen. There was, however, no significant correlation between the changes in Δ_{max} GH response and age (Spearman's r=0. 27, P=0.52) or body weight (Spearman's r=0.29, P=0.69).

With regard to behavioral responses, baclofen administration significantly decreased concentration (Z=-1.96, P=0.05) but overall did not have a significant effect on nausea (Z=0.00, P=1.0) , headache (Z=-0.38, P=0.70) or drowsiness (Z=-0.48, P=0.62). DVP treatment did not significantly alter any of these behavioral responses. There were no correlations between Δ_{max} GH levels and Δ VAS scores of concentration, nausea, headache or drowsiness either before or after DVP treatment (data not shown). The adverse events with DVP, which were reported with open questionnaires, included stomach upset and diarrhea (12.5% with mild degree), headache (25% with mild degree), feeling tired (25% with moderate degree), feeling sleepy (12.5% with moderate degree), and feeling moody (12.5% with moderate degree). Three out of the 8 subjects did not report any significant side effects.

E.   Discussion

Our major findings in the present study included 1) administration of baclofen, a $GABA_B$ receptor agonist, significantly increased plasma GH levels in healthy male humans 2) one week treatment with DVP significantly attenuated the GH response to baclofen, and 3) there

Table 2. Growth hormone responses to baclofen before and after treatment with divalproex sodium

Subject	Pre-Divalproex				Post-Divalproex			
	Baseline GH	Peak GH	Δ_{max} GH	AUC GH	Baseline GH	Peak GH	Δ_{max} GH	AUC GH
1	0.94	3.44	2.50	211.1	0.67	1.44	0.77	46.9
2	0.08	2.11	2.03	185.3	0.98	1.42	0.44	-4.9
3	0.67	9.76	9.09	739.5	0.37	9.44	9.07	622.8
4	0.82	2.51	1.69	160.3	1.17	1.60	0.43	-22.8
5	0.33	4.61	4.28	317.4	0.90	6.40	5.50	133.3
6	0.17	1.03	0.86	57.7	0.40	0.80	0.40	39
7	0.80	5.70	4.90	388.6	2.60	6.40	3.80	97.9
8	0.70	10.10	9.40	502.8	0.80	2.70	1.90	125.8
Mean ±SD	0.56 ± 0.32^a	4.90 ± 3.42	4.34 ± 3.30^b	320.3 ± 219.8^c	0.98 ± 0.70^d	3.77 ± 3.19	2.78 ± 3.15^e	129.7 ± 207.2^f
9	1.39	1.34	-0.05	-83.55	1.04	3.90	2.86	136.6
10	1.05	4.55	3.50	164.2	6.72	5.69	-1.03	-504.3

GH: Growth hormone (ng / ml), Δ_{max} GH (ng / ml): Peak minus baseline GH levels
AUC GH (ng x min / ml): Growth hormone response to baclofen calculated as net area under curve (AUC_{0-180})
a vs. *d*: P=0.12, *b* vs. *e*: P=0.05, *c* vs.*f*: P<0.02 (Wilcoxon's Sign Rank Tests)
Subject 9 had the highest baseline GH pre-treatment (1.39 ng / ml) and did not have an increase GH response defined by Δ_{max} GH > 0.5 ng / ml
Subject 10 had a baseline GH level above 5 ng/ml post-treatment (6.72 ng / ml)

