Pterins and Affective Disorders

Rocco Hoekstra

Pterinen en Stemmingsstoornissen

Pterins and affective Disorders

Pterinen en stemmingsstoornissen

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. S.W.J. Lamberts

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 19 september 2007 om 13.45 uur

door
Rocco Hoekstra

geboren te Vlaardingen

ERASMUS UNIVERSITEIT ROTTERDAM

Promotiecommissie

Promotor:
Prof.dr. W.M.A. Verhoeven

Overige leden:
Prof.dr. H.A. Drexhage
Prof.dr. M.W. Hengeveld
Prof.dr. R.S. Kahn

Copromotoren:
Dr. D. Fekkes
Dr. S. Tuinier

The research described in this thesis was carried out at the Department of Psychiatry of the Erasmus Medical Center, Rotterdam, the Netherlands and at the Delta Psychiatric Center, Poortugaal, the Netherlands.

The study described in this thesis was supported by a grant of the Stichting Jos Schaap-Feering, Rotterdam, the Netherlands.

Voor Cindy
Voor Maxime en Carmen

graphic design: IJzersterk, Onne Schuilenburg
Illustration design: Yke Schotten
Printing: De Nieuwe Grafische, Rotterdam

Support of this thesis by AstraZeneca, Bristol-Myers Squibb, GlaxoSmithKline, Janssen-Cilag B.V., Lundbeck BV, Organon Nederland bv, Servier Nederland Farma BV and Wyeth Pharmaceuticals bv is greatly acknowledged.

Contents

Abbreviations

Arg	arginine
BDI	Beck Depression Inventory
BH4	tetrahydrobiopterin
Cit	citrulline
CNS	central nervous system
CSF	cerebrospinal fluid
ECT	electroconvulsive therapy
GABA	gamma-aminobutyric acid
GTP	guanosine triphosphate
GTPCH	guanosine triphosphate cyclohydrolase
HRSD	Hamilton Rating Scale for Depression
HPA	hypothalamus-pituitary axis
HPLC	high performance liquid chromatography
5-HT	serotonin
IDO	indoleamine 2,3-dioxygenase
LNAA	large neutral amino acids
NMDA	N-methyl-D-aspatate
NO	nitric oxide
NOS	nitric oxide synthase
Phe	phenylalanine
SAD	seasonal affective disorder
SCN	suprachiasmatic nucleus
Trp	tryptophan
Tyr	tyrosine
YMRS	Young Mania Rating Scale

Introduction

Chapter One

Introduction

[1]1. Introduction

After centuries with various ideas about the pathophysiological mechanisms of '$\mu\varepsilon\lambda\alpha\nu\chi o\lambda\iota\alpha$' (melancholia, from the Greek, 'black bile'), in the 1950's, neurochemical hypotheses of mood disturbances emerged after the development of antidepressants (Lehmann and Kline, 1983; Wong and Licinio, 2001). The first idea on the biochemical pathophysiology of depression was the so-called monoamine hypothesis (Schildkraut, 1965). Based on this hypothesis, the selective serotonin reuptake inhibitors were developed. Over the past decades, it has become obvious that alterations in monoamines are most probably related to an overdrive of the HPA-axis, induced e.g. by stress (Holsboer, 1995; Dinan, 1995; De Kloet et al., 1996; De Kloet et al., 1998).
Early neurochemical explanations of bipolar disorders have implicated catecholamines and other neurotransmitters like serotonin, acetylcholine and GABA. More recent hypotheses deal with intracellular signaling cascades (Anand and Charney, 2000; Massat et al., 2000; Manji et al., 2001; Kugaya and Sanacora, 2005).
Despite extensive research, however, biochemical abnormalities underlying the predisposition to and the pathophysiology of affective disorders have so far not clearly been elucidated. Another area of research focuses on the role of pterins in affective disorders. Pterins (also called pteridines) are derived from guanosine triphosphate (GTP) and these compounds are important for the metabolism of monoamines and the biosynthesis of the reactive free radical nitric oxide (NO) (Mayer and Werner, 1995; van Amsterdam and Opperhuizen, 1999; Koshimura et al., 2000).
In the next paragraphs the biosynthesis, localization and clinical relevance of pterins and NO will be concisely described.

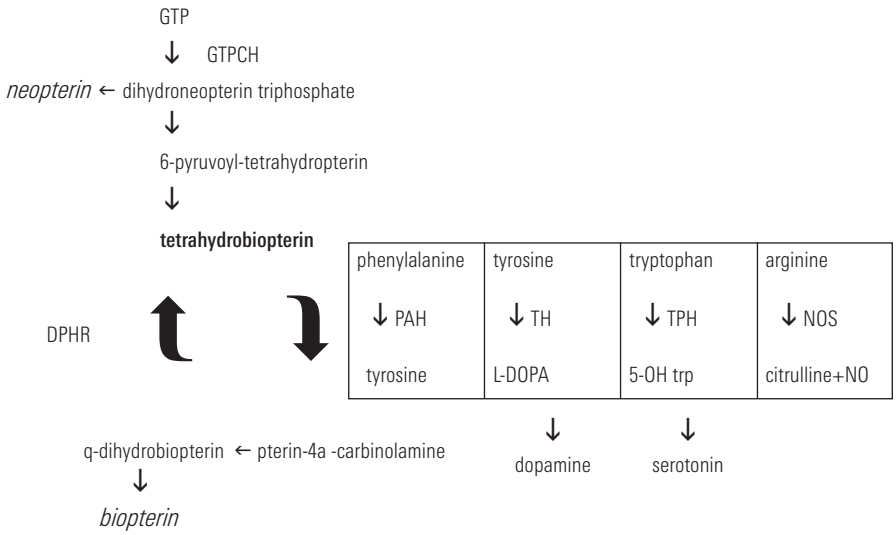
2. Biosynthesis of pterins

Pterins are compounds containing a 2-amino-4-oxo-pyrazine-pyrimidine (pterin) ring, which are derived from GTP via a magnesium-dependent cyclohydrolase (Nichol et al., 1985). GTP is converted to dihydroneopterin triphosphate and this substance can be converted to either neopterin or tetrahydrobiopterin (BH4) (Fig. 1). Neopterin is

1. Part of this Introduction has been published as: Pteridines and affective disorders. Hoekstra R., Fekkes D. Acta Neuropsychiatrica 2002;14:120-126 (addendum of this thesis)

synthesized primarily by human monocytes and macrophages after stimulation by interferon-gamma produced by helper T-cells (Huber et al., 1984). BH4 is the essential cofactor for the hydroxylation of phenylalanine to tyrosine, tyrosine to L-3,4-dihydroxyphenylanine (L-DOPA) and tryptophan to 5-hydroxytryptophan. The latter two reactions are rate-limiting steps in the synthesis of the catecholamines and serotonin, respectively. During these reactions, BH4 is transformed to quinoid dihydrobiopterin via pterin-4a-carbinolamine (Fig. 1). Concentrations of BH4 are maintained by de novo synthesis and by a salvage mechanism that reduces quinoid dihydrobiopterin back to BH4. BH4 is secreted in the urine together with its oxidative products dihydrobiopterin and biopterin (Levine, 1988; Auerbach and Nar, 1997; Thöny et al, 2000). Synthesis of BH4 is not dependent on dietary pterin, which is poorly absorbed from the gut (Leeming, 1980).

Figure 1.
Biosynthesis and function of tetrahydrobiopterin.



GTP = guanosine triphosphate
GTPCH = guanosine triphosphate cyclohydrolase
DHPR = dihydropterine reductase
PAH = phenylalanine hydroxylase
TH = tyrosine hydroxylase
TPH = tryptophan hydroxylase
NOS = nitric oxide synthase
L-DOPA = L-3,4-dihydroxyphenylanine
5-OH trp = 5-hydroxytryptophan
NO = nitric oxide

The reaction catalyzed by GTPCH is the rate-limiting step in the synthesis of tetrahydrobiopterin. Tetrahydrobiopterin is cofactor for the enzymatic reactions in the 4 squares. The cursive compounds are the excretion products of the pterin metabolism. Total biopterin is biopterin, q-dihydrobiopterin together with tetrahydrobiopterin.

3. Localization of pterins

BH4 is present in the liver, the gastrointestinal tract, the adrenal medulla, T-lymphocytes and macrophages. Brain areas like hypothalamus, pituitary gland and pineal gland contain relatively large concentrations of BH4 (Bullard et al., 1978; Levine, 1988; Thöny et al., 2000; Koshimura et al., 2000). Pterins are functionally closely related to the cellular immune system. Interferon-gamma, which is produced and secreted by activated T-lymphocytes, stimulates GTP cyclohydrolase activity, resulting in synthesis of neopterin in macrophages. Neopterin is therefore regarded as a validated marker of the activation of cell-mediated immunity (Dunbar et al., 1992; Maes et al., 1994; Matsuda et al., 1994; Bonaccorso et al, 1998). The close relationship between the cellular immune system and the HPA-axis, suggests a role of the pterins in stress related disorders, like depression.

4. BH4 metabolism and monoamines

BH4 is the natural cofactor of the aromatic amino acid hydroxylating enzymes tryptophan hydroxylase and tyrosine hydroxylase. The reactions catalyzed by these enzymes are the rate limiting steps in the formation of serotonin and the catecholamines dopamine and norepinephrine, respectively (Fig. 1.). Several in vitro studies showed that administration of BH4 results in an increased release of dopamine and serotonin as well as of glutamate (Miwa et al., 1985; Koshimura et al., 1990; Wolf et al., 1991). Independent of its role as a cofactor, BH4 seems to have a direct influence on the release mechanisms of these neurotransmitters (Mataga et al., 1991; Muguruma et al., 1996).
The essential role of BH4 in the synthesis of neurotransmitters is illustrated by congenital BH4 deficiencies. A disturbed BH4 synthesis causes atypical phenylketonuria (Pogson, 1997). About 2% of patients with phenylketonuria show a BH4-deficiency (Blau et al., 1996). In patients with congenital biopterin deficiency, decreased plasma and cerebrospinal fluid (CSF) concentrations of serotonin, dopamine and their metabolites were found next to hyperphenylalaninemia (Kaufman, 1998; Pogson, 1997). Without substitution this leads to severe neurological and psychiatric symptoms (Blaskovics and Giudici, 1988; Kaufman, 1998; Pogson, 1997).

5. Pterins and affective disorders

The relationship between pterins and affective disorders has been studied since the 1980's (Curtius et al., 1983; Levine and Lovenberg, 1984; Blair et al., 1984). This research strategy

was given impetus since it appeared that these compounds influence the synthesis and release of serotonin, dopamine and norepinephrine (Levine, 1988). Subsequent studies investigated the relationship between pterins and nitric oxide (NO) (Mayer and Werner, 1995; van Amsterdam and Opperhuizen, 1999).

5.1 Biochemical studies

As can be inferred from figure 1, the main product in the pterin synthesis is tetrahydrobiopterin (BH4), which is an essential cofactor for several enzymatic processes (Levine, 1988). Along with BH4, neopterin is synthesized from the same precursor. In this respect, several biochemical parameters have been studied of which BH4, total biopterin (the sum of BH4, dihydrobiopterin and biopterin) and neopterin are the most relevant (Hashimoto et al., 1987; Dunbar et al., 1992; Hashimoto et al., 1994). As indirect parameters of BH4 activity, the neopterin-biopterin ratio and/or the phenylalanine-tyrosine ratio are used (Coppen et al, 1989; Anderson et al., 1992; Anderson et al., 1994; Abou-Saleh et al., 1995). Measurements have been done in brain tissue, urine, plasma, serum and CSF. The equivocal results of these studies are depicted in table 1. In most studies an increase of total biopterin was found in depressed patients, whereas the activity of the active BH4 was decreased. The increased neopterin levels as demonstrated in some studies point towards an activation of cell-mediated immune activity (Dunbar et al., 1992; Maes et al., 1994; Bonaccorso et al., 1998).

5.2 Genetic studies

As presented in table 1, some genetic studies have recently been performed in search for mutations in the gene that codes for the enzyme guanosine triphosphate cyclohydrolase (GTPCH), which forms the rate-limiting step in the biosynthesis of BH4. Interestingly, in families with a polymorphism for this particular gene, there is an increased prevalence of psychiatric disorders (Hahn et al., 2001; Kealey et al., 2005; Van Hove et al., 2006).

5.3 Therapy with BH4

Sofar virtually no studies have been conducted using BH4 as a pharmacological agent. Preliminary results from older studies suggest that oral administration of BH4 may have some short lasting beneficial effects on symptoms of depression. (Curtius et al., 1982; Curtius et al., 1983; Woggon et al., 1984; Fleischhacker et al., 1985).

6. BH4 metabolism and Nitric Oxide

Apart from its role as an essential cofactor in the biosynthesis of monoamines, BH4 is required as an obligatory cofactor for the enzymatic conversion of L-arginine to L-citrulline, with nitric oxide (NO) as concomitant product (Fig. 2) (Mayer and Werner, 1995).
The enzyme responsible for the generation of NO is nitric oxide synthase (NOS). Three NOS isoforms have been described: neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS) that are coded by three distinct genes (Mayer and Werner, 1995; Prast and Philippu, 2001).
Endothelium-derived NO plays a crucial role in vascular function and homeostasis. Vascular NO production is almost exclusively attributable to the activity of eNOS and is involved in the vascular blood flow. The enzyme iNOS plays a key role in inflammatory reactions (Akyol et al., 2004; Bernstein et al., 2005).
In the central nervous system (CNS) nNOS is the major NOS isoform that accounts for about 90% of the overall NO production. In the human brain the highest levels of nNOS are measured in the substantia innominata, septal area, cerebellar cortex, nucleus accumbens, hypothalamus and subthalamus. In the cerebral cortex, nNOS is localized in 2% of the neurons, whereas in the human hypothalamic paraventricular nucleus, up to 20% of the neurons express this enzyme (Bernstein et al., 2005). The importance of BH4 in the metabolism of NO is reflected by the corresponding distribution of the activity of both BH4 and NO in the brain (Thöny et al., 2000). Functionally, NO may act as a hormone, neurotransmitter, paracrine messenger, mediator, cytoprotective and cytotoxic molecule (Bernstein et al., 2005).
In most brain structures NO modulates the release of the neurotransmitters acetylcholine, catecholamines, serotonin and GABA, and the amino acids glycine, glutamate and aspartate (Prast and Philippu, 2001). The effect of NO on the release of monoamines within a particular brain area seems to be modulated by glutamate. Glutamate binds to the N-methyl-D-Aspartate (NMDA) receptor, which results in Ca2+ influx, stimulation of nNOS and increased production of NO. NO in turn influences glutamatergic neurotransmission by directly inhibiting the NMDA receptor function (Prast and Philippu, 2001; Akyol et al., 2004; Bernstein et al., 2005).
NO may act as a free radical due to its unpaired electron and can be toxic at higher concentrations (Akyol et al., 2004; Bernstein et al., 2005). Under certain conditions, e.g. when the availability of arginine or BH4 is limited, NOS enzymes produce oxygen-derived radicals such as peroxynitrite (Koshimura et al., 2000; Akyol et al., 2004). It has been suggested that BH4 itself may act as a protecting factor for NO toxicity and that this compound may also protect dopaminergic neurons from oxidative stress (Koshimura et al., 2000; Nakamura et al., 2001).

Table 1.
Pterins in affective disorders

Authors (year)	Number of patients and controls	Measures	Results
Biochemical studies			
Leeming et al. (1982)	31 females with BD or MD using lithium, 156 HC	7,8-dihydrobiopterin in serum	increase of 7,8-dihydrobiopterin in patients using tricyclic antidepressants
Kellner et al. (1983)	11 MD (bipolar and unipolar), 24 HC	BH4 in CSF	no difference
Duch et al. (1984)	29 MD, 9 BD, 28 HC	total biopterin and neopterin in urine	increase of total biopterin in MD. Increase of neopterin in MD and BD
Blair et al. (1984)	13 with history of MD, 4 with history with BD, euthymic using lithium	total biopterin in urine	decrease of total biopterin in bipolar disorder
Blair et al. (1984)	4 patients with history of MD	BH4 in post mortem temporal cortex	decrease of BH4 synthesis in depression
Garbutt et al. (1985)	9 MD, 28 HC	total biopterin and neopterin in urine	increase of total biopterin in MD, no difference in neopterin, no change after clinical improvement
Hashimoto et al. (1987)	12 MD, 12 HC	total biopterin in plasma	increase of biopterin in MD, no effect of antidepressants
Hashimoto et al. (1988)	8 MD, 4 BD, 1 hypomania, 12 HC	total biopterin in plasma	increase of biopterin in MD and hypomania
Coppen et al. (1989)	48 with history of MD, 28 with history of BD, euthymic using lithium, 60 HC	total biopterin and neopterin in urine	increase of neopterin-biopterin ratio in patients. Decrease of total biopterin in female patients
Knapp et al. (1989)	20 MD, 20 HC	total biopterin in plasma	increase of total biopterin in MD
Hashimoto et al. (1990)	8 MD, 4 bipolar-II, 8 HC.	total biopterin and BH4 in plasma	increase of total biopterin in patients. Decrease of BH4 in depressive phase, increase of BH4 in hypomania
Bottiglieri et al. (1992)	34 MD, 10 controls with neurological disease	BH4 in CSF	decreased BH4 correlated with lower 5-HIAA and HVA in MD with low folate levels
Dunbar et al. (1992)	26 MD, 63 HC	neopterin in plasma and urine	increase of plasma neopterin in MD, no effect of psychotropic drugs
Anderson et al. (1992)	23 MD, 26 HC	total biopterin and neopterin in urine before and after ECT	increase of neopterin-biopterin ratio in psychotic MD and responders to ECT; reduction of neopterin-biopterin ratio after response to ECT
Hashimoto et al. (1994)	10 MD, 10 HC	total biopterin and BH4 in plasma	increase of total biopterin and decrease of BH4-biopterin ratio in MD; normalization in remission phase
Anderson et al. (1994)	26 MD	Phe-Tyr ratio in serum before and after ECT	decrease of Phe-Tyr ratio in responders to ECT.
Matsuda et al. (1994)	67 MD, 40 HC	neopterin in serum	increase of neopterin in MD
Maes et al. (1994)	16 minor depression, 31 MD, 30 HC	neopterin in plasma	increase of neopterin in MD
Abou-Saleh et al. (1995)	48 MD, 26 HC	total biopterin and neopterin in urine	Increase of neopterin-biopterin ratio in MD. No change after treatment
Landmann et al. (1997)	22 MD, 22 HC	neopterin in plasma	no difference
Bonaccorso et al. (1998)	10 MD, 17 HC	total biopterin and neopterin in 24-h urine	increase of neopterin in MD

O'Toole et al. (1998)	35 MD, 35 HC	neopterin in plasma	no difference
Bell et al. (1998)	10 MD, 11 HC	neopterin in serum	no difference
Lestra et al. (1998)	27 MD	total biopterin in urine before and after treatment	no change
Abou-Saleh et al. (1999)	62 postpartum women, 38 HC	total biopterin and neopterin in urine	decrease of neopterin-biopterin ratio in MD (n=5)
Capuron et al. (2003)	26 patients with malignant melanoma	neopterin in plasma	greater increase of neopterin after treatment with interferon-alpha in patients with MD (n=7)
Van Gool et al. (2003)	67 patients with malignant melanoma	total biopterin, neopterin and Phe-Tyr ratio in serum	increase of neopterin in patients with depression (n=6).
Stastny et al. (2003)	9 patients in remission of SAD and MD, 4 patients in remission of SAD and BD	neopterin in plasma after tryptophan depletion and catecholamine depletion	increase of neopterin after tryptophan and catecholamine depletion
Chrapko et al. (2004)	15 MD, 16 HC	total biopterin in plasma	no difference
Schins et al. (2005)	post myocardial patients, 57 with MD, 46 without MD	neopterin in serum	no difference
Genetic studies			
Hahn et al. (2001)	family with mutation in GTPCH1 gene	psychiatric symptomatology	psychiatric dysfunction including depression in 50% of family members
Kealey et al. (2005)	families with bipolar disorder	association between bipolar disorder and polymorphism in GTPCH-I	linkage of allele 14q22-24, with candidate gene of GTPCH-I, with bipolar disorder. Variant A allele preferentially transmitted in bipolar probands.
Van Hove et al. (2006)	3 families with GTPCH deficiency	psychiatric assessment	MD more frequent in mutation carriers

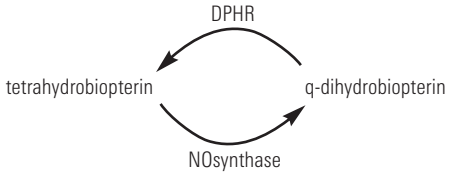
MD = major depression, BD = bipolar depression, HC = healthy controls, BH4 = tetrahydrobiopterin, CSF = cerebrospinal fluid,
ECT = electroconvulsive therapy, Phe-Tyr = phenylalanine-tyrosine, GTPCH = GTP cyclohydrolase.

Table 2.
NO in affective disorders

Authors (year)	Number of patients and controls	Measures	Results
Biochemical studies			
Elgun et al. (2000)	18 MD, 30 HC	arginase activity in serum	increase of arginase in MD and positive correlation between arginase and depression scores
Suzuki et al. (2001)	17 MD, 12 HC	NOx in plasma	increase of nitrate in MD and decrease after recovery
Savas et al. (2002)	44 BP-I mania, 21 HC	NOx in plasma	increase of NOx in mania
Srivastava et al. (2002)	66 MD, 114 HC	nitrite in PMNs	decrease of nitrite in MD
Suzuki et al. (2003)	17 MD, 129 controls, all after treatment with interferon-alpha	NOx in p	increase of nitrate in MD
Chrapko et al. (2004)	15 MD, 16 HC	NOx in plasma, platelet eNOS activity	decrease of NOx eNOS activity in MD
Ozcan et al. (2004)	22 mania, 8 MD, 21HC	NOx in plasma	decrease of NOx in MD
Selley et al. (2004)	25 MD, 25 HC	ADMA and NOx in plasma	increase of ADMA and decrease of NOx in MD
Chrapko et al. (2005)	17 MD, 12 HC	NOx in plasma, platelet eNOS activity	decrease of NOx and platelet eNOS activity in MD. After paroxetine treatment increase of NOx and no change of eNOS.
Savas et al. (2006)	27 BP-I euthymic, 20 HC	NOx in serum	increase of NOx in BP-I and positive correlation of NOx with number of mood episodes
Histochemical studies			
Bernstein et al. (1998)	8 MD, 13 controls	nNOS immunohistochemistry in hypothalamic paraventricular nucleus of post-mortem brain	reduction of NOS-containing neurons in MD
Bernstein et al. (2002)	11 recurrent mood episodes (MD or BP), 11 controls	nNOS immunohistochemistry in nucleus suprachiasmaticus of post-mortem brain	NOS activity decreased in MD
Xing et al. (2002)	15 BP, 15 MD, 15 controls	Constitutive NOS activity in prefrontal cortex of post-mortem brain	decrease of nNOS in MD, not in BD
Karolewicz et al., 2004	12 MD, 12 controls (cerebellum) (11 D)	nNOS immunohistochemistry in locus coeruleus tissue and locus coeruleus projection area	decrease of nNOS in MD
Bernstein et al., 2005	6 MD, 5 BP, 11 controls	nNOS immunohistochemistry in hypothalamic suprachiasmatic nucleus	decrease of nNOS in depressive subjects. Positive correlation between antidepressants and nNOS.
Genetic studies			
Yu et al., 2003	108 MD, 108 HC	association of nNOS polymorphism with MD and response to fluoxetine	no association

Reif et al., 2005	91 BP, 45 MD, 284 HC	association of eNOS polymorphisms with bipolar disorder and major depression	association of eNOS haplotypes in BD, not with MD.
Xu et al., 2005	35 BP, 35 HC	expression of gene for CAPON in dorsolateral prefrontal cortex in post mortem brain	increased expression of CAPON in bipolar disorder
Buttenschön et al., 2004	369 BP, 436 HC	association of nNOS polymorphism and bipolar disorder	no association

MD = unipolar major depression; BD = bipolar depression; BP = bipolar disorder; HC = healthy control; NOx = metabolites of NO, i.e. nitrates and nitrites; PMN = polymorphonuclear leukocytes; eNOS = endothelial nitric oxide synthase; nNOS = neuronal nitric oxide synthase; ADMA = asymmetric dimethylarginine (inhibitor of eNOS); CAPON = gene for carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase.



arginine → citrulline + NO

Figure 2.
Synthesis of nitric oxide (NO)

In animal experiments it was found that glucocorticoids inhibit induction of NO formation by a direct influence on the enzyme NO synthase as well as by reducing the availability of BH4 (Hattori et al., 1997). An overactivated HPA-axis, like in depression, could therefore decrease the BH4 concentration and consequently may affect both monoamine and NO metabolism.
The suprachiasmatic nucleus (SCN) is a small hypothalamic structure, considered to be a major circadian and circannual pacemaker of the mammalian brain. Disturbances in the biological clock function are characteristic for many disorders including depression. Since the NO/cGMP pathway is prominently involved in the mammalian circadian clock, it is conceivable that NOS expression may be disturbed in depression with or without atypical symptoms. In this respect the number of NOS immunoreactive neurons in depression has been demonstrated to alter with the seasons (Bernstein et al., 1998; Bernstein et al., 2005; Swaab et al., 2005).

7. NO and affective disorders

As can be inferred from table 2, in depressed patients mostly a decrease of NO was found in plasma or serum. The data, however, are far from unequivocal. The sparse findings on NO in bipolar disorder, all published by the same Turkish group of investigators, suggest higher levels of metabolites of NO (Savas et al., 2002; Savas et al., 2006). Since it is impossible to measure the gaseous compound NO directly, most investigators use the measurement of its metabolites nitrate and nitrite. However, the determination of nitrate/nitrite may be influenced by diet, environmental factors and even the glassware used. A more reliable parameter used as an indication of NO synthesis may be the ratio between the amino acids citrulline and arginine, which can easily be determined in the plasma or serum (Finkel et al., 1996; Fekkes et al., 2007).

Central question of the thesis

Since the late eighties of the past century there is a growing interest in the role of NO in the pathophysiology of a variety of neuropsychiatric disorders, particularly schizophrenia and affective disorders. The functional activity of this gas is closely linked to the synthesis and degradation of pterins. Therefore studies were conducted to evaluate the potential role of both pterins and parameters of NO activity in various types of affective disorders.
The first chapter of this thesis describes the differences between three major groups of affective disorders: major depression, seasonal affective disorder and bipolar-I disorder. In the symptomatic as well as in the recovered state several biochemical parameters related to the pterins and NO metabolism were measured.
The second chapter focuses on a group of patients with a severe, medication-resistant, depressive disorder. The effect of electroconvulsive therapy on various biochemical parameters was studied.
In the third chapter patients with seasonal affective disorder are described. These patients showed atypical depressive symptoms. Biochemical measures before and after light therapy are presented.
The fourth chapter describes NMDA receptor related amino acids in bipolar manic and bipolar euthymic patients.