Figure 12.   Mean (± S.E.M.) GH response to baclofen in healthy male humans before and after DVP treatment.
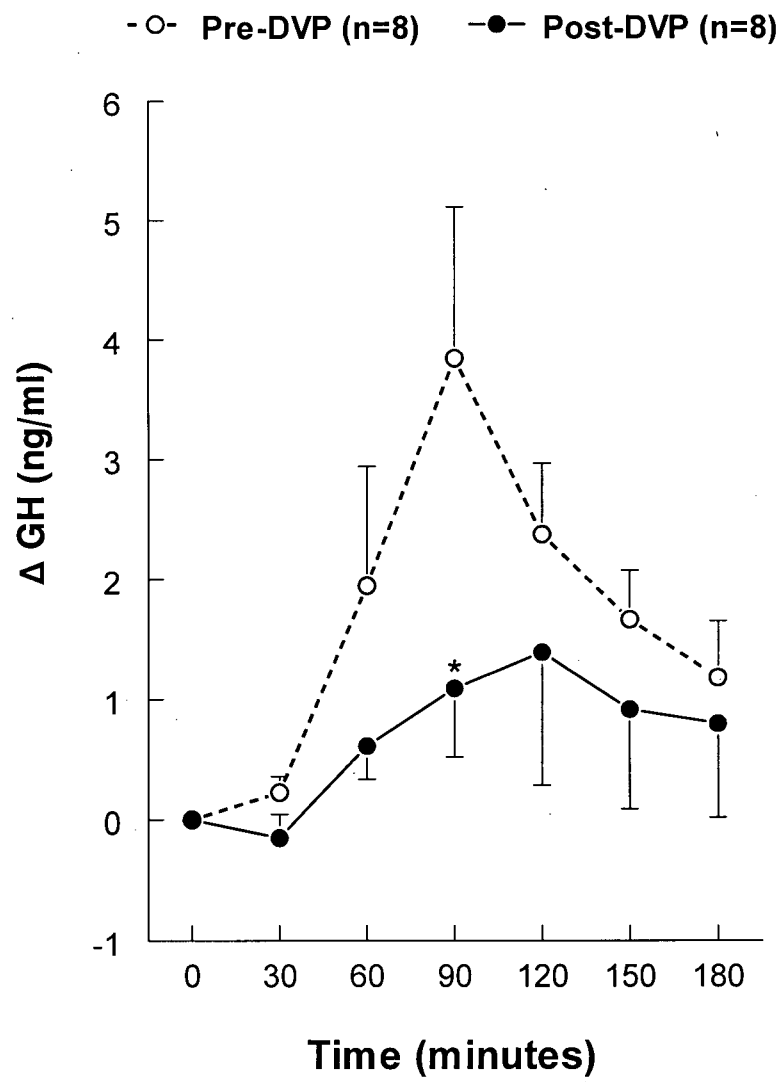
Figure 13.   The degree of attenuation effect of DVP on baclofen-induced GH response, measured as change in Δ max GH, and the blood levels of valproic acid in healthy male humans. The line indicates a significant positive correlation between change in Δ max GH and blood levels of valproic acid.

Spearman's r = 0.79, P < 0.03, N=8
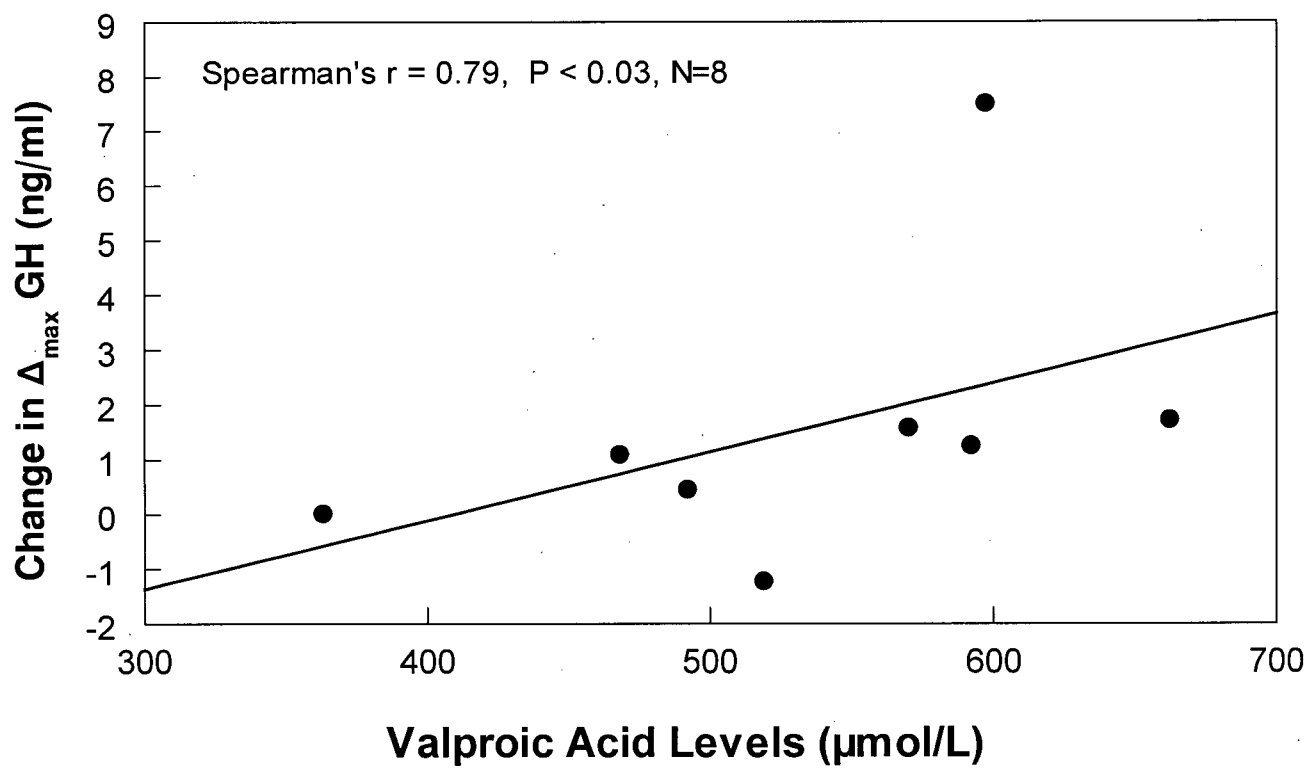
was a significant positive correlation between the degree of attenuation of GH responses and the blood levels of valproic acid.

Our finding of an increase in GH release in healthy males following administration of 20 mg of baclofen, is consistent with previous studies (Davis et al. 1996b; Lucey et al. 1994; Monteleone et al. 1990a; Monteleone et al. 1990b; O'Flynn and Dinan 1993), and further supports a role for $GABA_B$ receptors in modulating GH release in humans. One of our study subjects, however, failed to significantly increase GH response to baclofen. This was also reported in two previous studies that showed some healthy subjects did not have an increased GH response to 20 mg dose of baclofen (Davis et al. 1996b; Lucey et al. 1994). It is unclear why some normal subjects have little or no GH response to baclofen (Davis et al. 1996b). But, it is of interest to note that in our ten study subjects, the only subject who did not have an increase in GH response, had the highest baseline GH pre-treatment (1.39 ng / ml). Such a finding supports the view that subjects with high baseline GH levels may show blunted responses to a subsequent pharmacological challenge (Davis et al. 1996b; Laakmann et al. 1990).

Our finding of an attenuation of $GABA_B$-mediated endocrine response to DVP in healthy males, suggests that DVP down-regulated hypothalamus $GABA_B$ receptors in the study subjects. Although the sample size for this study was relatively small, the attenuating effect of DVP was further confirmed by the positive correlation between the degree of attenuation measured as change in Δ_{max} GH and the blood levels of valproic acid. Since we did not include a placebo control condition to minimize the effects of confounding variables such as

stress effects and variations in baseline hormone levels, we can not exclude the possibility of a Type I error of inference (Thompson, Maes, and Meltzer 1994). In addition, we did not measure the plasma levels of baclofen. We, therefore, can not exclude the possibility of a pharmacokinetic interaction between baclofen and DVP leading to lower plasma baclofen levels during post-treatment condition, and hence lower GH response. Such, however, seems unlikely because that pharmacokinetic data have shown that DVP and baclofen are metabolized through different mechanisms. DVP is metabolized via glucuronidation or oxidation (McNamara 1996), whereas baclofen is excreted unaltered in the urine (85%) or metabolized via deamination (Krogh 1995).

It is of interest to note that our finding is at variance with two rat studies which demonstrated that chronic treatment with sodium valproate significantly increased $GABA_B$ receptors in the rat hippocampus or frontal cortex (Lloyd, Thuret, and Pilc 1985; Motohashi 1992). Since these two animal studies did not examine the effect of valproate on the hypothalamic $GABA_B$ receptors, and that there is, to date, no other study that examined the effect of DVP on $GABA_B$ receptors in different brain regions, we do not know if valproate treatment alters (up- or down-regulates) the hypothalamic $GABA_B$ receptors in the rat. This raises the possibility that DVP may have differential effects on $GABA_B$ receptors in different brain regions. That is, DVP may up-regulate $GABA_B$ receptors in hippocampus or frontal cortex while down-regulating those in hypothalamus. In support of this hypothesis, some indirect evidence has been provided by regional brain studies of rats. Patsalos and Lascelles (1981) showed that that 10 days treatment with sodium valproate significantly elevated the brain GABA concentrations in several brain regions including hippocampus, cerebral cortex, pons

and medulla, and cerebellum, but produced little change in GABA levels in hypothalamus, striatum and mid-brain. Baf et al. (1994) examined the treatment effect of sodium valproate on monoamine levels in different regions of the rat brain. They found that after 45 days administration of sodium valproate, there was a significant increase in NE levels in hippocampus and brain stem, whereas a significant decrease was noted in hypothalamus. Serotonin levels were significantly increased in striatum-accumbens, brain stem, motor cortex, and hippocampus, but they were significantly decreased in hypothalamus and cerebellum. Furthermore, the only animal study that examined the effects of another mood stabilizer, lithium, on GABA receptor binding in hypothalamus, has shown a decrease in [^{3}H]-GABA binding sites in rat hypothalamus following chronic administration of lithium (Maggi and Enna 1980). This is compatible with the result of our study that showed a down-regulation of hypothalamic $GABA_B$ receptor function with DVP treatment.