References

- Abou-Saleh MT, Anderson DN, Collins J, Hughes K, Cattell RJ, Hamon CGB, Blair JA. The role of pterines in depression and the effects of antidepressive therapy. Biol Psychiatry 1995; 38:458-463.
- Abou-Saleh MT, Ghubash R, Karim L, Krymski M, Anderson DN. The role of pterins and related factors in the biology of early postpartum depression. Eur Neuropsychopharmacol 1999; 9:295-300.
- Akyol O, Zoroglu SS, Armutcu F, Sahin S, Gurel A: Nitric oxide as a physiopathological factor in neuropsychiatric disorders. In Vivo 2004;18:377-390.
- van Amsterdam JGC, Opperhuizen A. Nitric oxide and biopterin in depression and stress. Psychiatry Res 1999; 85:33-38.
- Anand, A, Charney, DS. Abnormalities in catecholamines and pathophysiology of bipolar disorder. In: Bipolar disorders: basic mechanisms and therapeutic implications. Soares JR, Gershon S (eds.). Marcel Dekker, New York, pp. 59-94; 2000.
- Anderson DN, Abou-Saleh MT, Collins J, Hughes K, Cattell RJ, Hamon CGB, Blair JA, Dewey ME. Pterin metabolism in depression: an extension of the amine hypothesis and

possible marker of response to ECT. Psychol Med 1992; 22: 863-869.
- Anderson DN, Wilkinson AM, Abou-Saleh MT, Blair JA. Recovery from depression after electroconvulsive therapy is accompanied by evidence of increased tetrahydrobiopterin-dependent hydroxylation. Acta Psychiatr Scand 1994; 90: 10-13.
- Auerbach G, Nar H. The pathway from GTP to tetrahydrobiopterin: three dimensional structures of GTP cyclohydrolase I and 6-pyruvoyl tetrahydropterin synthase. Biol Chem 1997; 378: 185-192.
- Bell IR, Patarca R, Baldwin CM, Klimas NG, Schwartz GER, Hardin EE. Serum neopterin and somatization in women with chemical intolerance, depressives, and normals. Biol Psychiatry 1998; 38:13-18.
- Bernstein H-G, Stanarius A, Baumann B, Henning H, Krell D, Danos P, Falkai P, Bogerts B. Nitric oxide synthase-containing neurons in the human hypothalamus: reduced number of immunoreactive cells in the paraventricular nucleus of depressive patients and schizophrenics. Neuroscience 1998; 83(3):867-875.
- Bernstein H-G, Heinemann A, Krell D, Mawrin C, Bielau H, Danos P, Diekmann S, Keilhoff G, Bogerts B, Baumann B. Further immunohistochemical evidence for impaired NO signaling in the hypothalamus of depressed patients. Ann N Y Acad Sci 2002; 973:91-93.
- Bernstein H-G, Bogerts B, Keilhoff G: The many faces of nitric oxide in schizophrenia. A review. Schizophr Res 2005a; 78:69-86.
- Bernstein H-G, Heinemann A, Krell D, Dobrowolny H, Bielau H, Keulhoff G, Bogerts B. Hypothalamic nitric oxide synthase in affective disorder: focus on the suprachiasmatic nucleus. Cell Mol Biol 2005; 51:279-284.
- Blair JA, Barford PA, Morar C, Pheasant AE, Hamon CGB, Whitburn SB, Leeming RJ, Reynolds GP, Coppen A. Tetrahydrobiopterin metabolism in depression. Lancet 1984; 21:163.
- Blaskovics M, Giudici TA. A new variant of biopterin deficiency. New Engl J Med 1988; 319: 1611-12.
- Blau N, Barnes I, Dhondt JL. International database of tetrahydrobiopterin deficiencies. J Inherit Metab Dis 1996;19(1):8-14.
- Bonaccorso S, Lin A, Verkerk R, van Hunsel F, Libbrecht I, Scharpe S, DeClerck L, Biondi M, Janca A, Maes M. Immune markers in fibromyalgia: comparison with major depressed patients and normal volunteers. J Affect Disord 1998; 48: 75-82.
- Bottiglieri T, Hyland K, Laundy M, Godfrey P, Carney MWP, Toone BK, Reynolds EH. Folate deficiency, biopterin and monoamine metabolism in depression. Psychol Med 1992; 22:871-876.
- Bullard, W.P., Guthrie, P.B., Russo, P.V., Mandell, A.J. Regional and subcellular distribution and some factors in the regulation of reduced pterins in rat brain. J Pharmacol

Exp Ther 1978; 206: 4-20.
- Buttenschön HN, Mors O, Ewald H, McQuillin A, Kalsi G, Lawrence J, Gurling H, Kruse TA. No association between a neuronal nitric oxide synthase (NOS1) gene polymorphism on chromosome 12q24 and bipolar disorder. Am J Med Genet B Neuropsychiatr Genet 2004; 124B:73-75.
- Capuron L, Neurauter G, Musselman DL, Lawson DH, Nemeroff CB, Fuchs D, Miller AH. Interferon-alpha-induced changes in tryptophan metabolism: relationship to depression and paroxetine treatment. Biol Psychiatry 2003; 54:906-914.
- Chrapko WE, Jurasz P, Radomski MW, Lara N, Archer SL, Le Melledo J-M. Decreased platelet nitric oxide synthase activity and plasma nitric oxide metabolites in major depressive disorder. Biol Psychiatry 2004; 56:129-134.
- Chrapko W, Jurasz P, Radomski MW, Archer SL, Newman SC, Baker G, Lara N, Le Melledo J-M. Alteration of decreased plasma NO metabolites and platelet NO synthase activity by paroxetine in depressed patients. Neuropsychopharmacology 2005; 31(6):1286-93.
- Coppen A, Swade C, Jones, Armstrong RA, Blair JA, Leeming RJ. Depression and tetrahydrobiopterin: the folate connection. J Affect Disord 1989; 16: 103-107.
- Curtius H-Ch, Müldner H, Niederwieser A. Tetrahydrobiopterin: efficacy in endogenous depression and Parkinson's disease. J Neural Transm 1982; 55: 301-308.
- Curtius H-Ch, Niederwieser A, Levine RA, Lovenberg W, Woggon B, Angst J. Successful treatment of depression with tetrahydrobiopterin. Lancet 1983; 19: 657-658.
- Dinan TG. Monoamines and steroids: a unifying hypothesis of mood disorder. J Serotonin Res 1995; 4: 233-242.
- Duch DS, Woolf JH, Nichol CA, Davidson JR, Garbutt JC. Urinary excretion of biopterin and neopterin in psychiatric disorders. Psychiatry Res 1984;11:83-89.
- Dunbar PR, Hill J, Neale TJ, Mellsop GW. Neopterin measurement provides evidence of altered cell-mediated immunity in patients with depression, but not with schizophrenia. Psychol Med 1992; 22: 1051-1057.
- Elgün S, Kumbasar H. Increased serum arginase activity in depressed patients. Prog. Neuro-Psychopharmacol & Biol Psychiat 2000;24:227-232.
- Fekkes D, Bannink M, Kruit WHJ, Van Gool AR, Mulder PGH, Sleijfer S, Eggermont AMM. Influence of pegylated interferon-α therapy on plasma levels of citrulline and arginine in melanoma patients. Amino Acids 2007; 32:121-126.
- Finkel MS, Laghrissi-Thode F, Pollock BG, Rong J. Paroxetine is a novel nitric oxide synthase inhibitor. Psychopharmacol Bull 1996; 32(4):653-8.
- Fleischhacker WW, Meise U, Schubert H. Re-evaluation of antidepressant effect of tetrahydrobiopterine. Lancet 1985; 17: 387.
- Garbutt JC, Duch DS, Nichol CA, Woolf JH. Urinary biopterin and neopterin excretion

and pituitary-adrenal activity in psychiatric patients. Psychiatry Res 1985;16:181-187.
- van Gool AR, Fekkes D, Kruit WHJ, Mulder PGH, Ten Hagen TLM, Bannink M, Maes M, Eggermont AMM. Serum amino acids, biopterin and neopterin during long-term immunotherapy with interferon-alpha in high risk melanoma patients. Psychiatry Res 2003; 119:125-132.
- Hahn H, Trant MR, Brownstein MJ, Harper RA, Milstien S, Butler IJ. Neurologic and psychiatric manifestations in a family with a mutation in exon 2 of the guanosine triphosphate-cyclohydrolase gene. Arch Neurol 2001; 58(5): 749-55.
- Hashimoto R, Ozaki N, Ohta T, Kasahara Y, Kaneda N, Nagatsu T. Total biopterin levels of plasma in patients with depression. Neuropsychobiology 1987; 17: 176-177.
- Hashimoto R, Ozaki N, Ohta T, Kasahara Y, Kaneda N, Nagatsu T. Plasma biopterin levels of patients with affective disorders. Neuropsychobiology 1988; 19:61-63.
- Hashimoto R, Ozaki N, Ohta T, Kasahara Y, Kaneda N, Nagatsu T. Plasma tetrahydrobiopterin levels in patients with psychiatric disorders. Neuropsychobiology 1990; 23:140-143.
- Hashimoto R, Mizutani M, Ohta T, Nakazawa K, Nagatsu T. Changes in plasma tetrahydrobiopterin levels of depressives in depressive and remission phases: reconfirmed by measurement with an internal standard. Biol Psychiatry 1994; 29: 57-60.
- Hattori Y, Akimoto K, Nakanishi N, Kasai K. Glucocorticoid regulation of nitric oxide and tetrahydrobiopterin in a rat model of endotoxic shock. Biochem Biophys Res Commun 1997; 240: 298-303.
- Holsboer, F. Neuroendocrinology of mood disorders. In: Psychopharmacology; the fourth generation of Progress. F.E. Bloom, D.J. Kupfer (eds.). Raven Press, New York, pp. 957-969; 1995.
- van Hove JL, Steyaert J, Matthijs G, Legius E, Theys P, Wevers R, Romstad A, Moller LB, Hedrich K, Goriounov D, Blau N, Klein C, Casaer P. Expanded motor and psychiatric phenotype in autosomal dominant Segawa syndrome due to GTP cyclohydrolase deficiency. J Neurol Neurosurg Psych 2006; 77(1): 18-23.
- Huber C, Batchelor JR, Fuchs D, Hausen A, Lang A, Niederwieser D, Reibnegger G, Swetly P, Troppmair J, Wachter H. Immune response-associated production of neopterin. Release from macrophages primarily under control of interferon-gamma. J Exp Medicine 1984; 160(1):310-316.
- Karolewicz B, Szebeni K, Stockmeier CA, Konick L, Overholser JC, Jurjus G, Roth BL, Ordway GA. Low nNOS protein in the locus coeruleus in major depression. J Neurochem 2004; 91:1057-1066.
- Kaufman S. Genetic disorders involving recycling and formation of tetrahydrobiopterin. Adv in Pharmacology 1998; 42: 41-43.
- Kealey C, Roche S, Claffey E, McKeon P. Linkage and candidate gene analysis of 14q22-

24 in bipolar disorder: support for GCHI as a novel susceptibility gene. Am J Med Genet B Neuropsychiatr Genet 2005; 136(1): 75-80.
- Kellner CH, Rakita RM, Rubinow DA, Gold PW, Ballenger JC, Post RM. Tetrahydrobiopterin levels in cerebrospinal fluid of affectively ill patients. The Lancet 1983; 2(8340):55-56.
- de Kloet ER, Rots NY, Cools AR. Brain-corticosteroid hormone dialoque: slow and persistent. In: Cellular and Molecular Neurobiology 1996; 16: 345-356.
- Knapp S, Irwin M. Plasma levels of tetrahydrobiopterin and folate in major depression. Biol Psychiatry 1989;26:156-162.
- Koshimura K, Miwa S, Lee K, Fujiwara M, Watanabe Y. Enhancement of dopamine release in vivo from the rat striatum by dialytic perfusion of 6R-L-erythro-5,6,7,8-tetrahydrobiopterin. J Neurochem 1990; 54: 1391-1397.
- de Kloet ER, Vreugdenhil M, Oitzl S, Joels M. Brain-corticosteroid receptor balance in health and disease. Endocr Rev 1998; 19: 269-301.
- Koshimura K, Murakami Y, Tanaka J, Kato Y. The role of 6R-tetrahydrobiopterin in the nervous system. Prog Neurobiol 2000; 61(4): 415-438.
- Kugaya A, Sanacora G. Beyond monoamines: glutamatergic function in mood disorders. CNS Spectr 2005; 10(10): 808-19.
- Landmann R, Schaub B, Link S, Wacker HR. Unaltered monocyte function in patients with major depression before and after three months of antidepressive therapy. Biol Psychiatry 1997; 41:675-681.
- Leeming RJ, Blair JA. The effects of pathological and normal physiological processes on biopterin derivative levels in man. Clin Chim Acta 1980; 108:103-111.
- Leeming RJ, Blair JA, Walters J. Serum dihydrobiopterin levels in patients on tricyclic antidepressants. Psychological Medicine 1982; 12:191-192.
- Lestra C, D'Amato T, Ghaemmaghami C, Perret-Liaudet A, Broyer M, Renaud B, Dalery J, Chamba G. Biological parameters in major depression: effects of paroxetine, volixazine, moclobemide, and electroconvulsive therapy. Relation to early clinical outcome. Biol Psychiatry 1998; 44:274-280.
- Levine RA, Lovenberg W. CSF tetrahydrobiopterin levels in patients with affective disorders. Lancet 1984; i:283.
- Lehmann, HE, Kline NS. Clinical discoveries with antidepressant drugs. In: Discoveries in pharmacology, Vol. 1: Psycho- and neuropharmacology. Parnham MJ, Bruinvels J (eds.) Elsevier Science Publishers, pp. 209-221; 1983
- Levine RA. Tetrahydrobiopterin and biogenic amine metabolism in neuropsychiatry, immunology, and aging. Ann N Y Acad Sci 1988; 521: 129-139.
- Maes M, Scharpe S, Meltzer HY, Okayli G, Bosmans E, D'Hondt P, Vanden Bossche B, Cosyns P. Increased neopterin and interferon-gamma secretion and lower availability of

L-tryptophan in major depression: further evidence for an immune response. Psychiatry Res 1994; 54: 143-160.
- Manji HK, Moore GJ, Chen G. Bipolar disorder: leads from the molecular and cellular mechanisms of action of mood stabilisers. Br J Psychiatry 2001; 178 (suppl. 41), s107-s119.
- Massat I, Souery D, Mendlewicz J, Papadimitriou GN. The GABAergic hypothesis of mood disorders. In: Bipolar disorders: basic mechanisms and therapeutic implications. Soares JR, Gershon S (eds.). Marcel Dekker, New York, pp. 143-165; 2000
- Mataga N, Imamura K, Watanabe Y. 6R-Tetrahydrobiopterin perfusion enhances dopamine, serotonin, and glutamate outputs in dialysate from rat striatum and frontal cortex. Brain Res 1991; 551: 64-71.
- Matsuda J, Gohchi K, Gotoh N. Serum concentrations of 2',5'-oligo-adenylate synthetase, neopterin, and Beta-glucan in patients with chronic fatigue syndrome and in patients with major depression. Lancet 1994; 1015-16.
- Mayer B, Werner ER. In search of a function for tetrahydrobiopterin in the biosynthesis of nitric oxide. Naunyn-Schmiedeberg's Arch Pharmacol 1995; 351:453-463.
- Miwa S, Watanabe Y, Hayaishi O. 6R-L-erythro-5,6,7,8-tetrahydrobiopterin as a regulator of dopamine and serotonin biosynthesis in the rat brain. Arch Biochem Biophys 1985; 239: 234-241.
- Muguruma K, Imamura K, Watanabe Y. Repeated peripheral administration of 6R-L-erythro-5,6,7,8-tetrahydrobiopterin alters the binding activities of 5-HT1A and 5-HT2 receptors in rat brain. Biochem Biophys Res Commun 1996; 227: 794-799.
- Nakamura K, Bindokas VP, Kowlessur D, Elas M, Milstien S, Marks JD, Halpern HJ, Kang UJ. Tetrahydrobiopterin scavenges superoxide in dopaminergic neurons. J Biol Chem 2001; 276(37):34402-34407.
- Nichol CA, Smith GK, Duch DS. Biosynthesis and metabolism of tetrahydrobiopterin and molybdopterin. Annu Rev Biochem 1985; 54:729-764.
- O'Toole SM, Chiappelli F, Rubin RT. Plasma neopterin in major depression: relationship to basal and stimulated pituitary-adrenal cortical axis function. Psychiatry Res 1998; 79:21-29.
- Ozcan ME, Gulec M, Ozerol E, Polat R, Akyol O. Antioxidant enzyme activities and oxidative stress in affective disorders. Int Clin Psychopharmacol 2004; 19:89-95.
- Pogson D. Issues for consideration in dihydropteridine reductase (DHPR) deficiency: a variant form of hyperphenylalaninaemia. J Intellectual Disabil Res 1997; 41: 208-214.
- Prast H, Philippu A. Nitric oxide as modulator of neuronal function. Prog Neurobiol 2001; 64(1): 51-68.
- Reif A, Strobel A, Jacob CP, Herterich S, Freitag CM, Töpner T, Mössner R, Fritzen S, Schmitt A, Lesch K-P. A NOS-III haplotype that includes functional polymorphisms is

associated with bipolar disorder. Int J Neuropsychopharmacol 2006;9:13-20.
- Savas HA, Herken H, Yurekli M, Uz E, Tutkun H, Zoroglu SS, Ozen ME, Cengiz B, Akyol O. Possible role of nitric oxide and adrenomedullin in bipolar affective disorder. Neuropsychobiology. 2002; 45(2):57-61.
- Savas HA, Gergerlioglu HS, Armutcu F, Herken H, Yilmaz HR, Kocoglu E, Selek S, Tutkun H, Zoroglu SS, Akyol O. Elevated serum nitric oxide and superoxide dismutase in euthymic bipolar patients: impact of past episodes. World J Biol Psychiatry. 2006; 7(1):51-5.
- Schildkraut, JJ. The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am J Psychiatry 1965; 122: 509-522.
- Schins A, Tulner D, Lousberg R, Kenis G, Delanghe J, Crijns HJ, Grauls G, Stassen F, Maes M, Honig A. Inflammatory markers in depressed post-myocardial infarction patients. J Psychiatr Res 2005; 39:137-144.
- Selley ML. Increased (E)-4-hydroxy-2-nonenal and asymmetric dimethylarginine concentrations and decreased nitric oxide concentrations in the plasma of patients with major depression. J Affect Disord 2004; 80:249-256.
- Srivastava N, Barthwal MK, Dalal PK, Agarwal AK, Nag D, Seth PK, Srimal RC, Dikshit M. A study on nitric oxide, ß-adrenergic receptors and antioxidant status in the polymorphonuclear leukocytes form the patients of depression. J Affect Disord 2002; 72:45-52.
- Stastny J, Konstantinidis A, Schwarz MJ, Rosenthal NE, Vitouch O, Kasper S, Neumeister A. Effects of tryptophan depletion and catecholamine depletion on immune parameters in patients with seasonal affective disorder in remission with light therapy. Biol Psychiatry 2003; 53(4):332-337.
- Suzuki E, Yagi G, Nakaki T, Kanba S, Asai M. Elevated plasma nitrate levels in depressive states. J Affect Disord 2001; 63:221-224.
- Suzuki E, Yoshida Y, Shibuya A, Miyaoka H. Nitric oxide involvement in depression during interferon-alpha therapy. Int J Neuropsychopharmacol 2003; 6:415-419.
- Swaab DF, Bao A-M, Lucassen PJ. The stress system in the human brain in depression and neurodegeneration. Ageing Res Rev 2005; 4:141-194.
- Thöny B, Auerbach G, Blau N. Tetrahydrobiopterin biosynthesis, regeneration and functions. Biochem J 2000; 347: 1-16.
- Woggon B, Angst J, Curtius H-Ch, Niederwieser A. Unsuccessful treatment of depression with tetrahydrobiopterin. Lancet 1984; 22/29: 1463.
- Wolf WA, Ziaja E, Arthur RA Jr., Anastasiadis PZ, Levine RA, Kuhn DM. Effect of tetrahydrobiopterin on serotonin synthesis, release, and metabolism in superfused hippocampal slices. J Neurochem 1991; 57:1191-7.
- Wong, M-L, Licinio J. Research and treatment approaches to depression. Nat Rev Neurosci 2001; 2(5): 343-51.

- Xing G, Chavko M, Zhang L-X, Yang S, Post RM. Decreased calcium-dependent constitutive nitric oxide synthase (cNOS) activity in prefrontal cortex in schizophrenia and depression. Schizophr Res 2002; 58:21-30.
- Xu B, Wratten N, Charych EI, Buyske S, Firestein BL, Brzustowicz LM. Increased expression in dorsolateral prefrontal cortex of CAPON in schizophrenia and bipolar disorder. PLoS Medicine 2005; 2:999-1007.
- Yu Y W-Y, Chen T-J, Wang Y-C, Liou Y-J, Hong C-J, Tsai S-J. Association analysis for neuronal nitric oxide synthase gene polymorphism with major depression and fluoxetine. Neuropsychobiology 2003; 47:137-140.

Nitric oxide and neopterin in bipolar affective disorder

Chapter Two

Nitric oxide and neopterin in bipolar affective disorder

R. Hoekstra, D. Fekkes, L. Pepplinkhuizen, A.J.M. Loonen, S. Tuinier and W.M.A. Verhoeven

Reprinted from Neuropsychobiology 2006;54:75-81, Copyright (2006), with permission from Elsevier.

Abstract

Background: There is an increasing interest in the role of nitric oxide (NO) and pterines in the pathophysiology of neuropsychiatric disorders. The results so far show an inconsistent pattern.

Methods: In the present study neopterin and a measure of NO synthesis in plasma of symptomatic and euthymic bipolar affective patients were compared to those of patients with a major depression and healthy controls. As an index of NO synthesis, the ratio of the amino acids citrulline and arginine (Cit-Arg ratio) was calculated. Neopterin is a bypass product in the synthesis of tetrahydrobiopterin, which is a cofactor of NO synthase.

Results: The results indicate that both neopterin and the Cit-Arg ratio are decreased in bipolar affective patients, irrespective their symptomatic status. In addition, an association between the values of the Cit-Arg ratio and the neopterin level was observed, which is suggestive for a low BH4 activity.

Conclusion: Nitric oxide formation may be endangered in bipolar affective disorder.

Introduction

Since two decades there has been an increasing interest in the role of nitric oxide (NO) in the pathophysiology of a variety of neuropsychiatric disorders. In the brain many processes are linked to NO, in that it influences the storage and uptake and/or release of most other neurotransmitters as well as certain neuropeptides and it exerts a strong influence on glutamatergic neurotransmission [1,2].

With respect to the neuropsychiatric disorders, indices of NO functionality have been investigated in schizophrenia, autism, obsessive-compulsive disorder, Alzheimer's disease and affective disorders, yielding contradictory results suggestive for an enhanced or a decreased synthesis of NO [3,4]. Much work has been done to elucidate the contribution of NO to the pathophysiology of mood disorders. As described by Bernstein and coworkers, a reduced synthesis of NO may be associated with major depression [5]. The results suggest a dichotomy between unipolar and bipolar affective disorder, in that in the blood of the latter increased concentrations of NO have been found [6,7]. Furthermore, NO synthase is highly expressed in hypothalamic neurons [8], the brain region that is implicated in the pathogenesis of stress-related disorders such as depression [9]. Concerning the latter, pteridines are also involved and a limited number of studies suggest an increased plasma concentration of neopterin in major depression [10,11].

Since both neopterin and NO have been implicated in depressive disorders, a study was conducted in patients with major depression or bipolar affective disorder. The NO synthesis was approximated by calculation of the ratio between the plasma levels of the amino acids citrulline and arginine (Cit-Arg ratio). Both NO and citrulline are formed from arginine, a reaction catalyzed by the enzyme NO synthase, and the Cit-Arg ratio is regarded as an index of the NO synthesis [12,13].

In a previous study conducted in patients suffering from a severe major depression, the ratio between the plasma levels of phenylalanine and tyrosine (Phe-Tyr ratio) appeared to be increased as compared to normal controls [14]. The Phe-Tyr ratio provides a reliable measure of the phenylalanine hydroxylase activity and may also be regarded as an indirect measure of tetrahydrobiopterin (BH4) activity [15]. Since neopterin and BH4 share the same precursor and plasma levels of BH4 were found to be decreased in depressed patients and increased in bipolar manic patients [16], the Phe-Tyr ratio was measured in the present study as well.

Materials and methods

Experimental procedures

The protocols were approved by official recognized medical ethics committees. The projects were performed in accordance with the Helsinki Declaration. Written informed consent was obtained from all patients.

Subjects

The first group comprised 20 patients, suffering from a Major Depressive Disorder with Melancholic Features (MDMF; n=20). The second group consisted of patients with a Major Depressive Disorder with Seasonal Pattern (MDSP; n=19). Patients from groups 1 and 2 were referred to the Department of Psychiatry of the Erasmus University Medical Centre for electroconvulsive treatment (ECT) and light therapy respectively. All patients were at least 1 week free of medication. The third group consisted of patients with a relapse of a Bipolar-I Disorder (BP-I) with either a Manic Episode (n=20) or a Depressed Episode (n=12) who were enrolled from the outpatient department of Delta Psychiatric Centre. The BP-I manic patients were treated with lithium (n=14) and/or mood stabilizing anticonvulsants (n=14). Twelve patients were treated with antipsychotics in addition to the mood stabilizer, whereas antidepressants were not prescribed. The BP-I depressed patients were treated with lithium (n=7) and/or moodstabilizing anticonvulsants (n=5). Six patients were treated with additional antidepressants and 4 patients with antipsychotics. Excluded were patients with clinically relevant somatic disorders or comorbid DSM axis-I disorders.

The first control group consisted of euthymic bipolar patients with lithium prophylaxis as monotherapy (BP-EU; n=12), who were free of symptoms for at least 6 months. The second control group comprised healthy persons (n=20), who were extracted from a large database. Demographic data and baseline scores of the 3 patient groups and the characteristics of the 2 control groups are presented in table 1.

Assessment

Diagnoses in the group of patients with MDMF were made on the basis of a semi-structured interview including the Schedule for Affective Disorders and Schizophrenia (SADS) [17]. The severity of depression in the groups with MDMF and BP-I depressed episode was assessed with the Hamilton Rating Scale for Depression (HAMD) [18]. Manic symptoms were rated with the Young Mania Rating Scale (YMRS) [19]. Depressive symptoms in the MDSP patients were established according to the Beck Depression Inventory (BDI) including the addendum for atypical symptoms (BDI-add) [20]. All patients met the criteria for the different DSM-diagnoses. In bipolar patients data from retrospective life charts [21] were included in the diagnostic procedure.

Table 1.
Demographic data and baseline scores of the different groups.

Group	Number (mean age and %males)	Depression Scores	Manic scores
MDMF	20 (52 yrs; 35%)	31.4 ± 7.7	--
MDSP	19 (41 yrs; 5%)	22.9 ± 12.1	--
BP-I manic	20 (50 yrs; 70%)	--	21.7 ± 6.1
BP-I depressed	12 (47 yrs; 58%)	20.6 ± 6.7	--
Healthy controls	20 (51 yrs; 40%)	--	--
BP-EU	12 (40 yrs; 67%)	--	--

Data are presented as mean ± SD.
MDMF=Major Depressive disorder with Melancholic Features
MDSP=Major Depressive disorder with Seasonal Pattern
BP-I=Bipolar-I disorder, Manic or Depressed
BP-EU=Bipolar-I disorder, euthymic controls
Depression scores: Hamilton Rating Scale for Depression in MDMF and BP-I depressed groups; Beck Depression Inventory with addendum for atypical features in MDSP group.
Manic Scores: Young Mania Rating Scale

Patients with MDMF underwent ECT (mean: 11 times; twice weekly) since they were all non-responders to a complete pharmacological treatment algorithm including lithium addition and MAO-inhibitors. MDSP patients received a standard regimen of light therapy on 5 consecutive mornings. Patients with a relapse of BP-I were treated, depending on the phase, with either addition of an antidepressant or a high potency benzodiazepine and/or an antipsychotic to the used mood stabilizing agents. In all groups the first assessment of symptomatology was performed directly before treatment (baseline scores). The second assessment of the patients with MDMF and MDSP was done after treatment with ECT or light therapy respectively, and for the BP-I patients this was done after recovery of clinical symptoms. The latter was defined as an outcome score of less than 10 on the HAMD or less than 6 on the YMRS or a more than 50% decrease from the baseline.

Blood drawing and biochemical assays

A venous blood sample was drawn before 10.00 a.m. and plasma was prepared by differential centrifugation (20 min at 2650 g and 20°C) within 2 hours. Blood of the patients was obtained before and after treatment. Plasma samples were stored at -80°C until assay. Amino acids and neopterin were assayed by high performance liquid chromatography with fluorometric detection according to the methods described previously [14,22]. The Cit-Arg and the Phe-Tyr ratios were calculated by dividing the plasma level of citrulline by that of arginine and the plasma level of phenylalanine by that of tyrosine respectively.

Table 2.
Biochemical parameters in the various groups at baseline.

	Phe-Tyr	Cit-Arg	Neopterin (nmol/l)
MDMF (n=20)	1.03 (0.14)	0.48 (0.14)	21.67 (8.18)
MDSP (n=19)	0.94 (0.21)	0.56 (0.19)	22.54 (7.62)
BP-I (n=32)	0.91 (0.21)	0.46 (0.25)	14.51 (9.98) *
Healthy controls (n=20)	0.94 (0.13)	0.51 (0.12)	18.84 (4.01)
BP-EU (n=12)	0.99 (0.13)	0.40 (0.16)	14.76 (6.0)

* MDMF vs. BP-I: p=0.02; MDSP vs. BP-I: p=0.007

Data are presented as mean (SD)
MDMF=Major Depressive disorder with Melancholic Features
MDSP=Major Depressive disorder with Seasonal Pattern
BP-I=Bipolar-I disorder, Manic and Depressed
BP-EU=Bipolar-I disorder, euthymic controls

Statistics

Data are presented as mean ± SD. BP-I depressed and manic were combined in the group BP-I symptomatic (n=32). Data were analyzed by one-way analysis of variance (ANOVA) and post-hoc comparisons were done by Bonferroni's multiple comparison test. Neopterin levels were correlated with the Cit-Arg ratio and Phe-Tyr ratio using the Pearson correlation test. Results were considered significant when $p < .05$.

Results

The baseline scores for the MDMF, MDSP, BP-I depressed and BP-I manic groups are shown in table 1. After treatment the scores on the depression and mania ratings in the different patient groups were significantly reduced (MDMF: 11.0 ± 10.1, $p<0.0001$; MDSP: 9.7 ± 8.0, $p<0.0001$; BP-I: depressed: 3.4 ± 3.3, $p<0.0001$ and BP-I manic: 1.6 ± 2.0, $p<0.0001$). The percentage symptomatic improvement was for the MDMF, MDSP, BP-I depressed and BP-I manic 65, 58, 83 and 93% respectively.