An alternative explanation for the discrepancy in findings between our study and the animal studies is species differences in $GABA_B$ receptor responsivity to valproate between humans and rodents. Consistent with this hypothesis, studies that examined the effects of valproate on $5-HT_{1A}$ receptor responsivity, have demonstrated conflicting results between humans and rats. In humans, we have recently found that DVP significantly attenuates the hypothermic response to ipsapirone, a $5-HT_{1A}$ receptor agonist, in ten healthy males (Shiah et al. 1997). In rats, however, Khaitan et al. (1994) reported that DVP did not alter the hypothermic response to 8-OH-DPAT (a prototypic $5-HT_{1A}$ receptor agonist). Further evidence in support of the possibility of species difference came from the studies that examined the antidepressant effects on $GABA_B$ receptor function. In humans, Monteleone et al. (1990a) reported that 28

days of amitriptyline (100 mg / day) treatment did not significantly alter the GH response to baclofen in 8 male depressed patients. A subsequent study by the same group (Monteleone et al. 1990b) reported that 15 and 35 days of treatment with amitriptyline (100 mg / day), imipramine (100 mg / day) and fluoxetine (20 mg / day) also did not modify this endocrine response in ten male depressed patients, even when a clear therapeutic effect was obtained. In contrast to the negative findings in human studies, rat studies showed an up-regulation of $GABA_B$ receptors following chronic administration of antidepressants (Lloyd and Pichat 1987; Lloyd, Thuret, and Pilc 1985).

In summary, we found that DVP treatment significantly attenuated baclofen-induced GH response in humans, and this attenuating effect was related to blood levels of valproic acid. Our findings may suggest that down-regulation of $GABA_B$ receptor function in the hypothalamus is involved in the mechanism of action of DVP in humans. The results of this study should, however, be viewed as preliminary, in light of the small sample size and the lack of placebo control. Further studies are required to replicate our findings before any firm conclusion can be drawn.

CHAPTER VI   GENERAL DISCUSSION, RECOMMENDATIONS FOR FUTURE STUDIES, AND CONCLUSIONS

A.   Major Findings and Limitations

The major findings of this thesis are summarized as follows:

1) manic patients have significantly enhanced ACTH and cortisol responses to ipsapirone when compared to normal controls, but the hypothermic response to ipsapirone did not differ between the two groups;

2) one-week treatment with DVP significantly attenuated hypothermic response to ipsapirone in healthy controls but this did not modify the ACTH or cortisol response to ipsapirone;

3) GH response to baclofen was significantly increased in manic patients when compared to normal controls;

4) one-week treatment with DVP significantly attenuated the GH response to baclofen in healthy controls, and this attenuating effect was related to the blood levels of valproic acid.

Although the limitations of individual experiments have been discussed in Chapters II to V, several other limitations for the whole thesis research need to be considered. First, we performed the experiments at different times over a period of three years, and blood samples from each experiment were assayed separately. This may have led to variations of hormone data between experiments. Second, the statistical methods we used to analyze data are not consistent throughout the thesis. We used parametric tests for calculating those data that fit

normal distributions whereas we employed non-parametric tests for those data that did not fit normal distributions. Third, we did not use dose-response study design in our experiments, which may provide more solid evidence to support or dispute the thesis hypotheses. However, we chose the 0.3 mg/kg dose of ipsapirone because dose-response and antagonist studies conducted in healthy subjects (Lesch et al. 1990c, 1990d) revealed that this dose produces robust and reliable hypothermic and hormonal responses. Similarly, the 20 mg dose of baclofen was chosen because a dose-response study (David et al. 1996b) showed that this dose induced a more robust GH release in healthy subjects than 10 mg of baclofen. Fourth, we did not control for seasonal effects on body temperature and hormonal release. This might have confounded the thesis findings. Finally, the experiments on the effects of DVP on 5-HT_{1A} and $GABA_B$ receptors were conducted in normal healthy volunteers and not in manic patients. It is therefore, unknown, if DVP will have the same effects on 5-HT_{1A} and $GABA_B$ receptors in manic patients. Further treatment studies of DVP on 5-HT_{1A} and $GABA_B$ receptor function in manic patients are needed to clarify this issue.

B. 5-HT Function in Mania and in the Mechanism of Action of DVP

As reviewed in the Chapter I, CSF, postmortem, platelet, and neuroendocrine challenge studies have provided some evidence to support the hypothesis of low 5-HT function in mania. Since 5-HT is involved in the regulation of mood, sleep, circadian pattern, vigilance, feeding, sexual behavior and motor output, disturbance of this neurotransmitter functioning can cause derangement of these behavioral and physiological responses, which are often observed in manic patients. In addition, it has also been suggested that low 5-HT function in

brain can lead to a compensatory up-regulation of postsynaptic 5-HT receptors (Stahl 1996) (see Figure 14). Consistent with this hypothesis, our first experiment showed that manic patients have enhanced ACTH and cortisol responses to ipsapirone, suggesting an increase in postsynaptic 5-HT_{1A} receptor sensitivity, presumably resulting from diminished 5-HT functioning in this condition.

With regard to the effects of DVP on 5-HT receptors, the findings of our second experiment suggest that DVP attenuates presynaptic 5-HT_{1A} autoreceptors but does not appear to have an effect on postsynaptic 5-HT_{1A} receptors. Presynaptic 5-HT_{1A} autoreceptors act as regulators of 5-HT release. Stimulation of these receptors causes a shutdown of 5-HT neuronal impulse flow and 5-HT release. When they are blocked, 5-HT release will increase. Given this, the attenuating effect of DVP on presynaptic 5-HT_{1A} autoreceptors would therefore lead to an increase in 5-HT release in 5-HT synapses (see Figure 14).

Taken together, the results of 5-HT neuroendocrine challenge studies in this thesis have provided evidence to support the contribution of 5-HT_{1A} receptors in mania and in the mechanism of action of DVP. Neuroendocrine challenge studies, however, provide the information on 5-HT activity in the hypothalamic region only but not in other brain regions. With the development of PET technique and suitable radioligands, it is now possible to examine distinct 5-HT receptor subtypes in vivo in different brain regions in humans. For example, suitable radioligands have been developed for quantitative

Figure 14. Depletion of 5-HT in synapses can lead to a compensatory up-regulation of postsynaptic 5-HT receptors (indicated by the empty arrow). DVP treatment can attenuate presynaptic 5-HT$_{1A}$ autoreceptors, which would in turn increase 5-HT release in synapses.

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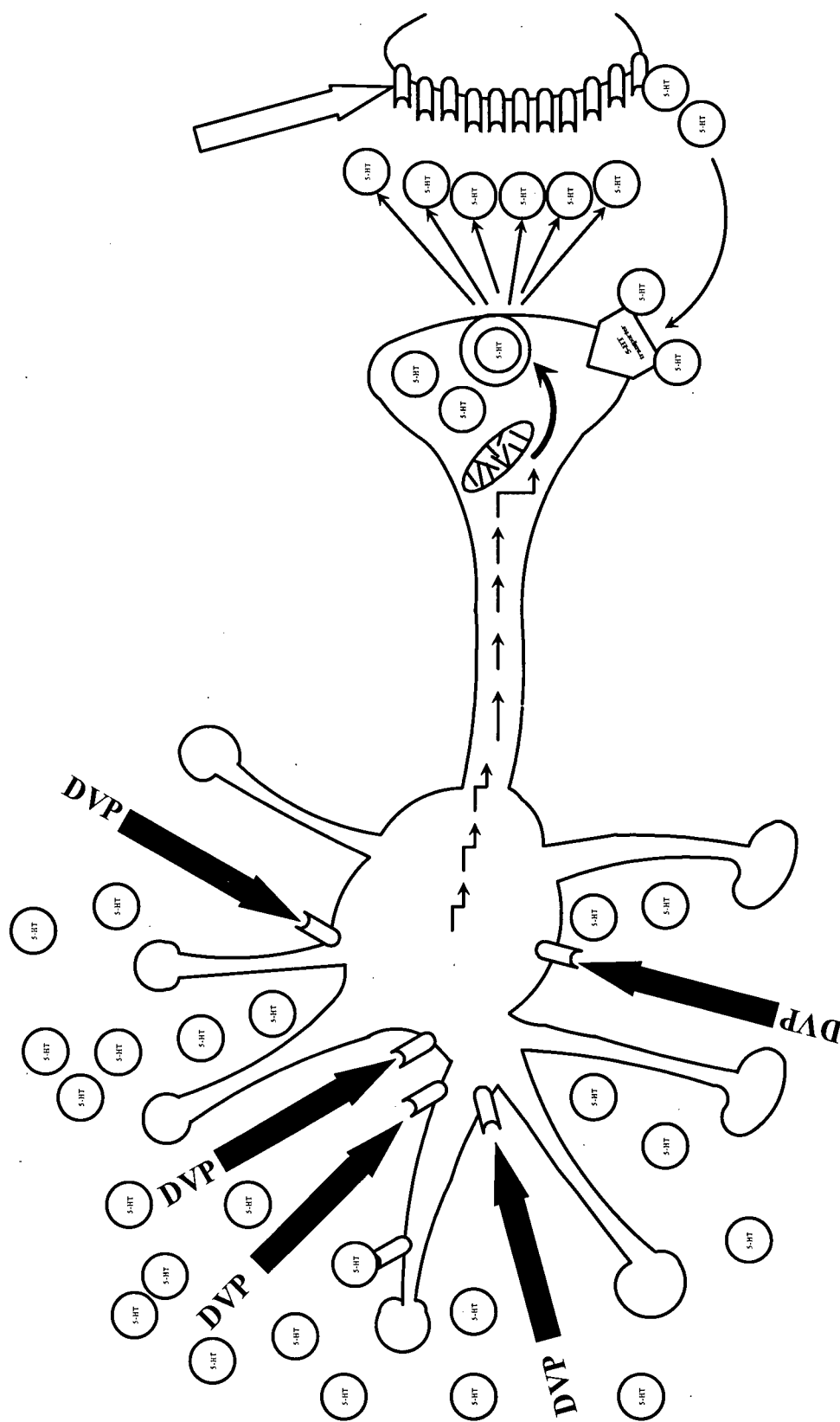
determination of 5-HT$_2$ (Nyberg et al. 1996) and 5-HT$_{1A}$ (Farde et al. 1998; Ito, Halldin, and Farde 1999) receptors in humans. There have been several PET studies that examined 5-HT receptors in patients with various psychiatric disorders (Attar-Levy et al. 1999; Biver et al. 1997; Blin et al. 1993; Lewis et al. 1999; Massou et al. 1997; Mayberg et al. 1988; Wang et al. 1995; Yatham et al. 1999) including major depression (Attar-Levy et al. 1999; Biver et al. 1997; Massou et al. 1997; Yatham et al. 1999). But, this has not been done in manic patients to date. Such receptor imaging studies will likely enhance our understanding of the role of 5-HT in mania and its treatment.