Values for the Phe-Tyr and Cit-Arg ratios and for neopterin are depicted in tables 2 (baseline) and 3 (after treatment). Good responders on ECT (n=15) or on light therapy (n=13) showed biochemical values that were not different from those of the less responding patients. When comparing the BP-I depressed with the BP-I manic patients no significant differences were found in the values for the Phe-Tyr ratio (0.95 ± 0.20 vs. 0.89 ± 0.21), the Cit-Arg ratio (0.44 ± 0.29 vs. 0.48 ± 0.23) and for neopterin (12.05 ± 4.94 vs.

Table 3.
Biochemical parameters in the various groups after treatment.

	Phe-Tyr	Cit-Arg	Neopterin (nmol/l)
MDMF (n=20)	0.89 (0.13)	0.59 (0.25)	23.62 (8.24)
MDSP (n=19)	0.95 (0.18)	0.52 (0.16)	21.95 (5.63)
BP-I (n=32)	0.91 (0.14)	0.45 (0.22)	14.57 (6.76) *
Healthy controls (n=20)	0.94 (0.13)	0.51 (0.12)	18.84 (4.01)
BP-EU (n=12)	0.99 (0.13)	0.40 (0.16)	14.76 (6.00) #

* MDMF vs. BP-I: p<0.0001; MDSP vs. BP-I: p=0.001

\# MDMF vs. BP-EU: p=0.004; MDSP vs. BP-EU: p=0.037

Data are presented as mean (SD)
MDMF=Major Depressive disorder with Melancholic Features
MDSP=Major Depressive disorder with Seasonal Pattern
BP-I=Bipolar-I disorder, symptomatic
BP-EU=Bipolar-I disorder, euthymic controls

15.99 ± 11.92 nmol/l). Therefore, the BP-I depressed and BP-I manic patients were combined in one symptomatic, BP-I group (n=32).

As can be inferred from table 2, the baseline value for neopterin in the symptomatic BP-I group is significantly lower as compared to both the MDMF and MDSP group. In addition, this value was decreased in the BP-EU controls. Furthermore, the Phe-Tyr ratio in the MDMF group is higher than in the groups with MDSP, symptomatic BP-I and healthy controls, albeit not significant. Although not reaching the level of significance, the Cit-Arg ratio is lower in the bipolar groups.

The biochemical parameters measured after treatment, are given in table 3. It can be seen that the differences in neopterin levels persisted after treatment, while the Phe-Tyr ratio in the MDMF group normalized (paired t-test; p<0.0001). In contrast to baseline, the Cit-Arg ratio in the MDMF group increased after treatment (paired t-test; p= 0.015), whereas this ratio did not change in the MDSP and symptomatic BP-I group.

Since psychotropics and mood stabilizers like e.g. antidepressants and valproic acid have been demonstrated to affect levels of amino acids [23,24], a group of 12 patients using only lithium (MBP-I) was composed from the symptomatic BP-I group. Interim analysis showed no effect of the plasma level of lithium on the biochemical parameters.

Figure 1 shows the Cit-Arg ratios before and after treatment in the symptomatic BP-I patients on lithium monotherapy (MBP-I) as well as in the MDMF and MDSP groups. In addition, these ratios are given for the BP-EU group and the healthy control group. Before treatment the Cit-Arg ratio in the MBP-I group (n=12) was significantly lower than in the MDSP group (p=0.002) and the healthy controls (p=0.024). After treatment these

Figure 1.

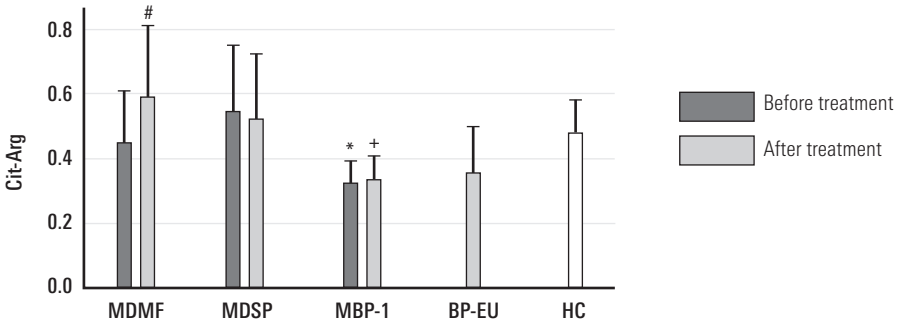
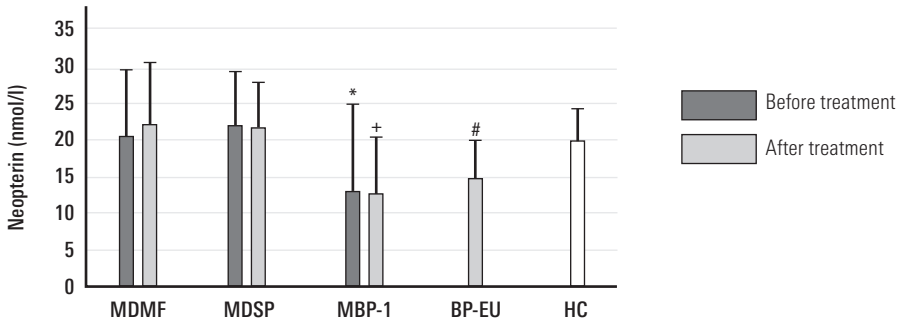


Figure 2.



Legends to the figures

Fig. 1. Cit-Arg ratios (± SD) in plasma of patients suffering from a Major Depressive Disorder with Melancholic Features (MDMF; n=20) or from a Major Depressive Disorder with Seasonal Pattern (MDSP; n=19) and in patients with a Bipolar-I Disorder with either a Manic Episode or a Depressed Episode on lithium monotherapy (MBP-I; n=12) before and after appropriate treatment. In addition, these ratios are given for the euthymic bipolar patients with lithium prophylaxis as monotherapy (BP-EU; n=12) and for the healthy control group (HC; n=20). *, p=0.002 and p=0.024 versus MDSP before treatment and HC, respectively. +, p=0.006 and p=0.007 versus MDSP after treatment and HC, respectively. #, p=0.004 and p=0.039 versus MBP-I after treatment and BP-EU, respectively.

Fig. 2. Neopterin concentrations (± SD) in plasma of patients suffering from a Major Depressive Disorder with Melancholic Features (MDMF; n=20) or from a Major Depressive Disorder with Seasonal Pattern (MDSP; n=19) and in patients with a Bipolar-I Disorder with either a Manic Episode or a Depressed Episode on lithium monotherapy (MBP-I; n=12) before and after appropriate treatment. In addition, these ratios are given for the euthymic bipolar patients with lithium prophylaxis as monotherapy (BP-EU; n=12) and for the healthy control group (HC; n=20). *, p=0.021 and p=0.009 versus MDMF and MDSP before treatment, respectively. +, p<0.0001 and p=0.002 versus MDMF and MDSP after treatment, respectively. #, p=0.003 and p=0.033 versus MDMF and MDSP after treatment, respectively.

differences persisted (p=0.006 and p=0.007, respectively). The Cit-Arg ratio was also significantly lower in both the MBP-I and BP-EU control group (n=12) as compared to the MDMF group (p=0.004 and p=0.039, respectively).
The results for the plasma neopterin levels are depicted in figure 2. Before treatment, neopterin was significantly lower in the MBP-I group (n=12) than in the MDMF (p=0.021) and MDSP group (p=0.009). After treatment these differences persisted (p<0.0001 and p=0.002, respectively). Neopterin levels were also significantly lower in the BP-EU group (n=12) than in the MDMF (p=0.003) and MDSP group (p=0.033). The neopterin levels as well as the Cit-Arg ratio in the MBP-I group (n=12) after treatment were not different from those in the BP-EU control group (n=12).
In the symptomatic BP-I group (n=32) the values of neopterin were positively correlated with the Cit-Arg ratio (r=0.44, p=0.012). A comparable positive correlation (r=0.31, p=0.044) was found after treatment in the total group of bipolar patients (n=44, including the 12 euthymic controls). No correlation was found between the Phe-Tyr ratio and neopterin levels.

Discussion

The present study demonstrates that patients with bipolar-I disorder show, irrespective of the presence of depressive or manic symptoms, a significantly lower plasma level of neopterin as compared to patients suffering from a major depressive disorder with melancholic features or from a major depressive disorder with seasonal pattern. In fact, neopterin is 33-35% lower in the bipolar patient groups with or without symptoms as compared to the groups with depressed patients, suggestive for a decreased activity of BH4. Since most of the patients from the bipolar groups were treated with psychotropics and mood stabilizers other than lithium, and these compounds are known to modify the here studied biochemical parameters [23-27], symptomatic bipolar patients on monotherapy with lithium were compared to the euthymic monolithium bipolar patient control group. Analyses of the biochemical parameters in the monolithium symptomatic subgroup revealed similar results with respect to decreased neopterin levels.
In addition, the symptomatic and euthymic subgroup of bipolar-I patients on lithium monotherapy both demonstrated a lower Cit-Arg ratio as compared to healthy controls as well as to the two patient groups with depressive disorders. This ratio is regarded as an index of the NO synthesis [13] and/or NO synthase activity [12] and thus, NO formation may be endangered in bipolar patients.
Given the absence of an effect of lithium on the plasma concentration of the biochemical parameters and the data from the literature demonstrating an enhancing effect of lithium

on NO synthesis [28,29], the results from the present study are unlikely to be influenced by lithium maintenance therapy.

The lower neopterin concentration in bipolar patients may be caused by a decreased activity of the enzyme GTP cyclohydrolase, resulting in a lower conversion of guanosine triphosphate to 7,8-dihydroneopterintriphosphate. The latter is not only the precursor of neopterin, but also of BH4, which is the essential cofactor in the production of citrulline and NO from arginine [30,31]. Therefore, the lower Cit-Arg ratio suggests not only a decreased formation of NO, but may also indicate a lower activity of BH4. The latter substance is also an essential cofactor for the enzymatic hydroxylation of phenylalanine, tyrosine and tryptophan, from which the major monoamine neurotransmitters are synthesized. However, a decreased activity of the enzyme NO synthase is also a possibility. Thus, both the lower values of neopterin and the Cit-Arg ratio and the observed association between these parameters found in bipolar affective disorders, indicates a reduced activity of BH4 and a decreased formation of NO in this group, in contrast to the group of major depressive disorders with melancholic features or a seasonal pattern. These findings are in agreement with those reported recently by Richardson et al.[32] in a large sample of patients with schizophrenia and support the idea that bipolar affective disorder and schizophrenia share common pathophysiological patterns [33].

With respect to NO, numerous studies have substantiated the view that this molecule has multiple cellular and molecular targets and that it may functionally act as a hormone, neurotransmitter, mediator and cytotoxic or cytoprotective molecule [1]. In addition, NO participates in the control of various neurosecretory processes, especially of the corticotropin releasing hormone system [34]. Given its participation in many physiological and chemical reactions in the brain, NO has been investigated in a variety of neuropsychiatric disorders [2]. In schizophrenia, data do not show a clear picture as to whether there is more or less NO synthesis. In a recent review, Bernstein and co-workers conclude that it is unlikely that alterations in NO metabolism are unique to or indicative of schizophrenia [1].

The role of NO in the pathophysiology of affective disorders has also been studied. In patients with major depression, a reduced NO synthase activity has been found in various brain regions [5,8,35-37]. Measurement of the NO metabolites nitrite and nitrate in blood of depressed patients revealed either decreased [38,39] or elevated levels [40]. In bipolar-I patients, plasma levels of NO were found to be higher [6,7]. Four studies with the SSRI paroxetine presented with conflicting results, in that Finkel et al. [12] and Goodnick and Goldstein [41] reported decreased serum nitrite and nitrate levels following paroxetine treatment, whereas Lara et al. [42] and Chrapko et al. [27] found the reverse.

From the current study it can be concluded that the NO synthesis is state independently impaired in patients with bipolar affective disorders. The results, however, have to be interpreted with some caution. First, no direct measurement of actual NO synthesis is

presented. As an index of NO synthase activity the plasma concentrations of arginine and citrulline were measured and the ratio of these amino acids was calculated. Although the amino acid citrulline is regarded a more stable marker of NO synthesis than measurement of the blood levels of nitrate/nitrite [43], it has to be mentioned that citrulline originates not only from NO production but also from the action of the enzyme ornithine carbamoyltransferase. Furthermore, the enzyme argininesuccinate synthase is capable of citrulline catabolism in mammals. Second, the question remains whether peripheral measurements of NO synthesis reflect NO activity in the central nervous system or not, although some investigators think it does [4,44]. Finally, the contradictory results from the literature may be an expression of the fundamental concern in neuropsychiatry, in that descriptive psychiatric diagnoses are by no means uniform neither biologically nor clinically.

Acknowledgements

The authors thank Mrs. A.C.C. Voskuilen-Kooyman, Mrs. E. Taal, Mrs. M. Mulder, Mrs. J. Hoek and Mrs. M. Waltmans for their skilled technical assistance. This study was supported by a grant from the Jos Schaap - Feering Foundation.

References

1. Bernstein H-G, Bogerts B, Keilhoff G: The many faces of nitric oxide in schizophrenia. A review. Schizophr Res 2005a; 78:69-86.
2. Keilhoff G: Basic research on nitric oxide (NO). Foreword in: Cell Mol Biol (Noisy-le-grand) 2005; 51(3):245.
3. Harvey BH: Affective disorders and nitric oxide: a role in pathways to relapse and refractoriness? Hum Psychopharmacol 1996; 11:309-319.
4. Akyol O, Zoroğlu SS, Armutcu F, Sahin S, Gurel A: Nitric oxide as a physiopathological factor in neuropsychiatric disorders. In Vivo 2004; 18:377-390.
5. Bernstein H-G, Heinemann A, Krell D, Dobrowolny H, Bielau H, Keilhoff G, Bogerts B: Hypothalamic nitric oxide synthase in affective disorder: Focus on the suprachiasmatic nucleus. Cell Mol Biol 2005b; 51:279-284.
6. Savas H, Herken H, Yurekli M, Uz E, Tutkun H, Zoroğlu SS, Ozen ME, Cengiz B, Akyol O: Possible role of nitric oxide and adrenomedullin in bipolar affective disorder. Neuropsychobiology 2002; 45(2):57-61.
7. Yanik M, Vural H, Tutkun H, Zoroğlu SS, Savaş HA, Herken H, Kocyiğit A, Keleş H, Akyol O: The role of the arginine-nitric oxide pathway in the pathogenesis of bipolar

affective disorder. Eur Arch Psychiatry Clin Neurosci 2004; 254:43-47.
8. Bernstein H-G, Stanarius A, Baumann B, Henning H, Krell D, Danos P, Falkai P, Bogerts B: Nitric oxide synthase-containing neurons in the human hypothalamus: reduced number of immunoreactive cells in the paraventricular nucleus of depressive patients and schizophrenics. Neuroscience 1998; 83(3):867-875.
9. Swaab DF, Bao AM, Lucassen PJ: The stress system in the human brain in depression and neurodegeneration. Ageing Res Rev 2005; 4:141-194.
10. Van Amsterdam JGC, Opperhuizen A: Nitric oxide and biopterin in depression and stress. Psychiatry Res1999; 85:33-38.
11. Hoekstra R, Fekkes D, Van de Wetering BJM, Pepplinkhuizen L, Verhoeven WMA: Effect of light therapy on biopterin, neopterin and tryptophan in patients with seasonal affective disorder. Psychiatry Res 2003; 120:37-42.
12. Finkel MS, Laghrissi-Thode F, Pollock BG, Rong J: Paroxetine is a novel nitric oxide synthase inhibitor. Psychopharmacol Bull 1996; 32(4):653-8.
13. Fekkes D, Bannink M, Kruit WHJ, Van Gool AR, Mulder PGH, Sleijfer S, Eggermont AMM: Influence of pegylated interferon-α therapy on plasma levels of citrulline and arginine in melanoma patients. Amino Acids 2006, in press.
14. Hoekstra R, Van den Broek WW, Fekkes D, Bruijn JA, Mulder PGH, Pepplinkhuizen L: Effect of electroconvulsive therapy on biopterin and large neutral amino acids in severe, medication-resistant depression. Psychiatry Res 2001; 103:115-123.
15. Anderson DN, Wilkinson AM, Abou-Saleh MT, Blair JA: Recovery from depression after electroconvulsive therapy is accompanied by the evidence of increased tetrahydrobiopterin-dependent hydroxylation. Acta Psychiatr Scand 1994; 90:10-13.
16. Hashimoto R, Ozaki N, Ohta T, Kasahara Y, Kaneda N, Nagatsu T: The plasma tetrahydrobiopterin levels in patients with affective disorders. Biol Psychiatry 1990;28:526-528.
17. Spitzer RL, Endicott J: Schedule for Affective Disorders and Schizophrenia (SADS), ed 3 revised. New York, Biometrics Research, New York State Psychiatric Institute, 1978.
18. Hamilton M: Standardised assessment of depressive symptoms. Psychiatry Neurol Neurosurg 1969; 72:210-205.
19. Young RC, Biggs T, Ziegler E, Meyer DA: A rating scale for mania: reliability, validity and sensitivity. Br J Psychiatry 1978; 133:429-435.
20. Meesters Y, Jansen JHC: Assessing atypical seasonal affective disorder complaints by means of self-rating. Acta Psychiatr Scand 1993; 88:361-363.
21. Leverich GS, Post RM: Life charting the course of bipolar disorder. Curr Rev Mood and Anxiety Disorders 1996; 1:48-61.
22. Fekkes D, van Dalen A, Edelman, E, Voskuilen A: Validation of the determination of amino acids in plasma by high-performance liquid chromatography using automated

derivatization with o-phtaldialdehyde. J Chromatogr B 1995; 699(2):177-186.
23. Verhoeven WMA, Tuinier S, Van den Berg YWMM, Coppus AMW, Fekkes D, Pepplinkhuizen L, Thijssen JHH: Stress and self-injurious behavior; hormonal and serotonergic parameters in mentally retarded subjects. Pharmacopsychiatry 1999; 32:13-20.
24. Hoekstra R, Fekkes D, Loonen AJM, Pepplinkhuizen L, Tuinier S, Verhoeven WMA: Bipolar mania and plasma amino acids: Increased levels of glycine. Eur Neuropsychopharmacol 2006; 16:71-77.
25. Suzuki E, Nakaki T, Shintani F, Kanba S, Miyaoka H: Antipsychotic, antidepressant, anxiolytic, and anticonvulsant drugs induce type II nitric oxide synthase mRNA in rat brain. Neurosci Lett 2002; 333:217-219.
26. O'Donnell T, Rotzinger S, Ulrich M, Hanstock CC, Nakashima TT, Silverstone PH: Effects of chronic lithium and sodium valproate on concentrations of brain amino acids. Eur Neuropsychopharmacol 2003; 13:220-227.
27. Chrapko W, Jurasz P, Radomski MW, Archer SL, Newman SC, Baker G, Lara N, Le Mellédo JM: Alteration of decreased plasma NO metabolites and platelet NO Synthase activity by paroxetine in depressed patients. Neuropsychopharmacology 2005;1-8.
28. Bagetta G, Rodinò P., Paoletti AM, Arabia A, Massoud R, Nisticò G: Systemic administration of lithium chloride and tacrine but not kainic acid augments citrulline content of rat brain. Eur J Pharmacol 1995; 294:341-344.
29. Feinstein DL: Potentiation of astroglial nitric oxide synthase type-2 expression by lithium chloride. J Neurochem 1998; 71:883-886.
30. Levine RA: Tetrahydrobiopterin and biogenic amine metabolism in neuropsychiatry, immunology, and aging. Ann N Y Acad Sci 1988;521:129-139.
31. Koshimura K, Murakami Y, Tanaka J, Kato Y: The role of 6R-tetrahydrobiopterin in the nervous system. Prog Neurobiol 2000; 61(4):415-438.
32. Richardson MA, Read LL, Taylor Clelland CL, Reilly MA, Chao HM, Guynn RW, Suckow RF, Clelland JD: Evidence for a tetrahydrobiopterin deficit in schizophrenia. Neuropsychobiology 2005; 52(4):190-201.
33. Maier W, Zobel A, Wagner M: Schizophrenia and bipolar disorder: differences and overlaps. Curr Opin Psychiatry 2006; 19(2):165-70.
34. Riedel W: Role of nitric oxide in the control of the hypothalamic-pituitary-adrenocortical axis. Z Rheumatol 2000; 59(2):36-42.
35. Bernstein H-G, Heinemann A, Krell D, Mawrin C, Bielau H, Danos P, Diekmann S, Keilhoff G, Bogerts B, Baumann B: Further immunohistochemical evidence for impaired NO signaling in the hypothalamus of depressed patients. Ann N Y Acad Sci 2002; 973:91-93.
36. Xing G, Chavko M, Zhang LX, Yang S, Post RM: Decreased calcium-dependent consti-

tutive nitric oxide synthase (cNOS) activity in prefrontal cortex in schizophrenia and depression. Schizophr Res 2002; 58:21-30.
37. Karolewicz B, Szebeni K, Stockmeier CA, Koninck L, Overholser JC, Jurjus G, Roth, BL, Ordway GA: Low nNOS protein in the locus coeruleus in major depression. J Neurochem 2004; 91:1057-1066.
38. Chrapko WE, Jurasz P, Radomski MW, Lara N, Archer, SL, Le Mellédo JM: Decreased platelet nitric oxide synthase activity and plasma nitric oxide metabolites in major depressive disorder. Biol Psychiatry 2004; 56:129-134.
39. Selley ML: Increased (E)-4-hydroxy-2-nonenal and asymmetric dimethylarginine concentrations and decreased nitric oxide concentrations in the plasma of patients with major depression. Journal of Affective Disorders 2004; 80:249-256.
40. Suzuki E, Yagi G, Nakaki T, Kanba S, Asai M: Elevated plasma nitrate levels in depressive states. J Affect Disord 2001; 63:221-224.
41. Goodnick PJ, Goldstein BJ: Selective serotonin reuptake inhibitors in affective disorders-I. Basic pharmacology. J Psychopharmacol 1998; 12:S5-20.
42. Lara N, Archer SL, Baker GB, Le Mellédo JM: Paroxetine-induced increase in metabolic end products of nitric oxide. J Clin Psychopharmacol 2003; 23:408-412.
43. Pall ML: Levels of the nitric oxide synthase product, citrulline, are elevated in sera of chronic fatigue syndrome patients. J Chronic Fatigue Syndr 2002; 10:37-41.
44. Giovannoni G, Heales SJR, Silver NC, O'Riordon J, Miller RF, Land JM, Vlark JB, Thompson EJ: Raised serum nitrate and nitrite levels in patients with multiple sclerosis. J Neurol Sci 1997; 154:77-81.

Effect of electroconvulsive therapy on biopterin and large neutral amino acids in severe, medication-resistant depression

Chapter Three

Effect of electroconvulsive therapy on biopterin and large neutral amino acids in severe, medication-resistant depression.

Rocco Hoekstra, Walter W. van den Broek, Durk Fekkes, Jan A. Bruijn, Paul G. H. Mulder, Lolke Pepplinkhuizen.

Reprinted from Psychiatry Research 2001;103:115-123, Copyright (2001), with permission from Elsevier.

Abstract

Biopterin, neopterin and the large neutral amino acids (LNAA) i.e. phenylalanine, tyrosine, tryptophan, isoleucine, leucine and valine were measured in plasma of 20 severely depressed inpatients before and after a course of electroconvulsive therapy (ECT).

These patients showed a significantly lower plasma biopterin concentration at baseline in comparison with healthy controls. After treatment an increase in biopterin was found, which was statistically significant in the depressed patients with psychotic features. The plasma phenylalanine-tyrosine-ratio, which previously increased, normalised after ECT. Mean tryptophan concentration was lower in depressed patients than in normal controls. The patients who responded to ECT showed an increase in the tryptophan concentration and its ratio (tryptophan/LNAA) after treatment.

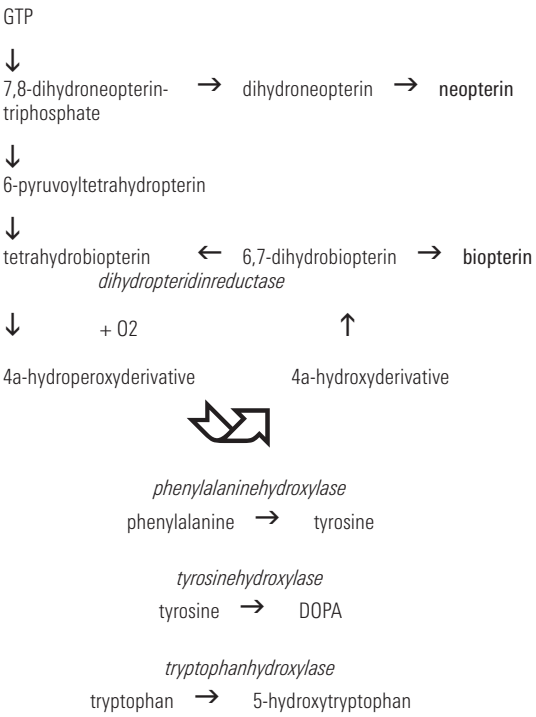
Our results suggest that ECT increases biopterin, which probably results in synthesis of amino acids, especially tyrosine. Furthermore, ECT seems to increase cerebral tryptophan availability because of less tryptophan catabolism parallel with biopterin activation. More research is required to see if biopterin could be useful as a biological marker for the depressive state in this subgroup of patients, because this compound seems to play an important role in the etiology and treatment of depression.

Introduction

The metabolism of biogenic amines has been hypothesised to play a key role in the pathophysiology of affective disorders. A functional deficiency of cerebral epinephrine and serotonin is still the most widely accepted model for understanding the biology of depression and the therapeutic action of antidepressant treatments (van Praag, 1982). Tetrahydrobiopterin (BH4) is the essential cofactor for the hydroxylation of phenylalanine, tyrosine and tryptophan, which is the rate limiting step in the formation of dopamine, norepinephrine and serotonin, respectively (Kapatos et al., 1993; Levine, 1988). Independent from its function as cofactor for the hydroxylation, BH4 also enhances the release of these neurotransmitters from nerve terminals (Mataga et al., 1991; Wolf et al., 1991). A link between BH4 and both nitric oxide, a neuroendocrine modulator of the HPA-axis, and the immune system has been hypothesised (van Amsterdam and Opperhuizen, 1999).
BH4 is synthesised de novo from guanosine triphosphate (GTP) which is converted to dihydroneopterin triphosphate (NHPT3). The latter is converted by a series of tetrahydro intermediates to BH4. The concentration of cellular BH4 is dependent on this pathway and a salvage mechanism that converts quinonoid dihydrobiopterin to BH4 by the enzyme dihydropteridine reductase. Hydrolysis of NHPT3 yields dihydroneopterin, which is excreted in urine with its oxidative product neopterin, as are BH4 and its oxidative products, dihydrobiopterin and biopterin (Levine, 1988) (figure 1).

Increased neopterin secretion is a sensitive marker of activation of cell-mediated immunity. Interferon-gamma, which is produced and secreted by activated T cells, is probably the most important activator of pteridine synthesis and release by activated cells of the monocyte / macrophage lineage (Hüber et al., 1984; Maes et al., 1994). Immune-activation not only increases synthesis of pteridines but also tryptophan catabolism through induction of indoleamine 2,3-dioxygenase (IDO) and will therefore influence serotonin metabolism (Maes et al., 1993). There is evidence for immune activation in depression (Maes et al., 1993). Indeed, plasma levels of tryptophan and the ratio of tryptophan to the sum of amino acids, which compete for the same cerebral uptake mechanism, i.e. phenylalanine, tyrosine, isoleucine, leucine and valine, have been found to be lower in depressed subjects than in normal control subjects (DeMyer et al., 1981; Møller, 1985).
Many studies have focused on the catecholamine hypothesis of depression (Schildkraut, 1965). Recent challenge studies have renewed interest in this theory (Berman et al., 1999). Levels of phenylalanine and tyrosine have been studied in depressed patients. A lower tyrosine level or a lower tyrosine ratio to competing amino acids is often reported. Antidepressant medication seems to have no effect on these measures (e.g. Møller et al.,

Figure 1.
Biosynthesis of tetrahydrobiopterin and its effect on the hydroxylation of phenylalanine, tyrosine and tryptophan.



1981). Anderson reported a reduction of the ratio of phenylalanine to tyrosine after response to ECT, suggestive of increased hydroxylation by BH4 after treatment (Anderson et al., 1994).