C. GABA Function in Mania and in the Mechanism of Action of DVP

As discussed in Chapter IV, the results of the third experiment suggest that patients with mania also have an up-regulated hypothalamic GABA$_B$ receptor function, presumably resulting from low GABA function in this condition. Further studies of GABA function in manic patients are needed to strengthen the hypothesis of low GABA function in mania. These would include 1) GABA$_A$ and GABA$_B$ receptor binding in postmortem brain in a pure group of bipolar patients; 2) brain GABA levels with magnetic resonance spectroscopy; 3) GABA$_A$ and GABA$_B$ receptors with PET or single photon emission computed tomography.

As discussed earlier, based on 1) our findings that GH response to baclofen was enhanced in mania and that DVP treatment attenuated the GH response to baclofen in healthy humans; 2) the animal study that showed a decrease in [^{3}H]-GABA binding sites in rat hypothalamus after chronic treatment with lithium; and 3) the assumption that mood stabilizers such as

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lithium and DVP enhance GABAergic neurotransmission in the brain, we hypothesize that chronic treatment with DVP restores the GABA deficit in mania by enhancing GABAergic neurotransmission, thus leading to a down-regulation of hypothalamic GABA receptors (see Figure 15). Further treatment studies of DVP on GABA function in manic patients are needed to verify this.

D.   Interaction between 5-HT, GABA and Other Neurotransmitters

Given that GABA is considered as an inhibitory neurotransmitter, one may expect a high rather than low GABA function in patients with mania, which in turn would result in a low 5-HT function. However, this is not necessarily true. As reviewed by Petty and his colleagues (Petty 1995; Petty, Kramer, and Hendrickse 1993), it is likely that much of GABA activity in brain pertinent to mood disorders is involved in local circuits and interneurons, which may actually lead to a facilitatory effect on serotonergic function. Evidence in support of this was provided by the studies involving interaction between GABA and 5-HT. For example, administration of a GABA agonist, γ-vinyl GABA was found to lead to a facilitation of basal 5-HT release in the prefrontal cortex of rodents (Petty, Kramer, and Hendrickse 1993). Stimulation of GABA receptors in suprachiasmatic nucleus of rat brain was also reported to induce an increase in 5-HT release (Francois-Bellan et al. 1988). Furthermore, it has been shown that chronic administration of the mixed $GABA_A$ and $GABA_B$ receptor agonist, progabide (Gray et al. 1986; Green et al. 1985; Green and Nutt 1983) and the $GABA_B$ receptor agonist baclofen (Metz, Goodwin, and Green 1985) increases the $5-HT_2$ receptor-mediated head twitch response and the density of cortical $5-HT_2$ receptors in rodents. This might suggest that $GABA_B$ receptors act as heteroceptors on serotonergic terminals in limbic