Because of its role in dopamine, norepinephrine and serotonin metabolism, BH4 has been studied in depressed patients. Contradictory results have been reported (Abou-Saleh et al., 1995; Anderson et al., 1992; Blair et al., 1984; Bonaccorso et al., 1998; Coppen et al., 1989; Duch et al., 1984; Garbutt et al., 1985). In these studies, however, urinary biopterin was measured. As can be expected from the metabolism and excretion of BH4, plasma measurements are not comparable with these results.

Hashimoto and Knapp found an increased plasma biopterin in depressed patients (Hashimoto et al., 1990; Knapp and Irwin, 1989). Methodological factors make these and several other studies difficult to compare. The population was often not well defined or not very restricted. 'Major depression' could represent too broad a spectrum of pathology for studying a biological marker. Besides, in these studies patients were using several psychotropic drugs.

The same problems arise when studying neopterin, as mentioned above a known marker of immunity. An increase in the concentration of neopterin could be expected in plasma of depressed patients. However, recently O'Toole found no significant difference in plasma neopterin between patients and normal controls, while Maes and Dunbar found increased levels in urine (Dunbar et al., 1992; Maes et al., 1994; O'Toole et al., 1998).
Studying strictly selected, severely depressed, medication free inpatients, we tried to answer the following questions: (1) Is the plasma concentration of biopterin lowered in this population, compared to normal controls, and is the neopterin concentration increased? (2) Are certain clinical features like psychotic symptoms or the response to treatment related to even lower concentrations of biopterin? (3) Is there an increase in plasma biopterin after effective treatment (ECT) with a reverse tendency for plasma neopterin? (4) Are the concentrations of the amino acids, on which tetrahydrobiopterin acts as a cofactor, changed before and after treatment? More specifically, is the phenylalanine-tyrosine-ratio, a known measure for hydroxylation activity of BH4, higher in the depressed population in comparison with normal controls and is this ratio lowered after successful treatment? And is tryptophan or the tryptophan-ratio decreased before treatment and increased after that?

Methods

The protocol of this study was approved by the Ethics Committee of the University Hospital Rotterdam. Informed consent was obtained from 20 patients, 7 male and 13 female, with a mean age of 52 (SD 13.1). Patients who were pregnant or suffered from serious diseases known to influence the biopterin metabolism, like infectious diseases, autoimmune disorders, neoplastic disorders, Parkinson disease or dementia, were excluded from this study. They had a DSM-IV diagnosis of major depression, which was assigned by two psychiatrists performing the depression part of the Schedule for Affective Disorders and Schizophrenia (SADS) (Spitzer and Endicott, 1977). Psychotic and melancholic features were assessed during this interview. The diagnosis of psychotic depression was only made when the patient was having definite mood-congruent delusions according to the SADS. The severity of the depression was scored using the Hamilton Rating Scale for Depression (HRS-D) (Hamilton, 1969). Improvement was measured with the HRS-D before and after the course of ECT and was defined as an outcome score of less than 10 or a more than 50% decrease from the baseline.
Furthermore, recommendation for inpatient ECT had been made by the treating psychiatrists and an independent psychiatrist. In The Netherlands this recommendation is almost exclusively made when a patient with a severe major depression has failed to respond to prior treatment with a tricyclic antidepressant, lithium addition or an irreversible

monoamine oxidase inhibitor. Twenty-nine healthy volunteers, 16 male and 13 female, with a mean age of 37 (SD 8.3), served as controls.

ECT was administered twice weekly. All patients were free of antidepressants for at least 7 days before the ECT course. In cases of severe agitation, only a short-acting benzodiazepine or droperidol was given on the morning of treatment. ECT was started with unilateral brief-pulse, constant current stimulation, using the Thymatron DGx. The initial dosage of the stimulus was based on the age of the patient. After intravenous administration of methylatropine, alfentanil and metoclopramide, anaesthesia was achieved with intravenous etomidate and succinylcholine in this order during all sessions. If there was no, or hardly any, clinical improvement after four sessions, as measured by the HRS-D, unilateral ECT was switched to bifrontotemporal ECT. In four patients ECT was started bilaterally because of the severity of the depression and/or the earlier good response to this mode of treatment. The decision to stop was made when no clinical improvement was observed by the treating psychiatrist after four to six bilateral treatments or when complete remission was obtained. Thus, this resulted in a different number of treatments.

A fasting, venous blood sample was taken in the morning before the first ECT administration. The morning after the last ECT administration the second sample was collected. The HRS-D score was also determined.

Immediately after venapuncture, plasma was prepared by a 20-min centrifugation step at 2650 g and stored at -80 ^{0}C. Neopterin and biopterin were measured after acid oxidation of the reduced forms of both pteridines as described earlier (Fukushima and Nixon, 1980). Plasma (0.4 ml) was oxidised in 0.1 ml 1 M trichloroacetic acid and 0.05 ml iodine solution (0.5% I2, 1%KI in 0.2 M trichloroacetic acid). For each assay, different amounts of neopterin and biopterin were added to a plasma pool for the determination of the percent recovery. After standing for 60 min under reduced light, excess iodine was reduced by the addition of 20 µl of 1% ascorbic acid solution and the mixture was centrifuged at 12 000 g for 15 min at 4 °C. The supernatant (0.4 ml) was transferred to an amber glass vial, and 10 µl was injected directly onto the analytical column using an HPLC system with an autosampler and a fluorescence detector (Hewlett Packard, Series 1100). We used a Hypersil C18 column (2.1 x 200 mm, 5 µm), which was protected by a narrow bore guard column (2.1 x 20 mm) of the same material (Hewlett Packard). The separation was achieved using an aqueous 15 mmol/l potassium phosphate buffer, pH 6.45 and a stepwise eluant gradient with methanol (from 1.5-2 min to 10%, from 4 to 5 min to 100%, and from 6 to 8.3 min to 0%). Total run time was 14 min, the flow rate was set at 0.4 ml/min, the column temperature was 50 °C and the excitation and emission wavelengths were 360 and 440 nm, respectively. Compounds were quantitated by their peak height in comparison with external standards. Neopterin and biopterin were eluted at approximately 2.2 and 4.8 min, and the detection limits of both compounds (at a signal-to-noise ratio of 3) were 2 and 3

fmol (0.2 and 0.3 nmol/l), respectively. Recoveries of neopterin and biopterin added to plasma samples were 100-140 and 95-110%, respectively. The intra-assay coefficients of variation for neopterin and biopterin were less than or equal to 2.4 and 2.1%, respectively. The interassay coefficients of variation for these compounds, determined on six different days, were 3.8 and 2.8%, respectively.
The amino acids phenylalanine, tyrosine, tryptophan, isoleucine, leucine and valine were measured by means of high performance liquid chromatography (Fekkes et al., 1995). The tryptophan ratio was calculated as 100 times the concentration of tryptophan divided by the summed concentration of the other large neutral amino acids, i.e. phenylalanine, tyrosine, isoleucine, leucine and valine (Fernstrom and Wurtman, 1972).
For statistical analysis a two-tailed Student t test was used. Data were expressed as mean and standard deviation. Statistical significance was defined as $p<0.05$.

Results

The mean score of the 20 patients on the HRS-D was 31, before the start of the ECT procedure, after unsuccessful medication treatment. After ECT, the mean HRS-D score was 11. According to the above-mentioned definition, 15 patients had a good clinical response. All patients showed melancholic features, and 10 patients showed mood-congruent psychotic features. Four patients got only unilateral and four patients received exclusively bilateral ECT. The total group received a mean of 11 ECT administrations. One patient used 2 mg of lorazepam and 10 mg of droperidol during the course of ECT, although the post-treatment blood sampling took place 5 days later while the patient was free of medication. Five patients had already restarted an antidepressant treatment, like imipramine, lithium or tranylcypromine, for 1-4 days before the post-treatment blood sample was taken. These five patients were free of medication before and during the ECT course.
Mean biopterin level in the depressed patients was 5.7 nmol/l while that in the normal controls was 7.0 nmol/l ($p=0.002$). After ECT, the mean biopterin concentration normalised to 6.3 nmol/l, but the difference between pre- and post-treatment was not significant (Table 1).
When only the subcategory of psychotic patients was studied, a significantly lower pre-treatment level was found compared to the normal controls, i.e. 5.5 nmol/l vs. 7.0 nmol/l ($p=0.04$). Psychotic depressed patients had a significantly increased level of biopterin after treatment, i.e. 6.5 nmol/l ($p=0.03$). Non-psychotic patients did not show any increase in the biopterin level (Table 2).
Lower pre-treatment levels were also found in patients who responded to ECT, i.e. 5.6

Table 1.
Biopterin, neopterin, P-T-ratio and tryptophan-ratio before and after a course of ECT in 20 severely depressed patients compared to 29 healthy controls

	Total pre-treatment (n=20)	Total post-treatment (n=20)	Controls (n=29)
biopterin(nmol/l)	5.7 (1.3) p=0.002	6.3 (1.4)	7.0 (1.4)
neopterin (nmol/l)	21.7 (8.2)	23.6 (8.2) p=0.03	19.6 (6.5)
P-T-ratio	1.0 (0.1) p=0.001	0.9 (0.1) p<0.0005	0.9 (0.2)
tryptophan	35.5 (9.0) p<0.0005	38.7 (7.7)	45.6 (6.1)
LNAA	489.7 (99.2)	506.7 (107.8)	549.4 (107.1)
Trp-ratio	7.4 (2.0)	7.7 (1.0)	8.3 (1.1)

P-T-ratio = phenylalanine-tyrosine-ratio
Trp-ratio = tryptophan ratio
LNAA = large neutral amino acids; i.e. phenylalanine, tyrosine, isoleucine, leucine and valine.

Measures are presented as mean (standard deviation). P-values for pre-treatment measures give significance levels compared to normal controls. P-values for post-treatment measures give significance levels compared to pre-treatment measures.

nmol/l (p=0.003). The responders showed an increase in biopterin from 5.6 nmol/l to 6.4 nmol/l, whereas this concentration did not change in the non-responders (Table 3).
Neopterin concentration was not significantly higher in the total group of depressed patients when compared to normals, viz. 21.7 nmol/l vs. 19.6 nmol/l. After treatment the neopterin level increased significantly to 23.62 nmol/l (p=0.03).
The neopterin-biopterin ratio was significantly increased before treatment compared to the controls. (4.0 (2.0) vs. 2.9 (1.1); p=0.01) However, ECT did not change this ratio.
The depressed patients had a mean phenylalanine level of 52.8 (8.9) µmol/l and, after treatment, the level slightly decreased to 49.4 (6.8) µmol/l. The mean level in healthy controls is 56.0 (6.9) µmol/l. Tyrosine concentrations increased from 52.7 (11.9) to 56.3 (13.5) µmol/l. The mean level in healthy controls is 62.0 (14.4) µmol/l. Although these pre- and post-values were not significantly different, the phenylalanine-tyrosine (P-T) ratio did show a significant decline after treatment from 1.0 to 0.9 (p<0.0005). The subgroups with psychotic features and the responders had comparable results with only the phenylalanine -tyrosine ratio being significantly different. Despite the decline of the P-T-ratio, normal values of the separate amino acids were not obtained.
The mean tryptophan concentration was 35.5 µmol/l in the depressed patients vs. 45.6 µmol/l in the controls (p<0.0005). After treatment, the tryptophan level increased non-significantly to 38.7 µmol/l. Psychotic depressed patients had a mean tryptophan level of 35.3 µmol/l rising to 39.6 µmol/l after ECT. The group that responded to treatment had an

Table 2.
Biopterin, neopterin, P-T-ratio and tryptophan-ratio before and after a course of ECT in 20 severely depressed patients, subdivided according to psychotic features.

	psychotic pre-treatment (n=10)	psychotic post-treatment (n=10)	non psychotic pre-treatment (n=10)	non psychotic post-treatment (n=10)
biopterin (nmol/l)	5.5 (1.1) p=0.04	6.5 (1.0) p=0.03	5.9 (1.4) p=0.04	6.1 (1.8)
neopterin (nmol/l)	19.8 (3.8)	21.8 (4.6)	23.5 (10.9)	25.4 (10.7)
P-T-ratio	1.0 (0.2) p=0.05	0.9 (0.1) p=0.001	1.0 (0.1) p=0.01	0.9 (0.2) p=0.001
tryptophan (µmol/l)	35.3 (11.1) p=0.02	39.6 (6.1)	35.7 (6.8) p=0.001	37.7 (9.3)
trp-ratio	7.0 (1.8) p=0.05	7.7 (0.5)	7.8 (2.2)	7.7 (1.4)

P-T-ratio = phenylalanine-tyrosine-ratio
Trp-ratio = tryptophan ratio

Measures are presented as mean (standard deviation). P-values for pre-treatment measures give significance levels compared to normal controls. Values of normal controls are mentioned in table 1. P-values for post-treatment measures give significance levels compared to pre-treatment measures.

even lower tryptophan level of 33.9 µmol/l rising significantly after ECT to 38.3 µmol/l (p=0.05). For the tryptophan ratio, the same trend was visible, except for a non-significantly lower ratio in the total group of patients compared to the controls.

The five patients who used medication a few days before the last sample was taken were distributed between the two subgroups: there were three responders and two non-responders; one non-psychotic and four psychotic patients. Exclusion of these patients did not essentially change the post-treatment results obtained. Without these patients post-treatment biopterin was 6.0 (1.3) vs. 6.3 (1.4) nmol/l with these patients included. The phenylalanine-tyrosine-ratio was 0.91 (0.14) vs. 0.89 (0.13) in the total group. This was significantly lower than the pre-treatment value, as it was in the total group. For the subgroup of responders, the same could be seen when these patients were excluded; a significant lowering of the P-T-ratio ($p<0.0005$) and an increase in the tryptophan-ratio (p=0.03).

Table 3.
Biopterin, neopterin, P-T-ratio and tryptophan-ratio before and after a course of ECT in 20 severely depressed patients, subdivided according to response on treatment.

	responders pre-treatment (n=10)	responders post-treatment (n=10)	non responders pre-treatment (n=10)	non responders post-treatment (n=10)
biopterin (nmol/l)	5.6 (1.2) p=0.003	6.4 (1.0)	5.8 (1.7)	5.9 (2.5)
neopterin (nmol/l)	21.8 (9.5)	24.3 (9.3) p=0.01	21.1 (1.9)	21.7 (3.6)
P-T-ratio	1.0 (0.1) p=0.001	0.9 (0.1) p<0.0005	1.0 (0.2)	0.9 (0.2) p=0.001
tryptophan (µmol/l)	33.9 (9.8) p<0.0005	38.3 (6.4) p=0.05	40.2 (4.3) p=0.005	37.7 (11.8)
Trp-ratio	6.6 (1.3) p=0.005	7.7 (1.1) p=0.003	9.8 (1.9)	7.7 (0.7)

P-T-ratio = phenylalanine-tyrosine-ratio
Trp-ratio = tryptophan ratio

Measures are presented as mean (standard deviation). P-values for pre-treatment measures give significance levels compared to normal controls. Values of normal controls are presented in table 1. P-values for post-treatment measures give significance levels compared to pre-treatment measures.

Discussion

Our main finding is that the total plasma biopterin concentration is lower than normal when studied in well selected, severely depressed inpatients, free of psychoactive medication. In most patients, treatment with ECT was successful (75%) and this was paralleled by an increase in biopterin levels. These findings were more pronounced in depressed patients with mood-congruent psychotic features and in ECT-responsive patients.

In children with phenylketonuria, it has been shown that the phenylalanine-tyrosine ratio is a useful measure for phenylalanine hydroxylase activity, better than phenylalanine alone (Rosenblatt and Scriver, 1968). As could be expected from the influence of biopterin on the hydroxylating enzymes, we found a lower phenylalanine-tyrosine ratio after treatment, indicating more hydroxylation of phenylalanine to tyrosine.

A lower pre-treatment concentration of tryptophan was observed both in the total group of patients and in the responders. Only successful ECT treatment resulted in a significant increase in tryptophan level. These seemingly contradictory results - successful ECT treatment gives rise to higher biopterin levels and a consequently higher conversion of tryptophan to serotonin - may be explained by a lower catabolism of tryptophan through

the kynurenine pathway, probably due to decreased cortisol levels. The tryptophan ratio may also be a predictor for successful ECT treatment because this parameter was significantly lower before treatment in the subgroup of responders and in the subgroup of psychotic patients. However, the increase in this ratio after ECT was only significant for the responders.

A limitation of our study is the use of medication before ECT was started. Perhaps the wash-out period of 7 days was too short to rule out every influence on the biopterin and amino acid metabolism. However, antidepressants seem to affect the plasma biopterin levels in a limited way (Hashimoto, 1987). The differences between the subgroups cannot be explained by such an influence because the same wash-out period was used for all patients. Although only short-acting anaesthetics were administered before ECT, we cannot rule out the possibility that this medication influenced the post-treatment measures. Since the same standardised anaesthesia procedure was used on all patients, it cannot explain the differences between the groups.

Our findings are consistent with the results of Blair (Blair et al., 1984). Anderson and Abou-Saleh also suggest a decrease in biopterin level with their finding of a higher neopterin-biopterin ratio in depressed patients (Abou-Saleh et al., 1995; Anderson et al., 1992). Patient selection, urinary measurements and concomitant use of medication could explain conflicting results found in other studies.

Neopterin levels in depressed patients differed non-significantly from levels in normal controls. Our plasma neopterin values are difficult to compare with the results of Maes and Dunbar. They found increased neopterin levels in depressed patients but did not use the same, medication-resistant, severely depressed inpatient population as we did and, above all, measured urinary neopterin (Dunbar et al., 1992; Maes et al., 1994). However, they are indeed comparable with those of O'Toole who also studied plasma concentrations (O'Toole et al., 1998). Perhaps this parameter is too non-specific to be a useful marker in depression.

Our results suggest that ECT influences biopterin metabolism, phenylalanine and tyrosine metabolism and, hence, catecholaminergic function, and the availability of tryptophan as can be concluded by the rise in the tryptophan-ratio. ECT apparently increases plasma concentrations of biopterin, which in turn increases the metabolism of the amino acids phenylalanine, tyrosine and tryptophan. This gives rise to interesting questions. Is effective treatment with antidepressants also related to an increase in biopterin? Does a low biopterin level predict a poor response to antidepressants? Or is ECT unique in its action on biopterin, which in turn, improves synthesis of mainly tyrosine and, hence, improves catecholaminergic function? More evidence that plasma biopterin can serve as a distinct marker for the necessity to start ECT has to be provided.

Acknowledgements:

The authors thank Mrs. A.C.C. Voskuilen-Kooyman for skilled technical assistance.

References

- Abou-Saleh, M.T., Anderson, D.N., Collins, J., Hughes, K., Cattell, R.J., Hamon, C.G., Blair, J.A., 1995. The role of pterins in depression and the effects of antidepressive therapy. Biological Psychiatry 38, 458-63.
- Amsterdam van, J.G.C., Opperhuizen, A., 1999. Nitric oxide and biopterin in depression and stress. Psychiatry Research 85, 33-38.
- Anderson, D.N., Abou-Saleh, M.T., Collins, J., Hughes, K., Cattell, R.J., Hamon, C.G., Blair, J.A., Dewey, M.E., 1992. Pterin metabolism in depression: an extension of the amine hypothesis and possible marker of response to ECT. Psychological Medicine 22, 863-9.
- Anderson, D.N., Wilkinson, A.M., Abou-Saleh, M.T., Blair, J.A., 1994. Recovery from depression after electroconvulsive therapy is accompanied by evidence of increased tetrahydrobiopterine-dependent hydroxylation. Acta Psychiatrica Scandinavica, 90, 10-13.
- Berman, R.M., Narasimhan, M., Miller, H.L., Anand, A., Cappiello, A., Oren, D.A., Heninger G.R., Charney, D.S., 1999. Transient depressive relapse induced by catecholamine depletion. Potential phenotypic vulnerability marker? Archives of General Psychiatry, 56, 395-403.
- Blair, J.A., Barford, P.A., Morar, C., Pheasant, A.E., Hamon, C.G., Whitburn, S.B., Leeming, R.J., Reynolds, G.P., Coppen, A., 1984. Tetrahydrobiopterin metabolism in depression. Lancet 2, 163.
- Bonaccorso, S., Lin, A.,Verkerk, R., Van Hunsel, F., Libbrecht, I., Scharpé, S., DeClerck, L., Biondi, M., Janca, A., Maes, M., 1998. Immune markers in fibromyalgia: comparison with major depressed patients and normal volunteers. Journal of Affective Disorders, 48, 75-82.
- Coppen, A., Swade, C., Jones, S.A., Armstrong, R.A., Blair, J.A., Leeming, R.J., 1989. Depression and tetrahydrobiopterin: the folate connection. Journal of Affective Disorders 16, 103-7.
- DeMyer, M.K., Shea, P.A., Hendric, H.G., Yoshimura, N.N., 1981. Plasma tryptophan and five other amino acids in depressed and normal subjects. Archives of General Psychiatry, 38, 642-646.

- Duch, D.S., Woolf, J.H., Nichol, C.A., Davidson, J.R., Garbutt, J.C., 1984. Urinary excretion of biopterin and neopterin in psychiatric disorders. Psychiatry Research 11, 83-9.
- Dunbar, P.R., Hill, J., Neale, T.J., Mellsop, G.W., 1992. Neopterin measurement provides evidence of altered cell-mediated immunity in patients with depression, but not with schizophrenia. Psychological Medicine 22, 1051-1057.
- Fekkes, D., Dalen van, A., Edelman, E., Voskuilen, A., 1995. Validation of the determination of amino acids in plasma by high-performance liquid chromatography using automated derivatization with o-phthaldialdehyde. Journal of Chromatography B, 669, 177-186.
- Fernstrom, J., Wurtman, R., 1972. Brain serotonin content: physiological regulation by plasma neutral amino acids. Science 178, 414-416.
- Fukushima, T., Nixon, J.C., 1980. Analysis of reduced forms of biopterin in biological tissues and fluids. Analyt. Biochemistry. 102, 176-188.
- Garbutt, J.C., Duch, D.S., Nichol, Ch.A., Woolf, J.H., 1985. Urinary biopterin and neopterin excretion and pituitary-adrenal activity in psychiatric patients. Psychiatry Research, 16, 181-187.
- Hamilton, M., 1969. Standardised assessment of depressive symptoms. Psychiatry, Neurology and Neurosurgery 72, 201-205.
- Hashimoto, R., Ozaki, N., Ohta, T., Kasahara, Y., Kaneda, N., Nagatsu, T., 1987. Total biopterin levels of plasma in patients with depression. Neuropsychobiology, 17, 176-177.
- Hashimoto, R., Ozaki, N., Ohta, T., Kasahara, Y., Kaneda, N., Nagatsu, T., 1990. The plasma tetrahydrobiopterin levels in patients with affective disorders. Biological Psychiatry 28, 526-528.
- Hüber, C., Batchelor, J.R., Fuchs, D., Hausen, A., Lang, A., Niederwieser, D., Reibnegger, G., Swetly, P., Troppmair, J., Wachter, H., 1984. Immune response-associated production of neopterin: release from macrophages primarily under control of interferon gamma. Journal of Experimental Medicine, 160, 310-316.
- Kapatos, G., Hirayama, K., Lentz, S.I., Zhu, M., Stegenga, S., 1993. Differential metabolism of tetrahydrobiopterin in monoamine neurons: a hypothesis based upon clinical and basic research. Advances in Experimental Medicine and Biology 338, 217-22.
- Knapp, S., Irwin, M., 1989. Plasma levels of tetrahydrobiopterin and folate in major depression. Biological Psychiatry 26, 156-162.
- Lentz, S.I., Kapatos, G., 1996. Tetrahydrobiopterin biosynthesis in the rat brain: heterogeneity of GTP cyclohydrolase I mRNA expression in monoamine-containing neurons. Neurochemistry International 28, 569-582.

- Levine, R.A., 1988. Tetrahydrobiopterin and biogenic amine metabolism in neuropsychiatry, immunology, and aging. Annals of the New York Academy of Sciences, 521, 129-139.
- Mataga, N., Imamura, K., Watanabe, Y., 1991. 6R-tetrahydrobiopterin perfusion enhances dopamine, serotonin, and glutamate outputs in dialysate from rat striatum and frontal cortex. Brain Research, 551, 64-71.
- Maes, M., Meltzer, H.Y., Scharpé, S., Bosmans, E., Suy E., De Meester I., Calabrese, J., Cosyns, P., 1993. Relationships between lower plasma L-tryptophan levels and immune-inflammatory variables in depression. Psychiatry Research, 49, 151-165.
- Maes, M., Scharpé, S., Meltzer, H.Y., Okayli, G., Bosmans, E., D'Hondt, P., Vanden Bossche, B., Cosyns, P., 1994. Increased neopterin and interferon-gamma secretion and lower availibility of L-tryptophan in major depression: further evidence for an immune response. Psychiatry Research 54, 143-160.
- Møller S.E., Reisby N., Ortmann J., Elley J., Krautwald O., 1981. Relevance of tryptophan and tyrosine availibility in endogenous and 'non-endogenous' depressives treated with imipramine or clomipramine. Journal of Affective Disorders 3 (3), 231-44.
- Møller S.E., 1985. Tryptophan to competing amino acids ratio in depressive disorder: relation to efficacy of antidepressive treatments. Acta Psychiatrica Scandinavica Suppl. 325, 3-31.
- O'Toole, S.M., Chiappelli, F., Rubin, R.T., 1998. Plasma neopterin in major depression: relationship to basal and stimulated pituitary-adrenal cortical axis function. Psychiatry Research 79, 21-9.
- Rosenblatt, D., Scriver, C.R., 1968. Heterogeneity in genetic control of phenylalanine metabolism in man. Nature, 218, 677-678.
- Schildkraut, J.J., 1965. The catecholamine hypothesis of affective disorders: a review of supporting evidence. American Journal of Psychiatry 122, 509-522.
- Spitzer, R.L., Endicott, J., 1978. Schedule for affective disorders and schizophrenia (SADS), 3rd edition, Biometric Research, New York State Psychiatric Institute, New York.
- Van Praag, H.M., 1982. Neurotransmitters and CNS disease: depression. Lancet ii, 1259-1264.
- Wolf, W.A., Ziaja, E., Arthur, R.A. Jr., Anastasiadis, P.Z., Levine, R.A., Kuhn, D.M., 1991. Effect of tetrahydrobiopterin on serotonin synthesis, release, and metabolism in superfused hippocampal slices. Journal of Neurochemistry 57, 1191-7.

Effect of light therapy on biopterin, neopterin and tryptophan in patients with seasonal affective disorder

Chapter Four

Effect of light therapy on biopterin, neopterin and tryptophan in patients with seasonal affective disorder.

Rocco Hoekstra, Durk Fekkes, Ben J.M. van de Wetering, Lolke Pepplinkhuizen, Willem M.A. Verhoeven.

Reprinted from Psychiatry Research 2003;120:37-42, Copyright (Year), with permission from Elsevier.

Abstract

The serotonergic system is believed to play a key role in the pathophysiology of seasonal affective disorder (SAD). Tetrahydrobiopterin is an essential cofactor in the hydroxylation of tryptophan and, therefore, in the synthesis of serotonin, while neopterin is known as marker of cell-mediated immune activity.

The present study was designed to measure levels of biopterin, neopterin and tryptophan in plasma of 19 depressed patients with a history of SAD, before and after light therapy as well as in a control group. In the group of patients a significantly lower plasma biopterin and tryptophan level and a higher neopterin level was demonstrated. After light therapy, the level of biopterin increased to that of the controls but lowered again in summer. Neopterin concentrations remained on the same level after light therapy, whereas tryptophan levels increased slightly after light therapy and reached normal values in summer.

It is concluded that the vulnerability for a depressive episode is enhanced by lowered levels of biopterin that, however, in SAD becomes symptomatically manifest in the presence of increased immune activity at the same time.

Introduction

Seasonal affective disorder (SAD) is characterized by recurrent episodes of winter depression with remission in the spring and summer. Patients with SAD differ from those with a major depressive episode with melancholic features in both clinical presentation and biological characteristics (Rosenthal et al., 1984). The efficacy of light therapy in SAD has been generally accepted (Partonen and Lonnqvist, 1998). In The Netherlands the prevalence of SAD is 3% (Mersch et al., 1999).

Although there is no consensus on the pathophysiology of SAD or on the mode of action of light therapy, serotonergic mechanisms seem to play an important role. Evidence is found not only for decreased activity of the serotonergic system, but therapeutic results of serotonergic agents are also described (Neumeister et al. 2001).

Pteridines are compounds that occur in human as skin and eye pigments and act as multifunctional cofactors such as folic acid and tetrahydrobiopterin (BH4) (Auerbach and Nar 1997). BH4 is the essential cofactor for the enzyme tryptophan hydroxylase. This enzyme hydroxylates tryptophan to 5-hydroxytryptophan, which is the rate-limiting step in the biosynthesis of serotonin. In the pineal gland serotonin is converted to melatonin (Levine, 1988). In addition, BH4 is an essential cofactor in the hydroxylation of phenylalanine and tyrosine from which the catecholamines are synthesized. Furthermore, this cofactor influences the release mechanism in nerve terminals of these neurotransmitters (Wolf et al., 1991).

BH4 is synthesized de novo from guanosine triphosphate (GTP) via the intermediate dihydroneopterin triphosphate (H2NTP). The latter substance may also be converted to neopterin, which is excreted in urine. The oxidative products of BH4 are dihydrobiopterin and biopterin, which are also excreted in urine (Levine, 1988).

Cytokine-mediated T-lymphocyte activation results in increased neopterin synthesis and its release from macrophages. Therefore, neopterin can be used as a marker for cell-mediated immune activation (Maes et al., 1994; Auerbach and Nar, 1997), and it has been suggested subsequently that alterations in cell-mediated and humoral immune function may play a role in the pathophysiology of depressive disorders (Maes et al., 1994). Support for this idea stems from the observation of altered immunologic parameters, such as neopterin, in depressed patients with SAD after monoamine depletion (Stastny et al., 2003).

Biopterin has been found to be decreased in depression (Abou-Saleh et al., 1995; Anderson et al., 1992; Coppen et al., 1989; Hoekstra et al., 2001). This could be explained by assuming that the intermediate H2NTP is mainly converted to the immunologic marker neopterin, more than to the cofactor BH4, resulting in less BH4-dependent hydroxylation of tryptophan or tyrosine and consequently in decreased synthesis of serotonin, dopamine and (nor) epinephrine. Since the pineal gland contains relatively high concentrations of

BH4, less activity of this substance may in turn result in a decrease of melatonin levels (Levine, 1988).
The present study was designed to measure plasma levels of biopterin and neopterin, and to investigate their relationship to plasma tryptophan in depressed patients with SAD, before and after light therapy. Given the crucial role of biopterin in the synthesis of serotonin and the diminished serotonergic activity in SAD, it was postulated that levels of biopterin and tryptophan, the precursor of serotonin, would be lower in depressed patients with SAD with an increase to normal values after light therapy. Since depression in general, and particularly SAD, is associated with immune activation, an increase in neopterin level before light therapy in depressed patients as compared to healthy controls, with normalization after light therapy, was hypothesized.

Methods

Subjects

The present study included 19 patients, one male and 18 females (mean age (±SD): 41.0 (±13.2) years) and was conducted in full accordance with the Helsinki principles. All had a history of affective disorders with a seasonal pattern, and all but one had been treated previously with light therapy. Excluded were the patients who were pregnant or suffered from serious diseases known to influence pteridine metabolism, like infectious diseases, autoimmune disorders, neoplastic disorders, Parkinson's disease or dementia. All patients were free of any psychoactive medication for at least 4 weeks prior to experimentation.

Assessment

Severity of the depression was assessed with the Beck Depression Inventory (BDI), a self-rating scale for depression. To ascertain the atypical symptoms of SAD, an addendum was used (BDI-add), which is highly correlated with the Hamilton Rating Scale for Depression - Add (Meesters and Jansen, 1993). This addendum includes item 12 (social withdrawal) and 17 (fatigability) from the BDI as well as items 16 (hypersomnia), 18 (appetite) and 19 (weight gain), which are inversely formulated. The presence of atypical symptoms was defined by a score of at least 4 on the BDI-add.
The period, in which light therapy was given ranged from 25 October to 8 March. Most patients were referred in November. Eleven patients could be re-evaluated in July or August of the following year. They did not differ in sex and age as compared to the 19 patients in winter.

Light therapy

All patients received a standard regimen of 10 000 lux cool-white fluorescent light therapy during 1 h on five consecutive mornings in our Outpatient Department. Light therapy was applied between 08.30 and 10.00 h.

Study design

On the first day of light therapy, an initial fasting venous blood sample was drawn. To obtain reliable values for the amino acids, blood sampling was performed before 10.00 h. Since light therapy was presented till Friday 10.00 h, the first possibility in practice to draw the post-treatment blood sample before 10.00 h was 3 days after treatment. The BDI and addendum were completed directly before light therapy and on the subsequent Friday post-treatment. All patients were asked to return for a re-evaluation of both biochemical and clinical parameters in the following summer period.

A total group of 31 healthy persons (16 males, 15 females; mean age (±SD): 36.8 (±8.3) years) was used as a control group. Blood samples were taken from them in October.

Biochemical determinations

Immediately after the venapuncture plasma was prepared by a 20 min centrifugation step at 2650 g and stored at -80 ^{0}C. The pteridines and the amino acids phenylalanine, tyrosine, tryptophan, isoleucine, leucine and valine were measured by means of high performance liquid chromatography (HPLC) as described elsewhere (Fekkes et al., 1995; Hoekstra et al., 2001). The neopterin-biopterin (N-B) ratio was calculated by dividing neopterin by biopterin concentration and is used as a measure for the production of BH4 (Barford et al., 1984; Abou-Saleh et al., 1995). The tryptophan ratio was calculated as 100 times the concentration of tryptophan divided by the summed concentration of the other large neutral amino acids, i.e. phenylalanine, tyrosine, isoleucine, leucine and valine (Fernstrom and Wurtman, 1972).

Statistical analysis

Changes in plasma levels of biopterin, neopterin and tryptophan before vs. after light therapy and before light therapy vs. summer were assessed with paired two-tailed t-tests. Differences between depressed patients and controls were examined with independent variables t-tests. The scores on the BDI-add in the depressed patients were correlated with the biochemical parameters using the two-tailed Pearson correlation test. Data are presented as mean ± S.D. Results were considered significant when $p<0.05$.

Table 1.
Plasma levels (mean and SD) of biopterin, neopterin, tryptophan and derived ratios in patients with SAD, measured in fall/winter before and after light therapy.

	controls in fall n=31	before light therapy n=19	after light therapy n=19
biopterin (nmol/l)	6.8 (1.4) #	6.0 (1.0)	6.8 (1.1) §
neopterin (nmol/l)	18.2 (5.9) #	22.5 (7.6)	22.0 (5.6)
N/B	2.8 (1.0) *	3.8 (1.4)	3.3 (0.9) §
tryptophan (µmol/l)	44.8 (6.6) ¶	39.5 (5.3)	41.4 (9.4)
trp-ratio	8.3 (1.1.)	8.3 (1.7)	8.3 (1.5)

p=0.03
§ p<0.05
* p=0.005
¶ p<0.0005

All significance levels shown are compared to the values measured before light therapy.

N/B is neopterin-biopterin-ratio
Trp-ratio is tryptophan-ratio

Results

Immediately before the first light therapy session, a value of 22.9 (±12.1) on the BDI was scored in the group of 19 patients. On the BDI-add, their score was 7.0 (±2.7). After light therapy, these values were 9.7 (±8.0), and 2.8 (±2.2), respectively.

Table 1 presents the main biochemical findings. As can be inferred, the concentration of biopterin in the patients before light therapy was significantly lower than in the winter control group and increased to the level of the control group after light therapy. The level of neopterin in the patients was higher than in controls and did not change after light therapy. In addition, the N-B ratio was higher in patients than in controls and decreased after therapy. Finally, patients showed lower plasma levels of tryptophan, which remained low during treatment. However, no differences between the groups were found regarding the tryptophan ratio. The mean tryptophan concentration in winter (39.5 (±5.3) µmol/l) was lower than in the summer (43.8 (±6.6) µmol/l) albeit this difference was not statistically significant (Fig. 1). The latter was within the range of the control group.

Neopterin level and the N-B-ratio were positively correlated with the BDI-add score before light therapy (r=-0.52, p=0.02 and r=-0.47, p=0.04, respectively). This association could not be demonstrated for biopterin levels.

Re-analysis by excluding the findings in the only male patient did not change the results; neither could a sex-dependent difference in pteridine or amino acid concentrations be demonstrated in the control group.

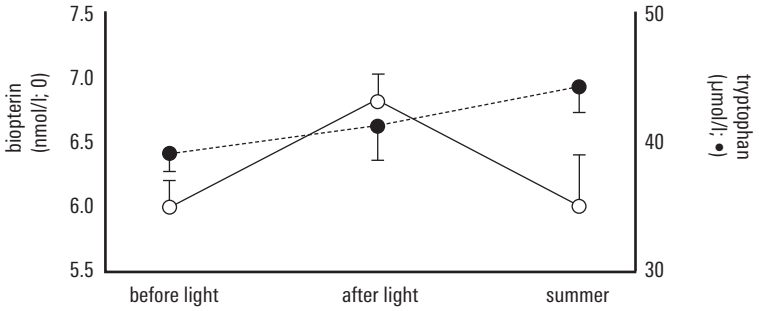


Figure 1.
Plasma biopterin and tryptophan concentrations before light therapy (n=19), after light therapy (n=19) and in summer (n=11) in depressed patients with seasonal affective disorder. Values are mean ± standard error.

Discussion

The present study demonstrated that in winter plasma biopterin and tryptophan levels in depressed patients with SAD are lower as compared to controls, while the opposite was found for plasma neopterin levels. After light therapy, biopterin was the only parameter that normalized. With respect to biopterin, lower levels in summer were found in SAD patients. Tryptophan levels did not alter in the various conditions.

Because of its role in dopamine, (nor) epinephrine and serotonin metabolism, BH4 has been studied in depressed patients. In most studies lower levels of biopterin were found in plasma, urine or CSF (Abou-Saleh et al., 1995; Anderson et al., 1992; Coppen et al., 1989; Hoekstra et al., 2001). It seems, therefore, plausible that decreased BH4 activity, because of its role as a cofactor, could result in a reduced serotonin synthesis and consequently to depressive symptoms.

The present observation of an increased neopterin level is in agreement with the findings in SAD patients by Stastny et al. (2003) and supports the hypothesis that activation of the immune system is involved in both SAD and depression in general (Dunbar et al., 1992; Maes et al. 1994). The fact that neopterin as a marker of immune activation is enhanced before and after light therapy suggests that an overactive cellular immune system may serve as a trait marker in this patient group.

The decreased tryptophan level in SAD has not been reported previously to our knowledge but is in line with the observation that tryptophan depletion may result in a relapse of depressive symptoms in remitted patients with SAD (Neumeister et al., 1998) and with the report by McGrath et al. (1990) that tryptophan may be effective in the treatment of this disorder.

Involvement of the serotonin system in SAD can also be derived from data of various studies, such as those on carbohydrate craving (Wallin and Rissanen, 1994), serotonin receptor challenge (Schwartz et al., 1999), brain imaging (Willeit et al., 2000) and the beneficial effects of serotonergic agents on the symptomatology of SAD (Lam et al., 1995). Interestingly, the observation in this study of low levels of biopterin and tryptophan before light therapy, with an increase of biopterin after treatment, is comparable to our results found before and after successful electroconvulsive therapy (Hoekstra et al., 2001). In the latter study, however, we found no significant differences in plasma neopterin between psychotic depressed patients and normal controls. These findings suggest not only immune activation in a subgroup of depressed patients, but also a relationship between biopterin and tryptophan and depressive symptomatology in general.

Because the tryptophan ratios across the groups studied did not change, there is no substantiation for decreased tryptophan levels in the CNS. The postulated impaired central serotonergic activity in SAD patients is, therefore, more likely due to reduced availability of the cofactor biopterin than to that of the precursor tryptophan. The increase of tryptophan in summer may point to a lower activity of indoleamine dioxygenase, which in turn may reflect a decreased cell-mediated immune activity (Fekkes and van Gool, 2003).

The biopterin level in patients with SAD in summer was lower than in normal controls and also lower than after light therapy. This indicates an aberrant pteridine metabolism that is apparently not sufficient to induce psychopathology. If, however, a second determinant like increased immune activity, resulting in lower tryptophan levels, is present, psychopathological symptoms may emerge. This phenomenon is illustrated in Fig. 1.

Since it is not known whether peripherally measured pteridines reflect their intracerebral levels, the results of the present study should be considered as preliminary. Although the number of included patients is rather small, the significant differences between patients and controls with respect to both neopterin and biopterin, may reflect actual changes in pteridine metabolism. It is highly unlikely that subclinical activation of the immune system with consequently increased neopterin levels did confound our results, since then both patients and controls would have been affected in a similar way.

In conclusion, the results of the present study support the hypothesis that pteridines may be involved in the pathogenesis of SAD, via their influence on the serotonergic system. Oral administration of BH4 in the treatment of SAD could further substantiate the role of pteridines in this disorder.

Acknowledgements

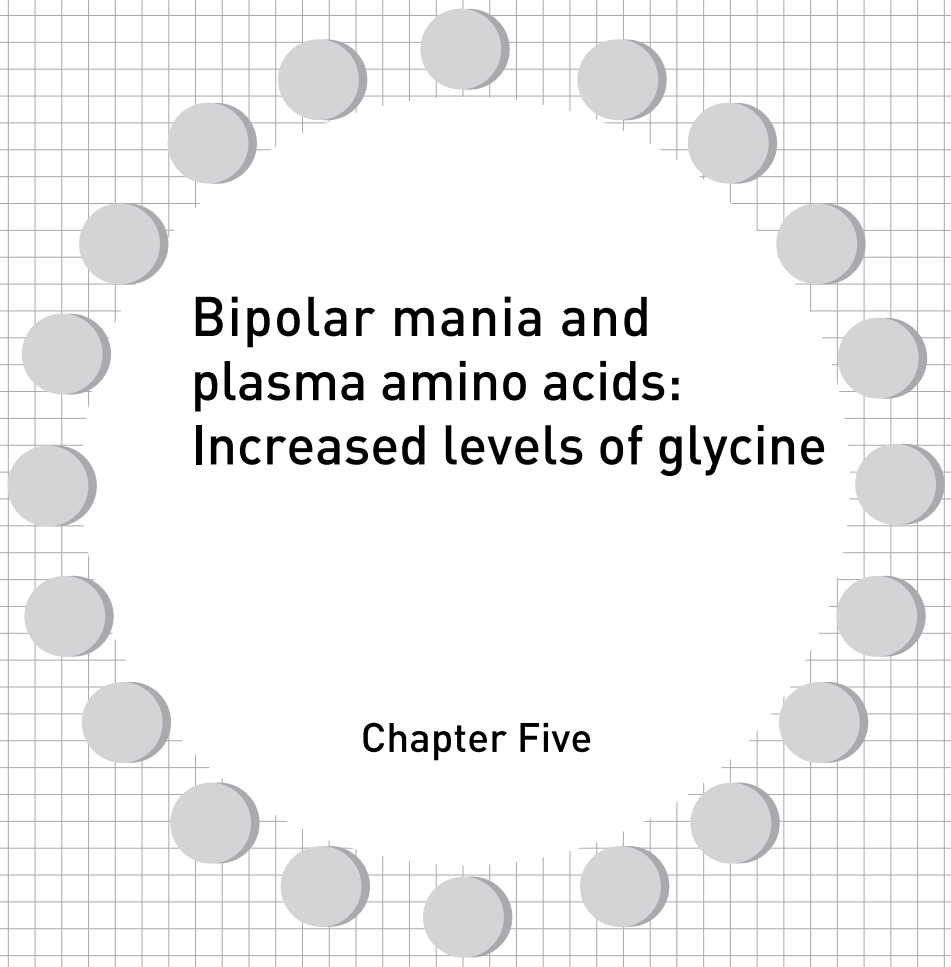
The authors are indebted to A.E.S. Sijben, MA, PhD, for his statistical analysis.
The authors also thank the collaborators of the Psychiatric Laboratory of Erasmus University Medical Center Rotterdam for expert technical assistance.

References

- Abou-Saleh, M.T., Anderson, D.N., Collins, J., Hughes, K., Cattell, R.J., Hamon, C.G., Blair, J.A., 1995. The role of pterins in depression and the effects of antidepressive therapy. Biological Psychiatry 38, 458-463.
- Anderson, D.N., Abou-Saleh, M.T., Collins, J., Hughes, K., Cattell, R.J., Hamon, C.G., Blair, J.A., Dewey, M.E., 1992. Pterin metabolism in depression: an extension of the amine hypothesis and possible marker of response to ECT. Psychological Medicine 22, 863-869.
- Auerbach, G., Nar, H., 1997. The pathway from GTP to tetrahydrobiopterin: three-dimensional structures of GTP cyclohydrolase I and 6-pyruvoyl tetrahydropterin synthase. Biological Chemistry 378, 185-192.
- Barford, P.A., Blair, J.A., Eggar, C., Hamon, C., Morar, C., Whitburn, S.B., 1984.
- Tetrahydrobiopterin metabolism in the temporal lobe of patients dying with senile dementia of Alzheimer type. Journal of Neurology, Neurosurgery and Psychiatry 47(7), 736-738.
- Coppen, A., Swade, C., Jones, S.A., Armstrong, R.A., Blair, J.A., Leeming, R.J., 1989. Depression and tetrahydrobiopterin: the folate connection. Journal of Affective Disorders 16, 103-107.
- Dunbar, P.R., Hill, J., Neale, T.J., Mellsop, G.W., 1992. Neopterin measurement provides evidence of altered cell-mediated immunity in patients with depression, but not with schizophrenia. Psychological Medicine 22, 1051-1057.
- Fekkes, D., Dalen van, A., Edelman, E., Voskuilen, A., 1995. Validation of the determination of amino acids in plasma by high-performance liquid chromatography using automated derivatization with o-phthaldialdehyde. Journal of Chromatography B 669, 177-186.
- Fekkes, D., van Gool, A.R., 2003. Interferon, tryptophan and depression. Acta Neuropsychiatrica 15, 8-14.
- Fernstrom, J., Wurtman, R., 1972. Brain serotonin content: physiological regulation by plasma neutral amino acids. Science 178, 414-416.

- Hoekstra, R., van den Broek, W.W., Fekkes, D., Bruijn, J.A., Mulder, P.G.H., Pepplinkhuizen, L., 2001. Effect of ECT treatment on biopterin and large neutral amino acids in severe, medication resistant depression. Psychiatry Research 103 (2-3), 115-123.
- Lam, R.W., Gorman, C.P., Michalon, M., Steiner, M., Levitt, A.J., Corral, M.R., Watson, G.D., Morehouse, R.L., Tam, W., Joffe, RT., 1995. A multi-centre, placebo-controlled study of fluoxetine in seasonal affective disorder. American Journal of Psychiatry 152, 1765-1770.
- Levine, R.A., 1988. Tetrahydrobiopterin and biogenic amine metabolism in neuropsychiatry, immunology, and aging. Annals of the New York Academy of Sciences 521, 129-139.
- Maes, M., Scharpé, S., Meltzer, H.Y., Okayli, G., Bosmans, E., D'Hondt, P., Vanden Bossche, B., Cosyns, P., 1994. Increased neopterin and interferon-gamma secretion and lower availability of L-tryptophan in major depression: further evidence for an immune response. Psychiatry Research 54, 143-160.
- McGrath, R.E., Buckwald, B., Resnick, E.V., 1990. The effect of L-tryptophan on seasonal affective disorder. Journal of Clinical Psychiatry 51 (4), 162-163.
- Meesters, Y., Jansen, J.H.C, 1993. Assessing atypical seasonal affective disorder complaints by means of self-rating. Acta Psychiatrica Scandinavica 88, 361-363.
- Mersch, P.P.A., Middendorp, H.M., Bouhuys, A.L., Beersma, D.G.M., van den Hoofdakker, R.H., 1999. The prevalence of seasonal affective disorder in the Netherlands: a prospective and retrospective study of seasonal mood variation in the general population. Biological Psychiatry 45, 1013-1022.
- Neumeister, A., Konstantinidis, A., Praschak-Rieder, N., Willeit, M., Hilger, E., Stastny, J., Kasper, S., 2001. Monoaminergic function in the pathogenesis of seasonal affective disorder. International Journal of Neuropsychopharmacology 4 (4), 409-420.
- Neumeister, A., Praschak-Rieder, N., Hesselmann, B., Vitouch, O., Rauh, M., Barocka, A., Kasper, S., 1998. Effects of tryptophan depletion in fully remitted patients with seasonal affective disorder during summer. Psychological Medicine 28 (2), 257-264.
- Partonen, T., Lonnqvist, J., 1998. Seasonal affective disorder. Lancet 352 (9137), 1369-1374.
- Rosenthal, N.E., Sack, D.A., Gillin, J.C., Lewy, A.J., Goodwin, F.K., Davenport, Y., Mueller, P.S., Newsome, D.A., Wehr, T.A., 1984. Seasonal affective disorder. A description of the syndrome and preliminary findings with light therapy. Archives of General Psychiatry 41, 72-80.
- Schwartz, P.J., Turner, E.H., Garcia-Borreguero, D., Sedway, J., Vetticad, R.G., Wehr, T.A., Murphy, D.L., Rosenthal, N.E., 1999. Serotonin hypothesis of winter depression: behavioral and neuroendocrine effects of the 5-HT (1A) receptor partial agonist

ipsipirone in patients with seasonal affective disorder and healthy control subjects. Psychiatry Research 86(1), 9-28.
- Stastny, J., Konstantinidis, A., Schwarz, M.J., Rosenthal, N.E., Vitouch, O., Kasper, S., Neumeister, A., 2003. Effects of tryptophan depletion and catecholamine depletion on immune parameters in patients with seasonal affective disorder in remission with light therapy. Biological Psychiatry 53(4), 332-337.
- Wallin, M.S., Rissanen, A.M., 1994. Food and mood: relationship between food, serotonin and affective disorders. Acta Psychiatrica Scandinavica, suppl. 377, 36-40.
- Willeit, M., Praschak-Rieder, N., Neumeister, A., Pirker, W., Asenbaum, S., Vitouch, O., Tauscher, J., Hilger, E., Stastny, J., Brucke, T., Kasper, S., 2000. [123]-Beta-CIT SPECT imaging shows reduced brain serotonin transporter availability in drug-free depressed patients with seasonal affective disorder. Biological Psychiatry 47, 482-489.
- Wolf, W.A., Ziaja, E., Arthur, R.A. Jr., Anastasiadis, P.Z., Levine, R.A., Kuhn, D.M., 1991. Effect of tetrahydrobiopterin on serotonin synthesis, release, and metabolism in superfused hippocampal slices. Journal of Neurochemistry 57, 1191-1197.



Bipolar mania and plasma amino acids: Increased levels of glycine

Chapter Five

Bipolar mania and plasma amino acids: Increased levels of glycine.

R. Hoekstra, D. Fekkes, A.J.M. Loonen, L. Pepplinkhuizen, S. Tuinier, W.M.A. Verhoeven.

Published in European Neuropsychopharmacology 2006; 16:71-77

Abstract

Previous studies have suggested that the N-methyl-D-aspartate (NMDA) glutamate receptor complex is implicated in the pathophysiology of several neuropsychiatric disorders. Especially the glycine coagonist site of this receptor has been proposed as a therapeutic target. It has been hypothesized that the NMDA receptor and the serotonergic system, which function is compromised in affective disorders, are functionally coupled. Furthermore, several studies suggest that peripheral levels of amino acids are associated with psychotic symptomatology. We therefore measured plasma levels of glutamate, glycine, tryptophan and the tryptophan ratio in 20 bipolar-I patients during the manic phase and at remission of symptomatology. Data were compared to a matched group of healthy controls and a group of euthymic bipolar-I patients.

During the manic phase a significant increase of both glutamate and glycine was found, that persisted at remission. Tryptophan and the tryptophan ratio were decreased in manic patients. Subsequent analysis showed that changes in glutamate, tryptophan and tryptophan ratio could be attributed to the use of anticonvulsants. The increased glycine, however, was not related to the use of mood stabilizers. Although the exact relationship between peripheral measures of amino acids e.g. glycine is not fully clear, the results of this study suggest an involvement of glycine and/or its coagonist site of the NMDA-receptor in a manic relapse of patients with a bipolar-I disorder.

Introduction

Dysfunction of the glutamatergic neuronal systems has been postulated to be involved in the pathogenesis of schizophrenia. Antagonism of the N-methyl-D-aspartate (NMDA) glutamate receptor complex induces behavioural and cognitive deficits in normal subjects with a broad range of central nervous system symptoms such as psychotic phenomena, agitation and disorientation (Goff and Coyle, 2001). Enhancement of the NMDA receptor activity has been demonstrated to induce symptomatic improvement in schizophrenia (Coyle et al., 2002). Hypofunction of the NMDA-receptor complex is associated with negative symptoms and cognitive deficits in patients with schizophrenia (Coyle et al., 2003). In addition to the binding site for the agonist glutamate, a glycine-coupled modulatory site is present at the NMDA receptor complex (Javitt et al., 1999; Coyle et al., 2003). This modulatory site must be occupied by glycine before glutamate is able to open the calcium channel of the NMDA receptor (D'Souza et al., 2000; Coyle et al., 2003). Several studies have described a relation between dysfunction of the glycine modulatory site and schizophrenia. It is therefore considered to be a target for symptomatic treatment of patients with schizophrenia (Coyle and Tsai, 2004; Heresco-Levy and Javitt, 2004; Javitt, 2004).
Recently, increased plasma levels of glutamate were found in patients with schizophrenia as compared to normal controls. Glutamate levels correlated significantly with negative symptoms. In the same study it was shown that poor response to atypical antipsychotics is associated with lower levels of peripheral serotonin (Van der Heijden et al., 2004). Furthermore, it was demonstrated that the central availability of tryptophan, determined by calculating the ratio between the plasma levels of tryptophan and the other large neutral amino acids, is reduced in poor responders (Van der Heijden et al., 2005). With respect to plasma concentrations of amino acids, lower levels of tryptophan have been demonstrated repeatedly in patients with schizophrenia (Rao et al., 1990; Tortorella et al., 2001) and with affective disorders (Lucca et al., 1995; Johnson et al., 2001; Neumeister, 2003). Although not investigated extensively, plasma amino acid levels have been used as indirect measures for central monoaminergic functionality (Finkelstein et al., 1982; Van der Mast and Fekkes, 2000).
In contrast to schizophrenia, the role of the NMDA receptor in mood disorders has been much less studied, although recent preclinical and clinical research suggests that this receptor complex may not only be involved in synaptic plasticity but may also be connected with the long-term treatment of severe mood disorders (Krystal et al., 2002; Manji et al., 2003; Zarate et al., 2003). Only few studies are known on the role of the NMDA receptor in specifically the bipolar affective disorder. Increased plasma glutamate levels have been found in depressed bipolar patients (Altamura, 1993). Furthermore, altered mRNA expression of glutamic acid decarboxylase in hippocampal tissue of bipolar patients has been demonstrated (Heckers et

al., 2002). Finally, elevated glutamate/glutamine levels in the frontal lobe and basal ganglia in children with bipolar disorder have been suggestive for disturbances in the glutamate metabolism (Castillo et al., 2000). One study focussed on bipolar mania and showed elevated glutamate levels in the left dorsolateral prefrontal cortex (Michael et al., 2003).
Furthermore, mood stabilizers like lithium and valproic acid influence cerebral uptake and release of glutamate (Dixon an Hokin, 1997, 1998; Zarate et al., 2002; Ketter and Wang, 2003; O'Donnell et al., 2003). In addition, antiglutamatergic agents like lamotrigine have been demonstrated to have some beneficial effect on depressive symptoms in bipolar disorders (Ernst and Goldberg, 2003).
With respect to glycine in bipolar disorder, only studies from the eighties have demonstrated an elevation of glycine in red blood cells of bipolar patients using lithium, which often returned to the normal range after chronic treatment. Plasma glycine, however, was not affected by lithium (Peselow et al., 1982; Rosenblatt et al., 1982).
The role of serotonin (5-HT) in the pathogenesis of mood disorders has been widely studied. However, the precise role of serotonin in bipolar mania is much less studied. Raised levels of 5-hydroxyindoleacetic acid, the metabolite of serotonin, in cerebrospinal fluid of manic female patients have been found (Swann et al., 1983), as well as diminished serotonin responsiveness after neuroendocrine challenge tests (Thakore et al., 1996) and increased platelet uptake of serotonin (Meagher et al., 1990). Recent studies suggest a relationship between glutamate receptors and the serotonergic system. Glutamate and 5HT-2A receptors are anatomically and functionally coupled in e.g. the prefrontal cortex. In addition, glutamate agonists activate 5-HT2A receptors, whereas serotonin activation results in inhibition of the NMDA response (Pralong et al., 2002).
The aim of this study was to investigate the plasma concentrations of amino acids related to the NMDA receptor, i.e. glutamate and glycine, in patients with a manic episode in the course of a bipolar affective disorder and after recovery from manic symptoms. To investigate the role of the serotonergic system and its relation with the NMDA receptor, we also assessed the central availability of the precursor amino acid of 5-HT by determining tryptophan and the other large neutral amino acids, e.g. tyrosine, phenylalanine, valine, isoleucine and leucine.

Experimental procedures

The protocol was approved by the "Toetsingscommissie Patientgebonden Wetenschappelijk Onderzoek" in Arnhem, The Netherlands, an officially recognized Medical Ethics Committee. The project was performed in accordance with the Helsinki declaration. Written informed consent was obtained from all patients.