Figure 15.   DVP restores the GABA deficit in mania by enhancing GABAergic neurotransmission, thus leading to a down-regulation (indicated by empty shapes with broken lines) of hypothalamic GABA receptors (Triangles: $GABA_A$ receptors, Ovals: $GABA_B$ receptors).
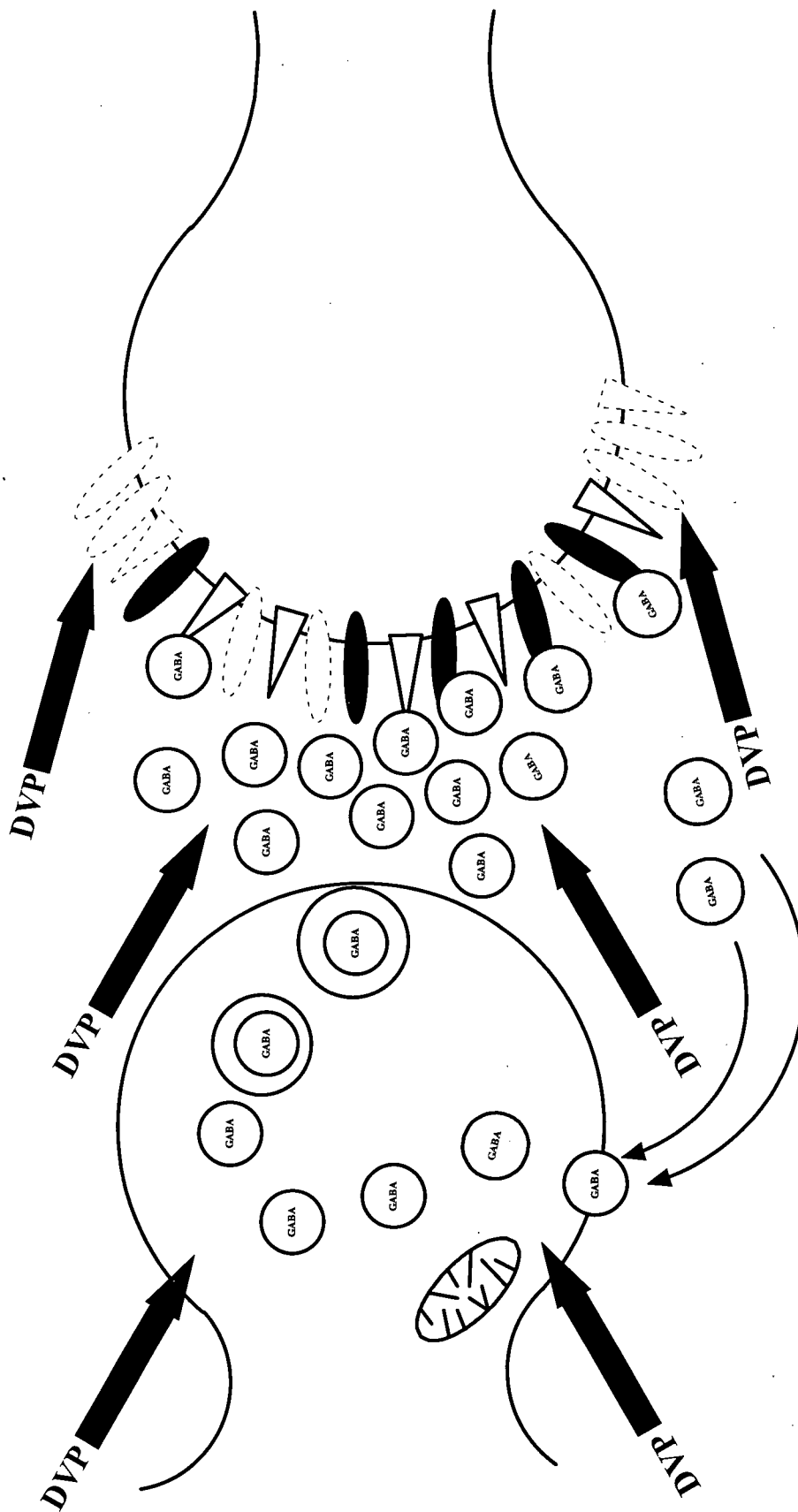
regions of the rat brain (Leonard 1994). Taken together, the studies of interaction between GABA and 5-HT suggest that GABA facilitates serotonergic function in some ways. This, therefore, might explain why low and not high GABA activity could be associated with mania. If this is true, the hypothesis of low GABA function can be integrated into the monoaminergic theories of mood disorders. In other words, low GABA function in brain may be a primary abnormality for mood disorders and can lead to an alteration in 5-HT, NE and DA functioning. In the case of depression, low GABA function is associated with low 5-HT, NE and DA function. In the case of mania, low GABA function is accompanied by low 5-HT and high NE and DA function (see Figure 16). There has been some evidence in the literature to support the hypothesis of excess catecholamine (NE and DA) function in mania (Buki and Goodnick 1998, for review) and the hypothesis of low catecholamine function in depression (Anand and Charney 1997, for review). Further studies of simultaneous measurement of activity of different neurotransmitter systems in patients with mood disorders are needed to verify this.

Another issue that is related to the interaction between different neurotransmitters and needs to be addressed in future studies is diagnostic subtypes. Acute mania can be subdivided into classic pure mania, mania with mood-congruent or mood-incongruent psychotic features, and mixed state (Kusumakar et al. 1997). It is possible that patients with different subtypes of mania may have alterations in different neurotransmitter activity and/or interaction. For example, Swann et al. (1987) found that female mixed manic patients had higher values for CSF 5-HIAA and homovanillic acid (HVA) than pure manics. Similarly, in a subsequent study by the same group (Swann et al. 1994), patients with mixed mania had higher urinary

Figure 16.   5-HT, GABA, NE, DA function and their interaction are involved in depression and mania.