Table 1.
Main demographic and clinical characteristics of patients with bipolar mania (n=20).

sex	age (yrs)	duration of illness (yrs)	number of relapses (manic and depressive)	sympton free interval (yrs)	severity of previous episode	family load	psychotropics used at initial blood draw (mg)	serum level of moodstabilizer (lithium in mmol/l and others in mg/l)	YMRS at inclusion	YMRS at recovery
m	56	14	>5	1	D2	-	lithium 800, valproate 1500	0.68, 43	24	4
m	38	12	>10	0.5	M3	+	lithium 800	0.53	23	1
m	55	34	>5	2	M4	-	lithium 400, valproate 1500, risperidone 4, lorazepam 3	0.17, 78	26	2
m	62	21	>10	0.5	D2	-	lithium 800 zuclopenthixol 300/3 wks lorazepam 2	0.48	33	0
m	54	29	>10	1	D3	?	valproate 2500 lorazepam 5.5	64	24	2
f	57	23	5	16	M3	?	lithium 800, lorazepam 2.5 lormetazepam 2	0.68	23	3
m	31	5	>5	1	M3	+	lithium 800, valproate 1500 olanzapine 20	0.77, 74	20	6
m	38	19	>5	2	M3	-	lithium 800, valproate 1500 clonazepam 0.5	0.85, 29	18	0
m	72	40	>5	2	M4	?	haloperidol 3 lorazepam 2.5		32	5
m	68	12	2	12	D2	?	valproate 1000 olanzapine 5, lorazepam 5.5	54	26	0
f	49	33	>10	0.25	M2	+	lithium 800, carbamazepine 1600, risperidone 6, promethazine 200, flunitrazepam 2, lorazepam 4.5	0.72, 8.2	13	0
m	60	20	>10	0.5	M2	+	lithium 1000, valproate 1000, clonazepam 1.5	0.88, 0.5	9	0

f	61	22	>5	3	D2	?	lithium 400, lorazepam 2.5	0.58	25	0
f	63	27	>5	5	M4	?	carbamazepine 600 zuclopenthixol 200/4wks lorazepam 2	5.3	19	3
f	21	7	>5	1	M3	+	lithium 1200, valproate 1100, risperidone 4, lorazepam 4.5	0.75, 51	19	0
m	44	13	>5	0.25	M2	?	lithium 1800, carbamazepine 400, risperidone 6, promethazine 25, lormetazepam 4, diazepam 15	0.88, 6.8	10	0
m	52	33	>5	1	M2	?	valproate 1500, olanzapine 20, lorazepam 6	81	22	0
m	26	9	2	1	M4	?	lithium 1600, valproate 1750, olanzapine 10, zopiclon 7.5	0.79, 62	25	0
m	43	17	5	3	D3	-	lithium 800, lorazepam 2.5, oxazepam 20	0.71	22	2
f	53	25	>5	4	M2	+	carbamazepine 1000, zuclopenthixol 8, levomepromazine 37.5	7.9	20	4

'severity of previous episode': D=depressive and M=manic; 1=slight, 2=moderate, 3=severe and 4=severe with psychotic features (according to DSM-IV).

Subjects

The patients were recruited from the specialist outpatient department for bipolar disorders of the Delta Psychiatric Teaching Hospital in Rotterdam, The Netherlands. All met the DSM-IV criteria for a bipolar-I disorder. The inclusion criterion was a relapse of (hypo)manic symptoms as assessed by the treating psychiatrist who used the elements of the Comprehensive Psychiatric Rating Scale (CPRS; Åsberg et al., 1978) and the severity of manic symptoms was measured by means of the Young Mania Rating Scale (YMRS). Patients were consecutively recruited over a period of 24 months. Main demographic and clinical characteristics, including the illness history, family load and treatment at inclusion are presented in Table 1. Excluded were patients with clinically relevant somatic disorders of any kind or comorbid DSM-IV axis-I disorders.

The study included a total of twenty consecutively referred patients, 14 males and 6 females with a mean (± SD) age of 50.2 (± 13.8) years. All received maintenance treatment with psychotropics (Table 1). During the manic phase treatment was adjusted according to plasma levels and clinical status.

Instrument

The severity of manic symptoms was assessed with the 11-item YMRS that ranges from 0 till 60 (Young et al., 1978). The YMRS was completed at inclusion and when the patient was judged to be clinically recovered. According to the factor analysis as described by Gonzalez-Pinto et al. (2003) the YMRS has three dimensions: 'activation' (items 2 [increased motor activation, energy], 6 [accelerated speech] and 7 [altered thinking and speech]), 'hedonism' (items 1 [euphoria], 3 [sexual interest], 4 [sleep] and 10 [appearance]), and 'dysphoria' (items 5 [irritability], 9 [aggressive behaviour] and 11 [lack of insight]).

Control subjects

Control values of amino acids were obtained from a large database of healthy subjects from which an age and sex matched control group was composed (males: 14; females 6; mean age ±SD 50.1 ±13.5 years). These values did not differ from those of the very large control group from which they were extracted.

In order to investigate the effect of other psychotropics than lithium, a second control group of 12 euthymic bipolar patients (mean age 40.2 ± 15.5; 8 males and 5 females) with lithium prophylaxis as monotherapy was recruited from the same outpatient department for bipolar disorders. Their demographic and clinical characteristics are depicted in Table 2. Assessments were identical to those applied in the patient group and revealed no symptoms of affective illness.

Table 2.
Demographic and clinical characteristics of the euthymic control group (n=12).

sex	age	duration of illness	number of relapses	symptom free interval	severity of previous episode	family load	serum level of lithium
M	19	1	1	1	M3	+	0.29
M	37	15	>5	0.75	M4	+	0.72
M	35	13	>5	2	M4	-	0.73
F	57	27	2	9	M4	+	0.83
F	51	24	3	9	D3	-	0.95
F	42	14	5	4	D2	+	1.06
M	66	9	4	6	D3	-	0.66
F	23	2	3	1	M3	+	0.71
M	52	8	4	6	D2	-	0.99
F	30	9	6	3	M4	-	0.82
F	41	13	4	8	D3	-	0.67
M	54	21	>5	0.5	M2	-	0.63

'Severity of last episode': D=depressive and M=manic; 1=slight, 2=moderate, 3=severe and 4=severe with psychotic features (according to DSM-IV).

Amino acid analysis

To measure amino acids a venous blood sample was drawn before 10 a.m. After remission of the manic episode, as established with the YMRS, a second venous blood sample was taken.

Immediately after the venapuncture, plasma was prepared by a 20 min centrifugation step at 2650 g and stored at -80 ^{0}C. The large neutral amino acids: tryptophan (Trp), phenylalanine, tyrosine, isoleucine, leucine and valine, and the amino acids glutamate, glycine, serine, methionine, alanine and taurine were measured by means of high performance liquid chromatography (HPLC) as described elsewhere (Fekkes et al., 1995; Hoekstra et al., 2001). The tryptophan ratio (Trp-ratio) was calculated as 100 times the concentration of Trp divided by the summed concentrations of the other large neutral amino acids (Fernstrom and Wurtman, 1972).

Statistical analysis

Changes in plasma levels of amino acids between the manic state and after recovery were assessed with the paired two-tailed Student t-test. Differences between patients and controls were examined with the two sample Student t-test. Change in YMRS scores, the three dimensions and the plasma levels of the mood stabilizers lithium, valproic acid and carbamazepine were correlated with the biochemical parameters using the Pearson correlation test. Data are presented as mean (± SD). Results were considered significant when $p<0.05$.

Table 3.
Plasma levels (SD) of amino acids in patients with bipolar mania (n=20) and matched controls (n=20).

	glutamate (µmol/l)	glycine (µmol/l)	tryptophan (µmol/l)	trp-ratio
manic phase	46.5 (19.4)	283.3 (102.7)	36.5 (10.9)	6.7 (1.6)
	p=0.03	p=0.02	p=0.0005	p=0.002
at remission	56.8 (27.4)	288.8 (93.6)	37.9 (12.2)	6.3 (1.7)
	p=0.04	p=0.69	p=0.53	p=0.24
healthy controls	36.1 (8.6)	224.0 (51.5)	47.8 (7.9)	8.2 (1.3)

P-values in row of 'manic phase' indicate comparison with healthy controls. P-values in row 'at remission' indicate comparison to manic phase.

Results

The mean (±SD) score on the YMRS at inclusion was 21.7 (± 6.1), which is compatible with moderately severe manic symptoms. After recovery, a score within the normal range was found (1.6 ± 2.0). With respect to the three dimensions of the YMRS, the scores decreased from 8.6 (± 1.7) to 0.1 (± 0.3) for 'activation', from 5.5 (± 2.3) to 0.5 (± 0.9) for 'hedonism' and from 4.5 (± 2.7) to 0.6 (± 1.2) for 'dysphoria'.

At remission, the mean (±SD) plasma level of lithium was significantly higher than during the manic episode (0.84 ± 0.13 versus 0.64 ± 0.2 mmol/l; p= 0.002). No changes in the plasma concentrations of valproic acid and carbamazepine neither in the doses of antipsychotics or other psychotropics were observed. This quite conservative medication policy was possible because the patients got an intensive psychosocial coaching.

As can be inferred from table 3, the mean glutamate concentration in the group of manic patients was significantly higher as compared to the group of healthy controls, with a further increase at remission. In addition, a significant and persistent higher concentration of glycine was found. Trp and Trp-ratio were significantly lower in the group of patients under both conditions as compared to healthy controls.

Patients using valproic acid or carbamazepine had significantly higher glutamate levels than the patients on lithium monotherapy only (52.3 ± 19.4 µmol/l (n=14) versus 34.0 ± 12.5 µmol/l; n=5; two sample t-test, p=0.049). In this group (n=14), Trp and its ratio as well as glycine were lower (Trp 34.2 ± 10.3 µmol/l versus 43.6 ± 11.5 µmol/l; Trp-ratio 6.4 ± 1.8 versus 7.7 ± 0.4; glycine 254.6 ± 84.1µmol/l versus 342.4 ± 122.4 µmol/l).

By comparing the manic patients who used lithium only (n=5) with the euthymic bipolar control group with lithium monotherapy (n=12), a significantly higher concentration of glycine and a significantly lower value for the Trp-ratio were found during the manic phase (Table 4).

Table 4.
Plasma levels (SD) of glutamate, glycine, tryptophan and tryptophan ratio in manic patients with lithium (n=5) and euthymic controls (n=12).

	glutamate (µmol/l)	glycine (µmol/l)	tryptophan (µmol/l)	trp-ratio
manic phase	34.0 (12.5)	342.4 (122.4)	43.6 (11.5)	7.7 (0.4)
	p=0.74	p=0.049	p=0.47	p=0.02
at remission	39.4 (19.3)	319.2 (105.7)	46.4 (14.9)	7.7 (0.6)
	p=0.56	p=0.60	p=0.55	p=0.96
euthymic controls	30.8 (19.1)	239.1 (75.6)	47.3(8.6)	9.8 (1.7)

P-values in row of 'manic phase' indicate comparison to euthymic controls. P-values in row of 'at remission' indicate comparison to manic phase.

With respect to the relationship between symptomatology and amino acid levels in the total group of manic patients, the plasma concentration of glutamate during the manic phase was negatively correlated with the total YMRS-score (r=-0.5; p=0.04) and with the dimension 'activation' (r=-0.6; p=0.005). In addition, the Trp-ratio was negatively correlated with the dimension 'hedonism' (r=-0.4; p<0.05). No significant correlations were found between the scores on the YMRS or its dimensions and any of the other amino acids.
In the lithium subgroup (n=5), the shift from the manic phase to the remitted state resulted in a slight decrease in the plasma concentration of glycine, which was correlated significantly with the change in the dimension hedonism (r=0.468; p=0.04).

Discussion

The present study with a group of bipolar-I patients with a manic relapse, demonstrates increased plasma levels of the amino acids glutamate and glycine during both the manic state and the remission phase as compared to the healthy control group. Furthermore, both Trp and its ratio are decreased in this group. In addition, glycine levels are higher in the subgroup with lithium monotherapy as compared to the euthymic bipolar control group using lithium.
The higher level of glutamate can be fully attributed to the use of anticonvulsants, an effect that has also been demonstrated in mainly preclinical studies (Vriend and Alexiuk, 1996; Dixon and Hokin, 1997; Petroff et al., 1999; O'Donnell et al., 2003). Thus, these findings do not support an involvement of a glutamatergic dysfunction in mania. This is different from the observations in patients with schizophrenia, in whom higher plasma glutamate

levels do indicate an involvement of glutamatergic dysfunction in the pathophysiology of schizophrenia (Macciardi et al., 1990; Goff and Coyle, 2001; Tortorella et al., 2001; Van der Heijden et al., 2004).

With respect to plasma Trp and its ratio, the observed decreased values in the group of bipolar patients compared to the control group, appears to be the result of treatment with anticonvulsants. Similar observations have been reported by other investigators (Vriend and Alexiuk, 1996; Verhoeven et al., 1999). Trp and Trp-ratio in the patient group on lithium monotherapy were not different from those in the healthy control group. In the group of euthymic bipolar patients, the Trp-ratio was found to be even higher than in the control group (9.8 ± 1.7 vs. 8.2 ± 1.3; two sample t-test, p=0.008; Tables 3 and 4). This difference is probably the result of chronic treatment with lithium. The exact mode of action of lithium is unclear. Most studies concerning its influence on the serotonergic system show an increased serotonin release after chronic treatment with lithium. Increased central availability of the serotonin precursor Trp may indicate enhanced synthesis of serotonin in the brain (Lenox and Hahn, 2000; Mahmood and Silverstone, 2001). The negative correlation between the Trp-ratio and the dimension hedonism indicates a possible influence of the cerebral availability of Trp to euphoria and other mood related symptoms.

The main finding of the present study is the sustained increase of the plasma glycine concentration in the group of patients with relapsing mania. The observed enhanced values of glycine could not be attributed to the use of lithium, neither to the use of any other mood stabilizing or psychotropic compound. This increase was no longer present in stable euthymic patients with lithium monotherapy. The latter is in agreement with two other studies (Rosenblatt et al., 1979; Rosenblatt et al., 1982), showing no effect of chronic lithium treatment on the plasma levels of glycine. Whether long-term treatment with valproic acid affects the plasma levels of glycine is unknown since the limited studies on this topic stem from the eighties and concern either acute effects in epileptic patients (Verity et al., 1983; Iinuma et al., 1988) or animal experiments (Mortensen et al., 1980). In this study no effect of valproic acid on plasma glycine was found. The change in glycine was correlated with the change in the dimension 'hedonism' from the YMRS, which forms the classic symptom profile of mania.

Despite the limitations of this study, like relatively small patient groups and the use of peripheral biochemical parameters, the observed increased plasma level of glycine may be relevant since animal as well as human experiments have demonstrated that peripherally administered glycine increases brain levels of this amino acid (Toth and Lajtha, 1986; Javitt et al., 1999; D'Souza et al., 2000). Besides, in schizophrenic patients a dose-dependent treatment response after glycine administration was reported (Javitt et al., 1994; Heresco-Levy and Javitt, 2004).

In conclusion, the reported data on glycine suggest a role for the glycine coupled modulatory site of the NMDA receptor not only in schizophrenia, but also in relapsing mania. It could therefore be postulated that dysfunction of the NMDA receptor complex plays a role in the vulnerability for the emergence of psychotic relapses in general. Although the exact relationship between peripheral measures and central functionality is not exactly clear, association studies like the present may be of heuristic value for the pathophysiology of mental disorders.

Acknowledgements

The authors thank A.C.C. Voskuilen-Kooyman, E. Taal, J. Hoek and M. Waltmans for skilled technical assistance. The authors are indebted to A.E.S. Sijben, M.A., Ph.D., for his assistance in the statistical analysis.

References

- Altamura, C.A., Mauri, M.C., Ferrara, A., Moro, A.R., D'Andrea, G., Zamberlan, F. (1993) Plasma and platelet excitatory amino acids in psychiatric disorders. Am. J. Psychiatry 150, 1731-1733.
- Åsberg, M., Montgomery, S.A., Perris, C., Schalling, D., Sedvall, G. (1978) A comprehensive psychopathological rating scale. Acta Psychiatr. Scand. Suppl. 271, 5-27.
- Castillo, M., Kwock, L., Courvoisie, H., Hooper, S.R. (2000) Proton MR spectroscopy in children with bipolar affective disorder: preliminary observations. Am. J. Neuroradiol. 21, 832-838.
- Coyle, J.T. Tsai G., Goff, D.C. (2002) Ionotropic glutamate receptors as therapeutic targets in schizophrenia. Curr. Drug Targets CNS Neurol. Disorders 1(2), 183-189.
- Coyle, J.T., Tsai, G., Goff, D. (2003) Converging evidence of NMDA receptor hypofunction in the pathophysiology of schizophrenia. Ann. N. Y. Acad. Sci. 1003, 318-27.
- Coyle, J.T., Tsai, G. (2004) The NMDA receptor glycine modulatory site: a therapeutic target for improving cognition and reducing negative symptoms in schizophrenia.
- Psychopharmacology 174, 32 - 38.
- D'Souza, D.C., Gil, R, Cassello, K., Morrissey, K., Abi-Saab, D., White, J., Sturwold, R., Bennett, A., Karper, L.P., Zuzarte, E., Charney, D.S., Krystal, J.H. (2000) IV glycine and oral D-cycloserine effects on plasma and CSF amino acids in healthy humans. Biol Psychiatry. 47(5), 450-62.

- Dixon, J.F., Hokin, L.E. (1997) The antibipolar drug valproate mimics lithium in stimulating glutamate release and inositol 1,4,5-triphosphate accumulation in brain cortex slices but not accumulation of inositol monophosphates and biphosphates. Proc. Natl. Acad. Sci. USA 94(9), 4757-60.
- Dixon, J.F., Hokin, L.E. (1998) Lithium acutely inhibits and chronically up-regulates and stabilizes glutamate uptake by presynaptic nerve endings in mouse cerebral cortex. Proc. Natl. Acad. Sci. USA 95, 8363-8368.
- Ernst, C.L., Goldberg, J.F. (2003) Antidepressant properties of anticonvulsant drugs for bipolar disorder. J. Clin. Psychopharmacol.23(2), 182-92.
- Fernstrom, J., Wurtman, R. (1972) Brain serotonin content: physiological regulation by plasma neutral amino acids. Science 178, 414-416.
- Fekkes, D., Dalen van, A., Edelman, E., Voskuilen, A. (1995) Validation of the determination of amino acids in plasma by high-performance liquid chromatography using automated derivatization with o-phtaldialdehyde. Journal of Chromatography B 669, 177-186.
- Finkelstein, J.A., Chance, W.T., Fischer, J.E. (1982) Brain serotonergic activity and plasma amino acid levels in genetically obese Zucker rats. Pharmacol. Biochem. Behav. 17(5), 939-944.
- Goff, D.C., Coyle, J.T. (2001) The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. Am. J. Psychiatry 158, 1367-1377.
- Gonzalez-Pinto, A., Ballesteros, J., Aldama, A., Perez de Heredia, J.L., Gutierrez, M., Mosquera, F., Gonzalez-Pinto, A. (2003) Principal components of mania. J. Affect. Disord. 76(1-3), 95-102.
- Heckers, S., Stone, D., Walsh, J., Shick, J., Koul, P., Benes, F.M. (2002) Differential hippocampal expression of glutamic acid decarboxylase 65 and 67 messenger RNA in bipolar disorder and schizophrenia. Arch. Gen. Psychiatry 59, 521-529.
- Heresco-Levy, U., Javitt, D.C. (2004) Comparative effects of glycine and D-cycloserine on persistent negative symptoms in schizophrenia: a retrospective analysis. Biol. Psychiatry 55, 165-71.
- Hoekstra, R., van den Broek, W.W., Fekkes, D., Bruijn, J.A., Mulder, P.G.H., Pepplinkhuizen, L. (2001) Effect of ECT treatment on biopterin and large neutral amino acids in severe, medication resistant depression. Psychiatry Res. 103 (2-3), 115-123.
- Iinuma, K., Hayasaka, K., Narisawa, K., Tada, K., Hori, K. (1988) Hyperamino-acidaemia and hyperammonaemia in epileptic children treated with valproic acid. Eur. J. Pediatr. 148(3), 267-9.
- Javitt, D.C., Zylberman, I., Zukin, S.R., Heresco-Levy, U., Lindenmayer, J.P. (1994) Amelioration of negative symptoms in schizophrenia by glycine. Am. J. Psychiatry 151, 1234-1236.

- Javitt, D.C., Balla, A., Sershen, H., Lajtha, A. (1999) Reversal of phencyclidine-induced effects by glycine and glycine transport inhibitors. Biol. Psychiatry 45, 668-679.
- Javitt, D.C. (2004) Glycine modulators in schizophrenia. Schizophr. Res. 66(2-3), 89-96.
- Johnson, L., El-Khoury, A., Aberg-Wistedt, A., Stain-Malmgren, R., Mathe, A.A. (2001) Tryptophan depletion in lithium-stabilized patients with affective disorder. Int. J. Neuropsychopharmacol. 4(4), 329-336.
- Ketter, T.A., Wang, P.W. (2003) The emerging differential roles of GABAergic and antiglutamatergic agents in bipolar disorders. J. Clin. Psychiatry 64 (suppl 3), 15-20.
- Krystal, J.H., Sancora, G., Blumberg, H., Anand, A., Charney, D.S., Marek, G., Epperson, C.N., Goddard, A., Mason, G.F. (2002). Glutamate and GABA systems as targets for novel antidepressant and mood-stabilizing treatments. Mol. Psychiatry 7 (suppl. 1), S71-S80.
- Lenox, R.H., Hahn, C-G. (2000) Overview of the mechanism of action of lithium in the brain: fifty-year update. J. Clin. Psychiatry; 61 (suppl 9), 5-15.
- Lucca, A., Lucini, V., Catalano, M., Smeraldi, E. (1995) Neutral amino acid availability in two major psychiatric disorders. Prog. Neuropsychopharmacol Biol Psychiatry 19(4), 615-626.
- Macciardi, F., Lucca, A., Catalano, M., Marino, C., Smeraldi, E. (1990) Amino acid pattern in schizophrenia : some new findings. Psychiatry Res. 32, 63-68.
- Mahmood, T., Silverstone, T. (2001) Serotonin and bipolar disorder. J. Affect. Disord. 66, 1-11.
- Manji, H.K., Quiroz, J.A., Sporn, J., Payne, J.L., Denicoff, K., Gray, N.A., Zarate Jr., C.A., Charney, D.S. (2003) Enhancing neuronal plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression. Biol. Psychiatry 53, 707-742.
- Meagher, J.B., O'Halloran, A., Carney, P.A., Leonard, B.E. (1990) Changes in platelet 5-hydroxytryptamine uptake in mania. J. Affect. Disord. 19(3): 191-6.
- Michael, N., Erfurth, A., Ohrmann, P., Goessling, M., Arolt, V., Heindel, W., Pfleiderer, B. (2003) Acute mania is accompanied by elevated glutamate/glutamine levels within the left dorsolateral prefrontal cortex. Psychopharmacology 168, 344-346.
- Mortensen, P.B., Kolvraa, S., Christensen, E. (1980) Inhibition of the glycine cleavage system: hyperglycinemia and hyperglycinuria caused by valproic acid. Epilepsia 21(6), 563-9.
- Neumeister, A. (2003) Tryptophan depletion, serotonin, and depression: where do we stand? Psychopharmacol. Bull. 37(4), 99-115.
- O'Donnell, T., Rotzinger, S., Ulrich, M., Hanstock, C.C., Nakashima, T.T., Silverstone, P.H. (2003) Effects of chronic lithium and sodium valproate on concentrations of brain amino acids. Eur. Neuropsychopharmacol. 13(4), 220-7.

- Peselow, E.D., Deutsch, S.I., Stanley, M., Banay-Schwartz, M., Virgilio, J., Fieve, R.R. (1982) Erythrocyte glycine in depressed, hypomanic, and euthymic bipolar patients treated with lithium carbonate. Psychopharmacology 78 (4), 314-6.
- Petroff, O.A., Rothman, D.L., Behar, K.L., Hyder, F., Mattson, R.H.. (1999) Effects of valproate and other antiepileptic drugs on brain glutamate, glutamine, and GABA in patients with refractory complex partial seizures. Seizure 8 (2), 120-7.
- Pralong E., Magistretti P., Stoop, R. (2002) Cellular perspectives on the glutamate-monoamine interactions in limbic lobe structures and their relevance for some psychiatric disorders. Prog. Neurobiol. 67(3) 173-202.
- Rao, M.L., Gross, G., Strebel, B., Braunig, P., Huber, G., Klosterkötter, J. (1990) Serum amino acids, central monoamines, and hormones in drug-naive, drug-free, and neuroleptic-treated schizophrenic patients and healthy subjects. Psychiatry Research 34, 243-257.
- Rosenblatt, S., Gaull, G.E., Chanley, J.D., Rosenthal, J.S., Smith, H., Sarkozi, L. (1979) Amino acids in bipolar affective disorders: increased glycine levels in erythrocytes. Am. J. Psychiatry 136(5), 672-4.
- Rosenblatt, S., Leighton, W.P., Chanley, J.D. (1982) Elevation of erythrocyte glycine levels during lithium treatment of affective disorders. Psychiatry Res. 6(2), 203-14.
- Swann, A.C., Secunda, S., Davis, J.M., Robins, E., Hanin, I., Koslow, S.H., Maas, J.W. (1983) CSF monoamine metabolites in mania. Am. J. Psychiatry 140(4), 396-400.
- Thakore, J.H., O'Keane, V., Dinan, T.G. (1996) D-fenfluramine-induced prolactin responses in mania: evidence for serotonergic subsensitivity. Am J Psychiatry 153(11):1460-3.
- Tortorella, A., Monteleone, P., Fabrazzo, M., Viggiano, A., De Luca, L., Maj, M. (2001) Plasma concentrations of amino acids in chronic schizophrenics treated with clozapine. Neuropsychobiology 44, 167-171.
- Toth, E., Lajtha, A. (1986) Antagonism of phencyclidine-induced hyperactivity by glycine in mice. Neurochem. Res. 11(3), 393-400.
- Van der Heijden, F.M.M.A., Tuinier, S., Fekkes, D., Sijben, A.E.S., Kahn, R.S., Verhoeven, W.M.A. (2004) Atypical antipsychotics and the relevance of glutamate and serotonin. Eur. Neuropsychopharmacology 14 (3), 259-65.
- Van der Heijden, F.M.M.A., Fekkes, D., Tuinier, S., Sijben, A.E.S., Kahn, R.S., Verhoeven, W.M.A. (2005) Amino acids in schizophrenia: evidence for lower tryptophan availability during treatment with atypical antipsychotics? J. Neural Transm 112, 577-585.
- Van der Mast, R.C., Fekkes, D. (2000) Serotonin and amino acids; partners in delirium pathophysiology? Semin. Clin. Neuropsychiatry 5(2), 125-131.
- Verhoeven, W.M.A., Tuinier, S., van den Berg, Y.W.M.M., Coppus, A.M.W., Fekkes, D., Pepplinkhuizen, L., Thijssen, J.H.H. (1999) Stress and self-injurious behavior; hormonal

and serotonergic parameters in mentally retarded subjects. Pharmacopsychiat. 32, 13-20.

- Verity, C.M., Applegarth, D.A., Farrell, K., Kirby, L.T. (1983) The influence of anticonvulsants on fasting plasma ammonia and amino acid levels. Clin. Biochem. 16(6), 344-5.
- Vriend, J.P., Alexiuk, N.A. (1996) Effects of valproate on amino acid and monoamine concentrations in striatum of audiogenic seizure-prone Balb/c mice. Mol. Chem. Neuropathol. 27 (3), 307-24.
- Young, R.C., Biggs, T., Ziegler, E., Meyer, D.A. (1978) A rating scale for mania: reliability, validity and sensitivity. Br. J. Psychiatry 133, 429-435.
- Zarate, C.A., Quiroz, J., Payne, J., Manji, H.K. (2002) Modulators of the glutamatergic system: implications for the development of improved therapeutics in mood disorders. Psychopharmacol. Bull. 36(4), 35-83.
- Zarate Jr., C.A., Du, J., Quiroz, J., Gray, N.A., Denicoff, K.D., Singh, J., Charney, D.S., Manji, H.K. (2003) Regulation of cellular plasticity cascades in the pathophysiology and treatment of mood disorders: role of the glutamatergic system. Ann. N. Y. Acad. Sci. 1003, 273-91.