Mania

Depression
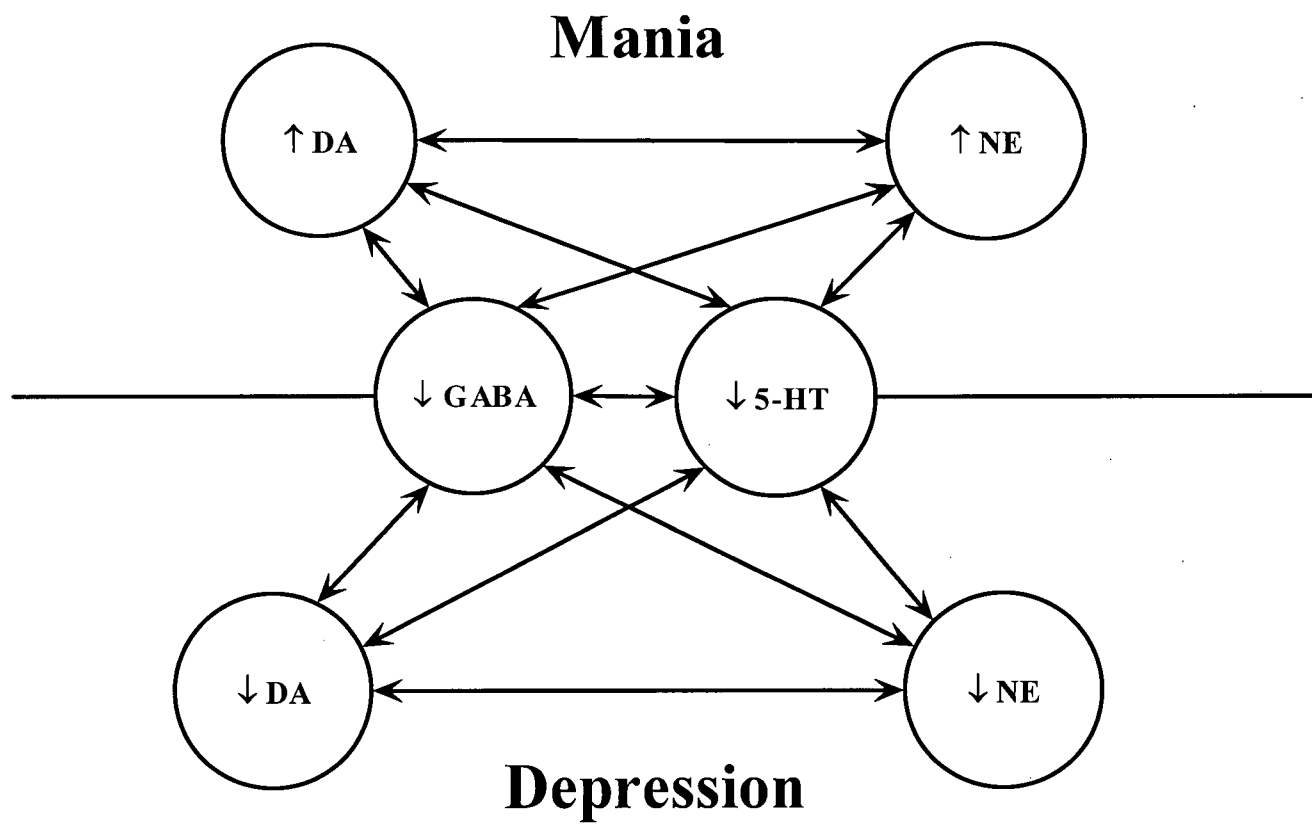
excretion of NE, and higher CSF 3-methoxy-4-hydroxyphenylglycol (MHPG) levels compared to patients with pure mania. Their findings suggest that mixed manic patients may differ from pure manics in the biochemical basis for their symptoms. Future biological studies delineating specific subtypes of mania will extend our current understanding of pathophysiology and ultimately lead to better treatment strategies for different subtypes of mania.

E. Conclusions

In conclusion, experiments in this thesis have shown that manic patients have an abnormality in the functioning of 5-HT_{1A} and $GABA_B$ receptors, which may result from deficits in 5-HT and GABA function. Studies in healthy controls in this thesis have shown that DVP administration for one week can modify the functioning of $GABA_B$ and presynaptic 5-HT_{1A} autoreceptors. These effects may be related to the efficacy of DVP in the treatment of mania. Taken together, our results support the involvement of 5-HT and GABA in mania and in the mechanism of action of DVP. However, alterations in the functioning of other neurotransmitters such as NE and DA and their interaction with 5-HT and GABA also likely contribute. More neurobiological studies on manic patients are warranted to enhance our understanding of biology of this illness and its treatment.

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