General Discussion

Chapter Six

General Discussion

1. The history and diagnosis of affective disorders

All patients included in the studies described in this thesis were suffering from various types of affective disorder: bipolar-I disorder, seasonal affective disorder and severe major depressive disorder with psychotic features. Although these clinical affective syndromes are distinct entities from the phenomenological viewpoint, it is by no means clear whether a distinct pathophysiology and aetiology corroborates their clinical differentiation.
The discussion about the symptomatic delineation of affective disorders goes back to the pre-Hippocratic area. In that time morbid states of depression and exaltation were known to the physicians. Hippocrates was the first to delineate melancholia, mania and paranoia but it is an anachronism to suppose that these words have the same meaning as the current disease entities. Our current notions about depression and mania date from the second half of the 19th century when the anatomo-clinical model of disease was embraced and subjective experiences were included in the symptomatology of mental disorders (Berrios, 1995). Until then, melancholia was used as a collective term for insanity states with a few delusions. Thus, states of non-psychotic depression would not have been called melancholia. In 1838 Jean-Etienne-Dominique Esquirol described the so called lypemania that can be considered as an early designation of a state characterized by debilitating and overwhelming sadness with delusions fixed on specific topics. Subsequently Billot (1856) defined lypemania on the basis of sad delusions and disordered affect. Berrios (1995) states that lypemania is an example of what historians would call a bridge category between the old notion of melancholia and the new conceptualization of a disorder of affect. As reviewed by Angst and Marneros (2001), the German psychiatrist Wilhelm Griesinger described in 1845 the change from melancholia to mania which he considered to be usual. The French psychiatrist Jean-Pierre Falret firstly described in 1851 the so-called folie circulaire, characterized by a continuous cycle of depression, mania and free intervals of various lengths. The description of this disease entity was supported by Karl Kahlbaum in his publication from 1882 about cyclisches Irresein. Subsequently, Emil Kraepelin (1893) grouped depressive and circular forms of depressive disorders in his concept of manic depressive illness. Kraepelin's unification of all affective disorders was challenged by the German psychiatrist Carl Wernicke (1906) and his fellow Karl Kleist who differentiated several types of affective syndromes, including unipolar and bipolar affective disorders. Their concepts were in 1957 completed by Karl Leonhard who classified the phasic psychoses into the pure and the polymorphic phasic disorders. Bipolar affective disorders and cycloid psychoses were classified within the polymorphic phasic psychoses and unipolar

depressive disorder within the pure phasic psychoses. In the mid sixties of the last century, both Jules Angst and Carlo Perris independently published their papers on the nosological differentiation of unipolar and bipolar affective disorders (Angst and Perris, 1968).
The history of the seasonal affective disorder is rather obscure. Although a seasonal rhythm of mental disorders was already described in the second half of the 19th century by the German and the French psychiatrists, it lasted until the eighties of the past century before the criteria for this subtype of depressive disorder were formulated by Rosenthal and co-workers (Rosenthal et al., 1984; Wehr and Rosenthal, 1989). Since then the operational criteria have undergone several changes and include vegetative symptoms that are the reverse of classical depressive vegetative symptoms e.g. increased sleep and appetite (Magnusson and Partonen, 2005).
The various types of affective disorder have been included in the current classification systems, the Diagnostic and Statistical Manual of Mental Disorders (DSM) and the International Classification of Diseases (ICD). The seasonal affective disorder was firstly incorporated in the DSM-III-R in 1987 as a specifyer of the longitudinal course of several types of affective disorders. In the DSM system, a 'psychotic depression' is a major depressive episode with a severity specifyer 'with psychotic features that can be mood congruent or incongruent', whereas the category bipolar-I disorder describes an affective disorder with at least one manic episode.
In order to include homogenous patient groups in the various studies as described in this thesis, patients had to meet the strict DSM-IV criteria for bipolar-I disorder, major depressive disorder with a seasonal pattern and severe depressive disorder with melancholic and/or psychotic features.

2. Biochemical parameters

2.1. Pterins

Measurement of neopterin levels with HPLC techniques is reliable and valid. Neopterin is the excretion product of 2,3-dihydroneopterine triphosphate. Cytokines like interferon-gamma stimulate the pterin synthesis through activation of GTP cyclohydrolase. In macrophages the enzyme 6-pyruvoyl tetrahydropterin synthase is not present. Therefore, the neopterin level is an accepted measure for cell-mediated immune activity (Levine, 1988; Dunbar et al., 1992; Maes et al., 1994; Murr et al., 2002).
A decrease of neopterin could be indicative for a general deactivation of the pterin metabolism resulting in less synthesis of tetrahydrobiopterin. Direct measurement of the active compound tetrahydrobiopterin is not feasible, because of its instability. After blood drawing, this substance is rapidly broken down to biopterin and pterin, which results in an

underestimation of the plasma levels of total biopterin (Fiege et al., 2004). Although several investigators have assessed total biopterin using comparable methods (e.g. Hashimoto et al., 1987; Knapp and Irwin, 1989; Anderson et al., 1992; Chrapko et al, 2004), measurement of total biopterin is considered to be a less valid indicator of the actual activity of BH4. A more precise measure is the Phe-Tyr ratio, which reflects the BH4-dependent hydroxylating activity (Anderson et al., 1994). The Cit-Arg ratio is also indicative for the activity of BH4 (Finkel et al., 1996; Fekkes et al., 2007). These amino acids, however, are also involved in other metabolic pathways, which makes this ratio less reliable.

2.2. Nitric oxide

Nitric oxide (NO) has a widespread function in the CNS and its synthesis is catalyzed by endothelial and neuronal NOS. After immune activation another isoform of NOS is induced, viz. iNOS, resulting in NO synthesis (Prast and Philippu, 2001; Akyol et al., 2004; Bernstein et al, 2005). Since NO plays a stimulating and mediating role in several neuronal processes, it is hypothesized to be implicated in various neuropsychiatric disorders (Prast and Philippu, 2001; Akyol et al, 2004).

Decreased activity of endothelial NOS may result in vasoconstriction and lowering of cerebral blood flow (Watkins, 1995; Li and Forsterman, 2000; Willmot et al, 2005).

Decreased activity of neuronal NOS has been implicated in a number of pathophysiological mechanisms. It may result in less release of mostly excitating neurotransmitters and in a disturbed balance of multiple cellular events. NO influences the activity of transcription factors, modulates upstream signalling cascades and affects both the stability and translation of mRNA (Bernstein et al., 2005). Targets of NO include guanylate cyclase, G proteins and amines, and neuropeptide release and transport (Akyol et al., 2004). Several of these processes are thought to be important in the regulation of mood (Akyol et al, 2004; Manji et al., 2000). Furthermore, NO is hypothesized to play a role in structural changes in the central nervous system through an influence on long term potentiation and long term desensitization, In addition, NO affects the NMDA receptor complex and influences the HPA-axis (Prast and Philippu, 2001; Bernstein et al, 2005; Akyol, 2004; Riedel, 2000).

2.3. Pterins and nitric oxide

The metabolism of pterins and nitric oxide is closely interrelated. The synthesis of NO from arginine is not only catalyzed by the enzyme NOS, but is also dependent on the presence of BH4 as a cofactor (Mayer and Werner, 1995; Koshimura et al., 2000). Thus, lower BH4 levels may result in decreased synthesis of NO. Moreover, if NOS is not fully saturated with BH4, potentially neurotoxic free radicals may be formed (Koshimura et al., 2000; Nakamura et al, 2001).

3. Main findings

Chapter 2 deals with bipolar-I patients who were compared with patients with either a major depressive disorder with melancholic features or with a seasonal pattern and with healthy controls. Manic-, depressed- and euthymic bipolar patients showed a decreased level of neopterin, which was correlated with a lower ratio of citrulline and arginine, indicative for less synthesis of NO. After symptomatic recovery the lowering of both neopterin and the citrulline-arginine ratio persisted.
These findings suggest that the NO and pterin metabolism play a role in a specific vulnerability of bipolar-I patients to develop mood changes.
Chapter 3 focuses on a group of patients with severe treatment-resistant depression with psychotic and/or melancholic features. In comparison to healthy controls, these patients showed before treatment significantly lower levels of biopterin, a higher Phe-Tyr ratio - indicative for a lower BH4 dependent hydroxylation -, and significantly lower plasma levels of tryptophan. Plasma levels of neopterin, however, were not different.
After treatment with electroconvulsive therapy (ECT), in the psychotic depressed patients an increase of biopterin was found and a decrease of the Phe-Tyr ratio. Responders to ECT showed an increase of both tryptophan and the tryptophan ratio.
These findings may be suggestive for a role of the hydroxylase function of tetrahydrobiopterin and probably of NO in the pathophysiology of severe major depression and the treatment effect of ECT.
Chapter 4 describes a group of patients with a major depression with a seasonal pattern. As compared to normal controls, the patients showed increased levels of neopterin and a decrease of biopterin and tryptophan. After light therapy, the higher level of neopterin persisted. In addition, an increase of biopterin and tryptophan was found. In summer, tryptophan levels normalized, whereas biopterin concentrations lowered again.
These results (lowering of biopterin) were originally interpreted as a vulnerability factor for the occurrence of a symptomatic period of this subtype of depression. The results of later studies, however, indicated that biopterin is a less precise measure of BH4 activity. The higher neopterin levels suggest increased cell-mediated immune activity in this disorder.
Chapter 5 investigates bipolar-I patients with a manic episode in comparison with healthy controls and bipolar-I patients in full remission. In the manic phase, a significant increase in glycine was demonstrated that persisted after remission of manic symptoms, suggestive for in involvement of the NMDA receptor complex in vulnerability for mania. Changes in other biochemical parameters could be attributed to the use of anticonvulsants.
In Summary:
The results of these studies reflect the functional activity of the essential cofactor BH4 in different patient groups with affective disorders as measured with indirect and relatively

simple biochemical methods. Neopterin, Phe-Tyr ratio, Cit-Arg ratio and the levels of the precursors and metabolites of dopamine and serotonin are all somehow related to BH4 activity. The observed disturbances suggest the presence of a biochemical endophenotype in affective disorders. This thesis comprises four studies with a relative small number of patients who were treated in normal daily clinical practice of a general psychiatric hospital. Although the research setting itself generates limitations, these findings demonstrate that with modest tools a contribution can be given to the scientific development in psychiatry.

4. Future research

In search for the biochemical endophenotype of affective disorders it could be of great interest to investigate the role of pterines and BH4 in other psychiatric diseases with an affective component such as schizoaffective and certain other psychotic disorders. To further investigate the role of pterines in the pathophysiology of affective disorders, interfering with this biochemical pathway, e.g. by oral administration of BH4, and close monitoring of changes in symptomatology might contribute to the development of novel therapeutic approaches. Since a polymorphism of the gene that encodes for GTPCH, the enzyme that is involved in the biosynthesis of BH4, has been demonstrated in a very limited number of recent studies to be associated with affective disorders, it is worthwhile to pursue this line of research in psychiatric disorders with affective symptoms.

References

- Akyol O, Zoroglu SS, Armutcu F, Sahin S, Gurel A: Nitric oxide as a physiopathological factor in neuropsychiatric disorders. In Vivo 2004;18:377-390.
- Anderson DN, Abou-Saleh MT, Collins J, Hughes K, Cattell RJ, Hamon CGB, Blair JA, Dewey ME. Pterin metabolism in depression: an extension of the amine hypothesis and possible marker of response to ECT. Psychological Medicine 1992; 22: 863-869.
- Anderson DN, Wilkinson AM, Abou-Saleh MT, Blair JA. Recovery from depression after electroconvulsive therapy is accompanied by evidence of increased tetrahydrobiopterin-dependent hydroxylation. Acta Psychiatr Scand 1994; 90: 10-13.
- Angst J. Marneros A. Bipolarity from ancient to modern times: conception, birth and rebirth. J. Affect Disord 2001 Dec;67(1-3):3-19.
- Angst J, Perris C. Zur Nosologie endogener Depressionen. Vergleich der Ergebnisse zweier Untersuchungen. Archiv für Psychiatrie und Zeitschrift f.d. ges. Neurologie 1968;210:373-386.

- Bernstein H-G, Bogerts B, Keilhoff G: The many faces of nitric oxide in schizophrenia. A review. Schizophr Res 2005a;78:69-86.
- Berrios GE. Mood disorders. Clinical section. In: Berrios GE and Porter R (eds.). A history of clinical psychiatry 1995, The Athlone Press, London. Pp. 385-408.
- Chrapko WE, Jurasz P, Radomski MW, Lara N, Archer SL, Le Melledo J-M. Decreased platelet nitric oxide synthase activity and plasma nitric oxide metabolites in major depressive disorder. Biol Psychiatry 2004; 56: 129-134.
- Dunbar PR, Hill J, Neale TJ, Mellsop GW. Neopterin measurement provides evidence of altered cell-mediated immunity in patients with depression, but not with schizophrenia. Psychological Medicine 1992; 22: 1051-1057.
- Fekkes D, Bannink M, Kruit WHJ, Van Gool AR, Mulder PGH, Sleijfer S, Eggermont AMM: Influence of pegylated interferon-α therapy on plasma levels of citrulline and arginine in melanoma patients. Amino Acids 2007;32:121-126.
- Fiege B, Ballhausen D, Kierat L, Leimbacher W, Goriounov D, Schircks B et al. Plasma tetrahydrobiopterin and its pharmacokinetic following oral administration. Mol Genet Metab 2004;81:45-51.
- Finkel MS, Laghrissi-Thode F, Pollock BG, Rong J: Paroxetine is a novel nitric oxide synthase inhibitor. Psychopharmacol Bull 1996;32(4):653-8.
- Hashimoto R, Ozaki N, Ohta T, Kasahara Y, Kaneda N, Nagatsu T. Total biopterin levels of plasma in patients with depression. Neuropsychobiology 1987; 17: 176-177.
- Knapp S, Irwin M. Plasma levels of tetrahydrobiopterin and folate in major depression. Biol Psychiatry 1989; 26: 156-162.
- Koshimura K, Murakami Y, Tanaka J, Kato Y. The role of 6R-tetrahydrobiopterin in the nervous system. Progress in Neurobiology 2000; 61(4): 415-438.
- Levine RA. Tetrahydrobiopterin and biogenic amine metabolism in neuropsychiatry, immunology, and aging. Ann N Y Acad Sci 1988; 521: 129-139.
- Li H, Forstermann U. Nitric oxide in the pathogenesis of vascular disease. J Pathol. 2000 Feb;190(3):244-54.
- Maes M, Scharpe S, Meltzer HY, Okayli G, Bosmans E, D'Hondt PD, Vanden Bossche B, Cosyns P. Increased neopterin and interferon-gamma secretion and lower availability of L-tryptophan in major depression: further evidence for an immune response. Psychiatry Research 1994; 54: 143-160.
- Magnusson A, Partonen T. The diagnosis, symptomatology, and epidemiology of seasonal affective disorder. CNS Spectr 2005;10(8):625-634.
- Manji HK, Moore GJ, Chen G. Clinical and preclinical evidence for the neurotrophic effects of mood stabilizers: implications for the pathophysiology and treatment of manic-depressive illness. Biol Psychiatry 2000; 48: 740-754.
- Mayer B, Werner ER. In search of a function for tetrahydrobiopterin in the biosynthesis

of nitric oxide. Naunyn-Schmiedeberg's Arch Pharmacol 1995; 351:453-463.
- Murr C, Widner B, Wirleitner B, Fuchs D. Neopterin as a marker for immune system activation. Curr Drug Metab 2002; 3: 175-187.
- Nakamura K, Bindokas VP, Kowlessur D, Elas M, Milstien S, Marks JD, Halpern HJ, Kang UJ. Tetrahydrobiopterin scavenges superoxide in dopaminergic neurons. J Biol Chem 2001;276(37):34402-34407.
- Prast H, Philippu A. Nitric oxide as modulator of neuronal function. Progress in Neurobiology 2001; 64(1): 51-68.
- Riedel W. Role of nitric oxide in the control of the hypothalamic-pituitary-adrenocortical axis. Z Rheumatol 2000; 59 (suppl 2): 39-42.
- Rosenthal NE, Sack DA, Gillin C, Lewy AJ, Goodwin FK, Davenport Y, Mueller PS, Newsome DA, Wehr TA. Seasonal affective disorder. Arch Gen Psychiatry 1984; 41: 72-80.
- Watkins LD. Nitric oxide and cerebral blood flow: an update. Cerebrovasc Brain Metab Rev 1995 Winter;7(4):324-337.
- Wehr TA, Rosenthal NE. Seasonality and affective illness. Am J Psychiatry 1989;146:829-839.
- Willmot M, Gray L, Gibson C, Murphy S, Bath PM. A systematic review of nitric oxide donors and L-arginine in experimental stroke; effects on infarct size and cerebral blood flow. Nitric Oxide 2005 May;12(3):141-9.

Summary

Chapter Seven

Summary

In this thesis patients with various affective disorders are described. Patients with a bipolar-I disorder, which is characterized by prominent affective symptomatology, were investigated. This disorder is characterized by cyclic lowered and elevated mood. In addition, patients with a severe, often psychotic, depressive disorder were studied as well as depressed patients with a seasonal pattern.

The central issue of this thesis deals with the role of pterins and nitric oxide (NO) in the pathophysiology of various types of affective disorders. NO is thought to be related to mood disorders because of its vascular and neuronal functional implications. The synthesis of NO is catalyzed by the enzyme nitric oxide synthase for which tetrahydrobiopterin is required as cofactor. In the synthesis of dopamine, norepinephrine and serotonin this cofactor is also required which underlines the putative role of this compound in neuropsychiatric disorders. In *chapter 1* the literature is reviewed.

Chapter 2 investigates the relationship between bipolar affective disorder and the pterin and NO metabolism. Bipolar-I patients were compared to those with a major depressive disorder with melancholic and/or psychotic features, with a seasonal affective disorder and with healthy controls. Irrespective the clinical state of the bipolar-I patients a lower concentration of both neopterin and the ratio of citrulline and arginine was found. In addition, these parameters were positively correlated. These results suggest that the bipolar-I disorder can be differentiated biochemically from other affective disorders. The lower ratio of citrulline and arginine reflects a decreased synthesis of NO. The decrease of neopterin is probably related to a lowered synthesis of pterins. These data suggest that NO and pterin metabolism are associated with the vulnerability for bipolar mood changes.

Chapter 3 describes the study on pterins and related amino acids in patients with major depression with melancholic and/or psychotic features. Medication-free patients were compared to healthy controls. Patients showed lower biopterin levels and an increase of the ratio of phenylalanine and tyrosine as well as a lower plasma tryptophan level. After electroconvulsive therapy (ECT) an increase of biopterin and a decrease of the phenylalanine tyrosine ratio were found in depressed patients with psychotic features, suggestive for an increased activity of the cofactor tetrahydrobiopterin. Responders to ECT showed an increase of tryptophan and tryptophan ratio. These findings suggest a disturbed pterin metabolism in this group.

Chapter 4 describes a study in patients with seasonal affective disorder. Plasma levels of

pterins and related amino acids were determined and compared to those obtained in healthy controls. A decrease of biopterin and tryptophan concentrations was found as well as an increase of neopterin. After treatment with light therapy the latter persisted, whereas the levels of biopterin and tryptophan increased. During the asymptomatic summer season, tryptophan normalized and biopterin decreased. This persistent increase of neopterin reflects enhanced cell mediated immune activity that, together with lower tryptophan levels may be associated with depressive symptoms in winter.

Chapter 5 focuses on bipolar-I manic patients who were compared with healthy controls and euthymic bipolar-I patients. The patients in a manic phase showed an increase of the plasma glycine levels which may indicate an involvement of the NMDA receptor complex in the pathophysiology of mania.

Chapter 6 discusses the main findings of this thesis. The delineation of the various affective disorders in history is outlined. In the present studies, the strict DSM-IV criteria for bipolar-I disorder, major depression with melancholic and/or psychotic features and seasonal affective disorder were applied.
The plasma level of neopterin seems to be a reliable and valid measure of the pterin metabolism. A decrease of neopterin suggests a general reduction of the pterin metabolism, resulting in a reduced synthesis of NO and of neurotransmitters such as dopamine and serotonin. The various abnormalities in the biochemical parameters most probably reflect a biochemical endophenotype in the different affective disorders.

Samenvatting in het Nederlands

(summary in dutch)

Chapter Eight

Samenvatting

Dit proefschrift gaat over patiënten met verschillende stemmingsstoornissen. Patiënten met een bipolaire stoornis type I, een aandoening waarbij bij uitstek de affectieve symptomatologie centraal staat, werden onderzocht. Deze psychiatrische ziekte wordt gekenmerkt door het voorkomen van zowel een verlaagde als een verhoogde stemming en heeft een karakteristiek episodisch beloop. Voorts zijn patiënten met ernstige, veelal psychotisch depressieve symptomatologie onderzocht. Patiënten met zogenaamde atypische depressieve klachten en een seizoensgebonden patroon zijn als aparte subgroep onderzocht.

De centrale vraag van dit proefschrift behelst de mogelijke betekenis voor de pathofysiologie van zowel pterines als stikstofoxide (NO) in de verschillende typen stemmingsstoornissen. NO wordt in toenemende mate in verband gebracht met affectieve stoornissen vanwege de invloed op zowel vasculaire als neuronale functies. Het enzym stikstofoxide synthase heeft tetrahydrobiopterine als cofactor nodig om NO te kunnen synthetiseren. Deze cofactor is daarnaast essentieel voor de synthese van dopamine, noradrenaline en serotonine en wordt daarom ook in verband gebracht met de pathofysiologie van neuropsychiatrische aandoeningen. In *hoofdstuk 1* wordt de literatuur over dit onderwerp kritisch beschouwd.

Hoofdstuk 2 onderzoekt de samenhang tussen een bipolaire stemmingsstoornis en het pterine metabolisme. De patiënten met een bipolaire stoornis type I werden vergeleken met patiënten met een depressieve stoornis met vitale en vaak psychotische kenmerken, met patiënten met een seizoensgebonden depressie en met gezonde controles. Ongeacht de klinische status van de bipolaire patiënt, d.w.z. zowel tijdens de depressieve, manische als euthyme fase, werd een verlaging van neopterine en van de citrulline-arginine ratio gevonden. Deze parameters waren bovendien positief gecorreleerd .
Deze bevindingen maken duidelijk dat op biochemisch niveau een onderscheid kan worden gemaakt tussen de bipolaire stoornis en andere affectieve aandoeningen. De verlaging van de citrulline-arginine ratio wijst op een verminderde synthese van NO. De verlaging van neopterine zou kunnen betekenen dat het verlaagde pterinemetabolisme hieraan gerelateerd is of zelfs oorzakelijk verbonden. Mogelijk is het veranderde NO metabolisme geassocieerd met een verhoogde kwetsbaarheid voor bipolaire stemmingswisselingen.

Hoofdstuk 3 beschrijft het onderzoek naar pterinen en daaraan gerelateerde aminozuren bij patiënten met een psychotische depressie. Medicatievrije patiënten werden vergeleken met gezonde controles. Bij de patiënten werd een verlaging van biopterine gevonden en een

daarmee samenhangende verhoging van de phenylalanine-tyrosine ratio. Voorts bleek het plasma tryptofaangehalte verlaagd te zijn. Het effect van behandeling met electroconvulsieve therapie (ECT) op deze parameters werd eveneens onderzocht. Vooral bij de psychotisch depressieve patiënten werd een toename van biopterine en een verlaging van de phenylalanine-tyrosine ratio gevonden, hetgeen wijst op een toegenomen activiteit van de cofactor tetrahydrobiopterine. Daarnaast werd een verhoging van tryptofaan en de tryptofaan ratio vastgesteld bij patiënten die verbeterden op ECT.
Deze bevindingen wijzen op een verstoring van het pterinemetabolisme bij ernstig depressieve patiënten. Behandeling met ECT is mogelijk van invloed op de synthese van pterines en wellicht ook op de vorming van NO.

Hoofdstuk 4 beschrijft een studie bij patiënten met een seizoensgebonden depressie. Plasmaconcentraties van pterines en daaraan gerelateerde aminozuren werden vergeleken met die van gezonde controles. Naast een verlaging van biopterine en tryptofaan werd een toename van neopterine gevonden. Na behandeling met lichttherapie bleef deze toename aanwezig, terwijl de concentraties van biopterine en tryptofaan stegen. Gedurende de asymptomatische zomerperiode trad een verdere normalisering van het tryptofaangehalte op, terwijl biopterine laag bleef. De persisterende verhoging van neopterine hangt mogelijk samen met een toegenomen celgemedieerde immuunactiviteit die, tezamen met een verlaging van tryptofaan, in de winter gepaard gaat met depressieve klachten.

Hoofdstuk 5 richt zich op patiënten met een bipolaire-I stoornis. De patiënten werden vergeleken met gezonde controles en euthyme bipolaire-I patiënten. De patiënten in een manische fase vertoonden een toegenomen plasma glycine concentratie, hetgeen wijst op een betrokkenheid van het NMDA receptor complex in de pathofysiologie van de manie.

Hoofdstuk 6 bespreekt de belangrijkste bevindingen uit dit proefschrift. Vanuit historisch perspectief wordt aangegeven hoe affectieve stoornissen een aparte nosologische categorie zijn geworden. De strikte DSM-IV criteria voor de bipolaire stoornis, ernstige depressieve stoornis met of zonder psychotische kenmerken en de depressieve stoornis met seizoensgebonden patroon zijn gehanteerd bij de selectie van de patiënten voor dit onderzoek.
Van de pterines is het gehalte aan neopterine de meest betrouwbare maat. Een afname wijst op een algehele verlaging van het pterinemetabolisme, hetgeen implicaties heeft voor de synthese van NO en neurotransmitters zoals dopamine en serotonine. De verschillende afwijkingen die gevonden zijn in pterines, NO en aminozuren zijn mogelijke een biochemisch endofenotype van de verschillende affectieve stoornissen.

Addendum

Pteridines and affective disorders

Pteridines and affective disorders

Rocco Hoekstra, Durk Fekkes

Published in Acta Neuropsychiatrica 2002; 14: 120-126

Abstract

The pteridine tetrahydrobiopterin (BH4) is an essential cofactor in the biosynthesis of dopamine, (nor)epinephrine, serotonin and nitric oxide (NO). Furthermore, BH4 has a direct influence on release mechanisms of these neurotransmitters and on serotonin receptor binding activity.

The synthesis of BH4 is stimulated by interferon-gamma and hence there is a close relationship with the immune system. In animal experiments it was also found that the hypothalamus-pituitary-adrenal-axis influences the pteridine metabolism. In clinical studies, so far, no evidence is found for this relationship.

A congenital biopterin deficiency results in atypical phenylketonuria with severe neuropsychiatric symptoms. In several neurological diseases, like Parkinson's disease, decreased levels of BH4 are found.

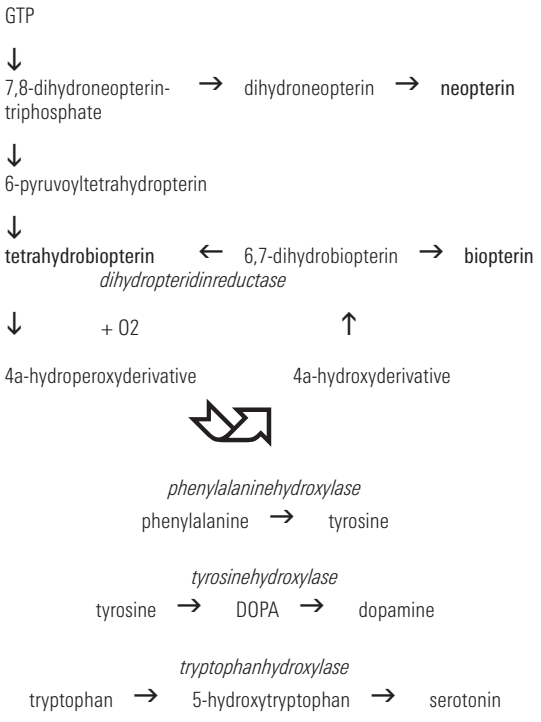
Since 1984 there have been reports on decreased biopterin and increased neopterin levels in urine and plasma of depressed patients. Conflicting results are also found however, probably due to methodological problems. Up to now, oral administration of BH4 to depressed patients has been performed by two investigators, which resulted in mostly temporal clinical improvement.

Understanding of biochemical mechanisms in which pteridines are involved may contribute to our knowledge of the pathogenesis and treatment of affective disorders. This article aims to give an overview of the relevant literature and warrant for further research on this intriguing compound.

Introduction

An aberrant metabolism of the monoamines norepinephrine, dopamine and serotonin in the central nervous system has been hypothesized to be related to the pathophysiology of affective disorders [1]. In the monoamine metabolism the pteridines, e.g. tetrahydrobiopterin (BH4), play an important role. BH4 is a reduced pterine molecule, which acts as the essential cofactor for the conversion of phenylalanine to tyrosine and the hydroxylation of tyrosine and tryptophan. These conversions are the rate limiting steps in the formation of serotonin, norepinephrine and dopamine [2,3] (figure 1). Independent of its cofactor-role, BH4 has a direct influence on the release mechanism of these neurotransmitters [4,5]. These are facts from which a pivotal role for the pteridines in the pathogenesis of affective disorders could be suggested. The aim of this review is to give an update of the relevant literature in this field. We searched for review articles about pteridines in general, and for original research articles about pteridines in relation to psychiatric disorders in specific.

Figure 1.
Biosynthesis of tetrahydrobiopterin and its effect on the hydroxylation of phenylalanine, tyrosine and tryptophan.



Biosynthesis

BH4 is synthesized de novo from guanosine triphosphate (GTP). GTP is converted to dihydroneopterin triphosphate and then by a series of tetrahydro intermediates to BH4. BH4 donates an electron in the process of hydroxylation of phenylalanine, tyrosine or tryptophan, which yields quinoid dihydrobiopterin. Cellular concentrations of BH4 are maintained by de novo synthesis and a salvage mechanism that reduces quinoid dihydrobiopterin to BH4 by the enzyme dihydropteridine reductase with NADH as a cofactor. Dihydroneopterin triphosphate is secreted in the urine after oxidation to neopterin. BH4 is secreted in the urine with its oxidative products dihydrobiopterin and biopterin [2,3,6] (figure 1). Synthesis is not dependent on dietary pterin, which is poorly absorbed from the gut [7].

Localization and function

BH4 is present in the liver, the gastrointestinal tract, the adrenal medulla, T-lymphocytes and macrophages. In brain, the hypothalamus, pituitary gland and pineal gland contain relatively large concentrations of BH4 [2,3]. Cerebral activity of BH4 seems to be related mainly to the activity of tyrosine hydroxylase and to a lesser extent to that of tryptophan hydroxylase. Thus, the influence on the catecholaminergic system seems to be more important than the influence on the serotonergic system [5, 8-12].
The best investigated function of BH4 is the above mentioned action as a natural cofactor of the aromatic amino acid hydroxylases, i.e. phenylalanine hydroxylase, tyrosine hydroxylase and tryptophan hydroxylase [2]. Furthermore, BH4 is required as an obligatory cofactor for the enzyme nitric oxide (NO)-synthase, which catalyses the synthesis of citrulline and NO from arginine [2,13,14].
BH4 has also been suggested to act as a self-protecting factor for NO-toxicity [2]. For example, in the endothelium activation of nitric oxide synthase, in the absence of BH4, causes increased formation of oxygen-derived radicals impairing vascular tone [15-17].
In this article we will focus on the sparse literature concerning the functions of BH4 in relation to psychiatric disorders.

Immunology

Pteridines are functionally closely related to the immune system [3]. Interferon-gamma, which is produced and secreted by activated T-cells, stimulates GTP cyclohydrolase activity, resulting in synthesis of pteridines.

Neopterin is an established, validated marker of the activation of cell-mediated immunity in biological processes. Neopterin detection is especially useful in screening for the presence of viral infections or in the management of particularly HIV infection [3,10,12,18]. Macrophages can secrete only neopterin and not biopterin because of the absence of the enzyme 6-pyruvoyl-dihydropterin (PPH4) synthase (figure 1). Activation of GTP cyclohydrolase in macrophages will therefore cause a large increase in the synthesis of neopterin [3].
Whether evidence for immune activation can be found in affective disorders remains debatable. Some groups did not find activated immune markers in depressed patients [19,20]. Other groups reported on signs of an activated Inflammatory Response System, like increased white blood cell count, monocytes, T-lymphocytes, prostaglandines and cytokines [21]. The increased neopterin secretion found in some studies of depressed patients is compatible with findings of an activated immune system in depression [12,18,22,23].

Hypothalamus-pituitary-adrenal-axis

The hypothalamus-pituitary-adrenal-axis seems to influence the pteridine metabolism. In animal experiments it was found that glucocorticoids inhibit induction of nitric oxide formation by direct influence on the enzyme NO synthase as well as by reducing the availability of BH4 [24]. An activated HPA-axis, as often found in depressed patients, could decrease the BH4 concentration and in this way affect the monoamine metabolism.
Furthermore, NO which is dependent on BH4 for its synthesis, plays a mediating role in the HPA-axis [13].
Only in a small number of patients the relationship between pteridines and the HPA-axis is studied. O'Toole reported no differences in neopterin concentration in 35 depressed patients before and after the CRH and ACTH stimulation tests [25]. Garbutt also found no differences in biopterin and neopterin concentrations in urine of 9 patients with major depression before and after a dexamethason suppression test [26]. Further research should focus on the link between biopterin in plasma and the HPA-axis in strictly selected patients.

Experimental research

The effect of administration of BH4 on the concentration of several neurotransmitters is studied in vitro. In samples of striatum and frontal cortex of rats, administration of BH4 caused an increase in the release of dopamine, serotonin and glutamate. The release of

glutamate is probably influenced by the catecholaminergic system [27]. After administration of BH4 in the ventricles of living rats, an increase in the concentrations of DOPA, dopamine, 5-hydroxytryptophan and serotonin was seen in vitro after decapitation, particularly in the diencephalon and the brainstem of rats [11].
Koshimura et al. also reported on an increase in dopamine release in striatum of rats after administration of BH4, but concluded that this was not caused by increased synthesis [4]. In a comparable study Wolf et al. showed that BH4 causes increased release of serotonin, probably by more influx of calcium or by increased sensibility of the release mechanism for calcium. These effects of BH4 occur independently of its cofactor role for tryptophan hydroxylase [5]. In rat brain a change in serotonin receptor binding activity was also seen after peripheral administration of BH4 in the living rat. A post mortem increase in binding activity of the 5-HT1a receptor in the hippocampus and decrease in the visual cortex was seen. Binding of ketanserin to the 5-HT2 receptor increased in the striatum and the hippocampus and decreased in the cerebellum and the visual cortex [28].

Diseases with changes in pteridine metabolism

A changed BH4 metabolism may lead to neurological and psychiatric symptoms. A disturbed BH4 synthesis causes atypical phenylketonuria. About 2% of patients with phenylketonuria show a BH4-deficiency [29]. Congenital biopterin deficiencies are the consequence of various enzyme defects. For example, deficiencies of dihydropteridine reductase and of 6-PPH4 synthase have been reported [29-31]. In case of BH4 deficiency mostly changes have been found in the liver and in the brain, although a BH4 deficiency solely in the liver has also been found [3]. It seems therefore plausible that a sole cerebral manifestation is possible. Probably several neuropsychiatric diseases with low BH4 concentrations can be explained in this way. In the brain there seems to be heterogeneity of BH4 activity in monoamine-containing neurons [8,9]. Therefore, it is not expected that peripherally measured, changed BH4 values are specific for one disease.
In patients with congenital biopterin deficiency, decreased plasma and cerebrospinal fluid (CSF) concentrations of serotonin, dopamine and their metabolites were found next to hyperphenylalaninemia. Unlike patients with classical phenylketonuria a phenylalanine restricted diet cannot prevent severe symptoms [9]. When substitution with the above-mentioned neurotransmitters is not started in the first postnatal weeks, which is very difficult, severe and progressive damage of the nervous system is seen. Clinical symptoms may be microcephaly, irritability, poor head control, feeding and swallowing problems, hypersalivation, temperature disturbance, truncal hypotonia and lethargy. Later signs include rigidity, convulsions and progressive mental retardation [32].

Decreased BH4 levels in CSF have been found in patients with Parkinson's disease, familial dystonia and some other rare neurological diseases [33,34]. Besides, in patients with Alzheimer's disease and other dementias lower biopterin levels were reported [35]. Kay et al. reported that the decrease of biopterin in CSF was not correlated with ventricular volume or with the severity of dementia, suggesting lowering of biopterin independent from atrophy of brain tissue [36].

Pteridines and affective disorders

Because of their influence on the synthesis and release of several neurotransmitters, the relationship between pteridines and affective disorders has been studied. In 1984 Blair already reported on a link between biopterin and recurrent affective disorders. Patients with a bipolar disorder, using lithium and being euthymic, had a significant decrease in total biopterin excretion compared with healthy controls and patients with a unipolar depression. It cannot be excluded that lithium is the cause of this lower excretion. On the other hand, he also found reduced BH4 synthesis in post mortem temporal cortex samples of four patients with a history of severe depression [37]. Coppen also found lowered biopterin concentrations in the urine of depressed patients [38]. However, Hashimoto reported an increase in total plasma biopterin levels in twelve patients with affective disorders, depressive as well as hypomanic. The active compound BH4, however, was decreased in depressed patients and increased in hypomanic patients. In patients with schizophrenia or panic disorder BH4 levels were not different from those in normal controls [39]. In a study of Anderson et al. it was shown that patients with a psychotic depression and depressed patients responding to electroconvulsive therapy (ECT) had a significantly higher urinary neopterin-biopterin (N-B) ratio compared to controls before starting ECT, while a positive therapeutic response was associated with a reduction of the N-B ratio towards control values [40]. A raised N-B ratio probably implies reduced availability of BH4 and hence less synthesis of serotonin, dopamine and norepinephrine [40]. Abou-Saleh et al. measured urinary neopterin and biopterin in 48 patients with depression, fulfilling DSM-III criteria, before and after treatment with placebo, antidepressants, or ECT. Patients prior to and post treatment had a significantly higher N-B ratio than control subjects and besides, a significant correlation was observed between the N-B ratio and severity of depression [41].

Our group has measured biopterin in plasma of 20 medication free patients, who had not responded to antidepressants and therefore were indicated for ECT. Pretreatment biopterin concentrations in these patients were decreased compared to normal controls. After - in most of the times - successful ECT treatment plasma biopterin levels in psychotic

depressed patients showed an increase [42].
However, some investigators found an increase in biopterin concentration in depressed patients. Knapp, for example, observed an increase in plasma biopterin in 20 mild to severely depressed patients with a history of alcohol abuse [43]. Duch and Garbutt found an increase in urinary levels of biopterin in small groups of depressed patients [26,44].

Neopterin, the secretory metabolite of a precursor of BH4, is measured by Dunbar et al. in plasma and urine of 26 depressed and 22 schizophrenic patients. These investigators found a significantly increased level of this compound in plasma of depressed patients, whereas no significant changes were seen in urine. The levels in schizophrenic patients were comparable with those in normal controls [18]. Maes et al. and Matsuda et al. found an increased serum neopterin concentration in depressed patients, while Bonaccorso reported on an increase of this substance in urine [12,22,23]. O'Toole and our group found no change in plasma neopterin in depressed patients compared to healthy controls [25,42].
Methodological deficits may explain these seemingly contradictory results. Measurements were often performed in urine samples and the symptomatology of the depressed patients was not always well described. Maybe the spectrum of pathology, when using DSM criteria, is too broad to find unequivocal results. Theoretically, using strictly selected depressed patients, an increased plasma neopterin level, suggesting an activated immune system, and a decreased plasma biopterin plasma level, leading to less biosynthesis of serotonin and catecholamines, should be expected.

Therapy

In vitro BH4 administration yields the same effects at neurotransmitter level as does standard antidepressant treatment [4,5,11,27,28]. Therefore, studying the effect of BH4 administration to depressed patients may be useful. Substantial amounts of BH4 can be detected in cerebrospinal fluid when BH4 is administered orally or intravenously [45,46]. After peripheral administration of BH4 some positive clinical effects can be observed in various neuropsychiatric disorders [47,48].

Curtius described in 1982 the effect of BH4 administration in three depressed patients and two patients with Parkinson's disease. Two of the depressed patients, especially those with so called inhibited endogenous depression, improved clinically after 4 to 5 hours and the symptoms reappeared approximately 10 hours after the loading [49]. Fleischhacker did a double-blind crossover trial with BH4 versus placebo in 8 therapy resistant depressed

patients. Previous and hitherto ineffective medication was continued. One patient improved after placebo. Another patient, who already used 5-hydroxytryptophan and tranylcypromine showed a positive response on BH4, which lasted for 11 days. In four patients signs of increased vigilance and drive were detected either by observation or in clinical EEG analyses for 1 to 6 days [50].
These results suggest that BH4 indeed is important in the pathogenesis of depression. We have not found any other reports in the literature on loading studies with BH4 in depressed patients.

Discussion

The pteridine metabolism plays an essential role in the biosynthesis of neurotransmitters, known to be related to affective disorders, such as serotonin, norepinephrine and dopamine [2]. Pteridines are functionally closely related to the cell-mediated immune system [3]. The link with the hypothalamus-pituitary-adrenal-axis is less clear as yet [13]. Furthermore, pteridines seem to act as a protecting factor for NO-toxicity. The role of NO in affective disorders is still unclear [13].
Although laboratory findings in animals and brain tissue point to an involvement of pteridines in the pathogenesis of depression, and although this would fit in most of the theories used for explaining depressive symptoms, so far contradictory results are reported in depressed patients. Most clinical studies, using severe endogenous depressed patients, point to a decrease in biopterin activity in depression [37-42]. The difference with biopterin values in other psychiatric disorders, like schizophrenia and panic disorder, is remarkable [39]. Probably this is indicative for a specific role of biopterin in depression.
It is not yet clear if plasma or urine biopterin reflects cerebral activity and can be used as a marker. In temporal cortex material of 4 patients with a history of severe depression less biopterin synthesis was found [37], probably corresponding with lowered biopterin plasma levels, found in depressed patients. In other diseases a decrease of biopterin in plasma as well as in CSF has been described [35,36,51,52]. In a few depressed patients an indication for low CSF biopterin or no change in CSF biopterin compared to healthy controls was found [53,54,55]. We think plasma levels could be an indicator of central activity but because pteridines are localized in many tissues, organic diseases can confound results easily. Disorders like Parkinson's disease, dementia, neoplastic disorders and immunological disturbances, known to influence pteridine metabolism, should be detected precisely and excluded in further studies. Furthermore results should be corrected for age, because there seems to be an age-related increase in biopterin derivatives levels [7]. Plasma and urine samples should be treated under standardized conditions, for example protected from light [18].

The most obvious results in peripheral measures were found in severely depressed patients [40-42]. Therefore, it seems necessary to include strictly selected patients with homogenous symptomatology. The pteridine metabolism could especially be disturbed in certain subpopulations, such as psychotic depressed patients or antidepressant resistant patients. Biopterin may also be specifically related to a certain dimension of depressive behaviour.

Peripheral values of neopterin seem to be less specific than biopterin. Even more conflicting results are found when neopterin is studied in depressed patients. Probably too much factors can influence neopterin, specifically age and somatic comorbidity.
A more dynamic approach, by interacting with several neuroendocrine systems through administration of biopterin, could give most interesting information. By studying the effect of biopterin on neurotransmitters, immunological parameters and the HPA-axis in vivo, much more information on the role of this intriguing compound in the pathophysiology of affective disorders will be obtained.

Conclusion

The pteridines seem to play an important role in affective disorders. There is still a big gap between theories about depression and in vitro laboratory findings on the one hand and clinical results on the other. This should be overcome by strict selection of patients and standardization of laboratory measurements. Probably biopterin can act as a marker for the depressive state in certain subpopulations. Results of biopterin loading studies could have therapeutic implications.

References

1. Praag van, HM. Neurotransmitters and CNS disease: depression. Lancet ii 1982; 1259-1264.
2. Thöny B, Auerbach G, Blau N. Tetrahydrobiopterin biosynthesis, regeneration and functions. Biochem J 2000; 347: 1-16.
3. Levine RA. Tetrahydrobiopterin and biogenic amine metabolism in neuropsychiatry, immunology, and aging. Annals of the New York Academy of Sciences 1988; 521: 129-139.
4. Koshimura K, Miwa S, Lee K, Fujiwara M, Watanabe Y. Enhancement of dopamine release in vivo from the rat striatum by dialytic perfusion of 6R-L-erythro-5,6,7,8-

tetrahydrobiopterin. Journal of Neurochemistry 1990; 54: 1391-1397.
5. Wolf WA, Ziaja E, Arthur RA Jr., Anastasiadis PZ, Levine RA, Kuhn DM. Effect of tetrahydrobiopterin on serotonin synthesis, release, and metabolism in superfused hippocampal slices. Journal of Neurochemistry 1991; 57:1191-7.
6. Auerbach G, Nar H. The pathway from GTP to tetrahydrobiopterin: three dimensional structures of GTP cyclohydrolase I and 6-pyruvoyl tetrahydropterin synthase. Biol Chem 1997; 378: 185-192.
7. Leeming RJ, Blair JA. The effects of pathological and normal physiological processes on biopterin derivative levels in man. Clinica Chimica Acta 1980;108:103-111.
8. Bullard, W.P., Guthrie, P.B., Russo, P.V., Mandell, A.J. Regional and subcellular distribution and some factors in the regulation of reduced pterins in rat brain. Journal of Pharmacology and Experimental Therapeutics 1978; 206: 4-20.
9. Kapatos G, Hirayama K, Lentz SI, Zhu M, Stegenga S. Differential metabolism of tetrahydrobiopterin in monoamine neurons: a hypothesis based upon clinical and basic research. Advances in Experimental Medicine and Biology 1993; 338: 217-22.
10. Lentz SI, Kapatos G. Tetrahydrobiopterin biosynthesis in the rat brain: heterogeneity of GTP cyclohydrolase I mRNA expression in monoamine-containing neurons. Neurochem Int 1996; 28: 569-582.
11. Miwa S, Watanabe Y, Hayaishi O. 6R-L-erythro-5,6,7,8-tetrahydrobiopterin as a regulator of dopamine and serotonin biosynthesis in the rat brain. Archives of Biochemistry and Biophysics 1985; 239: 234-241.
12. Maes M, Scharpé S, Meltzer HY, Okayli G, Bosmans E, D'Hondt P, Vanden Bossche B, Cosyns P. Increased neopterin and interferon-gamma secretion and lower availability of L-tryptophan in major depression: further evidence for an immune response. Psychiatry Research 1994;54: 143-160.
13. Amsterdam van JGC, Opperhuizen A. Nitric oxide and biopterin in depression and stress. Psychiatry Research 1999; 85: 33-38.
14. Mayer B, Werner ER. In search of a function for tetrahydrobiopterin in the biosynthesis of nitric oxide. Naunyn-Schmiedeberg's Arch Pharmacol 1995; 351: 453-463.
15. Maier W, Cosentino F, Lütolf RB, Fleisch M, Seiler C, Hess OM, Meier B, Lüscher TF. Tetrahydrobiopterin improves endothelial function in patients with coronary artery disease. Journal of Cardiovascular Pharmacology 2000;35:173-178.
16. Heitzer T, Krohn K, Albers S, Meinertz T. Tetrahydrobiopterin improves endothelium-dependent vasodilation by increasing nitric oxide actvitiy in patients with Type II diabetes mellitus. Diabetologica 2000;43:1435-1438.
17. Stroes E, Kastelein J, Cosentino F, Erkelens W, Wever R, Koomans H, Lüscher T, Rabelink T. Tetrahydrobiopterin restores endothelial function in hypercholesterolemia. J. Clinical Invest 1997; 99:41-46.

18. Dunbar PR, Hill J, Neale TJ, Mellsop GW. Neopterin measurement provides evidence of altered cell-mediated immunity in patients with depression, but not with schizophrenia. Psychological Medicine 1992; 22: 1051-1057.
19. Natelson BH, Denny T, Zhou XD, LaManca JJ, Ottenweller JE, Tiersky L, DeLuca J, Gause WC. Is depression associated with immune activation? Journal of Affective Disorders 1999; 53: 179-184.
20. Rapaport MH. Immune parameters in euthymic bipolar patients and normal volunteers. Journal of Affective Disorders 1994; 32: 149-156.
21. West van D, Maes M. Psycho-immunologie van affectieve stoornissen. In: den Boer JA, Ormel J, van Praag HM, Westenberg HGM, D'haenen H (eds). Handboek stemmingsstoornissen. Maarssen, Elsevier/De Tijdstroom, 1999, pp 179-183.
22. Matsuda J, Gohchi K, Gotoh N. Serum concentrations of 2',5'-oligo-adenylate synthetase, neopterin, and Beta-glucan in patients with chronic fatigue syndrome and in patients with major depression. The Lancet (letters to the editor) 1994; 1015-16.
23. Bonaccorso S, Lin A, Verkerk R, van Hunsel F, Libbrecht I, Scharpe S, DeClerck L, Biondi M, Janca A, Maes M. Immune markers in fibromyalgia: comparison with major depressed patients and normal volunteers. Journal of Affective Disorders 1998; 48: 75-82.
24. Hattori Y, Akimoto K, Nakanishi N, Kasai K. Glucocorticoid regulation of nitric oxide and tetrahydrobiopterin in a rat model of endotoxic shock. Biochem Biophys Res Commun 1997; 240: 298-303.
25. O'Toole SM, Chiappelli F, Rubin RT. Plasma neopterin in major depression: relationship to basal and stimulated pituitary-adrenal cortical axis function. Psychiatry Research 1998; 79: 21-29.
26. Garbutt JC, Duch DS, Nichol CA, Woolf JH. Urinary biopterin and neopterin excretion and pituitary-adrenal activity in psychiatric patients. Psychiatry Research 1985; 16: 181-187.
27. Mataga N, Imamura K, Watanabe Y. 6R-Tetrahydrobiopterin perfusion enhances dopamine, serotonin, and glutamate outputs in dialysate from rat striatum and frontal cortex. Brain Research 1991; 551: 64-71.
28. Muguruma K, Imamura K, Watanabe Y. Repeated peripheral administration of 6R-L-erythro-5,6,7,8-tetrahydrobiopterin alters the binding activities of 5-HT1A and 5-HT2 receptors in rat brain. Biochemical and biophysical research communications 1996; 227: 794-799.
29. Blau N, de Klerk JBC, Thöny B, Heizmann CW, Kierat L, Smeitink JAM, Duran M. Tetrahydrobiopterin loading test in xanthine dehydrogenase and molybdenum cofactor deficiencies. Biochemical and molecular medicine 1996; 58: 199-203.

30. Blaskovics M, Giudici TA. A new variant of biopterin deficiency. New Engl J Med 1988; 319: 1611-12.
31. Kaufman S. Genetic disorders involving recycling and formation of tetrahydrobiopterin. Adv in Pharmacology 1998; 42: 41-43.
32. Pogson D. Issues for consideration in dihydropteridine reductase (DHPR) deficiency: a variant form of hyperphenylalaninaemia. Journal of intellectual disability research 1997; 41: 208-214.
33. Williams AC. Dopamine, dystonia, and the deficient co-factor. The Lancet 1995; 345: 1130.
34. Curtius H-Ch, Müldner H, Niederwieser A. Tetrahydrobiopterin: efficacy in endogenous depression and Parkinson's disease. J Neural Transmission 1982; 55: 301-308.
35. Morar C, Whitburn SB, Blair JA. Tetrahydrobiopterin metabolism in senile dementia of Alzheimer type. Journal of Neurology, Neurosurgery, and Psychiatry 1983; 46: 582-590.
36. Kay AD, Milstien S, Kaufman S, Creasey H, Haxby JV, Cutler NR, Rapoport SI. Cerebrospinal fluid biopterin is decreased in Alzheimer's disease. Arch Neurol 1986; 43: 996-999.
37. Blair, J.A., Barford, P.A., Morar, C., Pheasant, A.E., Hamon, C.G., Whitburn, S.B., Leeming, R.J., Reynolds, G.P., Coppen, A. Tetrahydrobiopterin metabolism in depression. Lancet 1984; 2: 163.
38. Coppen A, Swade C, Jones SA, Armstrong RA, Blair JA, Leeming RJ. Depression and tetrahydrobiopterin: the folate connection. Journal of Affective Disorders 1989; 16: 103-107.
39. Hashimoto, R., Ozaki, N., Ohta, T., Kasahara, Y., Kaneda, N., Nagatsu, T. The plasma tetrahydrobiopterin levels in patients with affective disorders. Biological Psychiatry 1990; 28: 526-528.
40. Anderson, D.N., Abou-Saleh, M.T., Collins, J., Hughes, K., Cattell, R.J., Hamon, C.G., Blair, J.A., Dewey, M.E. Pterin metabolism in depression: an extension of the amine hypothesis and possible marker of response to ECT. Psychological Medicine 1992; 22: 863-9.
41. Abou-Saleh, M.T., Anderson, D.N., Collins, J., Hughes, K., Cattell, R.J., Hamon, C.G., Blair, J.A. The role of pterins in depression and the effects of antidepressive therapy. Biological Psychiatry 1995; 38: 458-63.
42. Hoekstra R, van den Broek WW, Fekkes D, Bruijn JA, Mulder PGH, Pepplinkhuizen L. Effect of ECT treatment on biopterin and large neutral amino acids in severe, medication resistant depression. Psychiatry Research 2001; 103 (2-3): 115-123.
43. Knapp, S., Irwin, M. Plasma levels of tetrahydrobiopterin and folate in major

depression. Biological Psychiatry 1989; 26: 156-162.
44. Duch, D.S., Woolf, J.H., Nichol, C.A., Davidson, J.R., Garbutt, J.C. Urinary excretion of biopterin and neopterin in psychiatric disorders. Psychiatry Research 1984; 11: 83-9.
45. Kaufman S, Kapatos G, McInnes RR, Schulman JD, Rizzo WB. Use of tetrahydropterins in the treatment of hyperphenylalaninemia due to defective synthesis of tetrahydrobiopterin: evidence that peripherally administered tetrahydropterin enter the brain. Pediatrics 1982; 70: 376-380.
46. Levine RA, Zoephel GP, Niederwieser A, Curtius H-C. Entrance of tetrahydropterin derivatives in brain after peripheral administration: effect on biogenic amine metabolism. Journal of Pharmacology and Experimental Therapeutics 1987; 242: 514-522.
47. Fernell E, Watanabe Y, Adolfsson I, Tani Y, Bergström M, Hartvig P, Lilja A, von Knorring A-L, Gillberg C, Långström B. Possible effects of tetrahydrobiopterine treatment in six children with autism - clinical and positron emission tomography data: a pilot study. Developmental Medicine & Child Neurology 1997; 39: 313-318.
48. Sakai T, Antoku Y, Matsuishi T, Iwashita H. Tetrahydrobiopterin double-blind, crossover trial in Machado-Joseph disease. Journal of Neurological Sciences 1996; 136: 71-72.
49. Curtius H-Ch, Niederwieser A, Levine RA, Lovenberg W, Woggon B, Angst J. Successful treatment of depression with tetrahydrobiopterin. Lancet 1983; 19: 657-658.
50. Fleischhacker WW, Meise U, Schubert H. Re-evaluation of antidepressant effect of tetrahydrobiopterine. Lancet 1985; 17: 387.
51. Aziz AA, Leeming RJ, Blair JA. Tetrahydrobiopterin metabolism in senile dementia of Alzheimer type. J Neurol Neurosurg Psychiatry 1983;46:410-413.
52. Nagatsu T, Yamaguchi T, Rashman MK, Trocewicz J, Oka K, Hirata Y, Nagatsu I, Narabayashi H, Kondo T, Iizuka R. Catecholamine-related enzymes and the biopterin cofactor in Parkinson's disease and related extrapyramidal diseases. Adv Neurol 1984;40:467-473.
53. Bottiglieri T, Hyland K, Laundy M, Godfrey P, Carney MWP, Toone BK, Reynolds EH. Folate deficiency, biopterin and monoamine metabolism in depression. Psychological Medicine 1992;22:871-876.
54. Van Kammen DP, Levine R, Sternberg D. Preliminary evaluation of hydroxylase cofactor in human spinal fluid: potential biochemical and clinical relevance for psychiatric disorders. Psychopharmacology Bulletin 1978;14:51-52.
55. Kellner CH, Rakita RM, RubinowDA, Gold PW, Ballenger JC, Post RM. Tetrahydrobiopterin levels in cerebrospinal fluid of affectively ill patients. Lancet 1980;ii:55-56.

Dankwoord

Dankwoord

Dit onderzoeksproject is uitgevoerd in de psychiatrische praktijk van alledag. Gegevens zijn verzameld en bewerkt op meerdere afdelingen in verschillende ziekenhuizen. Ik heb daardoor samengewerkt met veel mensen van allerlei disciplines, die allen op eigen wijze hun steentje hebben bijgedragen. Ik ben veel dank verschuldigd aan diegenen die mij behulpzaam zijn geweest, zowel door heel praktisch de helpende hand te bieden, maar ook door telkens weer oprechte belangstelling voor mijn bezigheden aan de dag te leggen.

Een speciaal woord van dank wil ik richten tot de patiënten die deelgenomen hebben aan mijn onderzoek. Het is heel stimulerend om zo'n grote bereidwilligheid te ontmoeten van mensen die in vaak nare omstandigheden verkeren.

Een aantal personen wil ik in het bijzonder noemen.

Prof. Dr. Verhoeven, mijn promotor. Beste Willem, jouw inbreng heeft op het juiste moment de benodigde stimulans en richting gegeven om de onderzoeksdata daadwerkelijk in een proefschrift te laten uitmonden. Je efficiënte en gedreven kritiek heb ik zeer gewaardeerd.

Dr. Fekkes, mijn copromotor. Beste Durk, al vele jaren werken we op een plezierige wijze samen. Jouw consciëntieuze, kritische blik heeft mij scherp gehouden.
Dr. Tuinier, mijn copromotor. Beste Siegfried, jij bent de stille kracht van dit proefschrift. Vele uren heb je doorgebracht om mijn manuscripten helder en to-the-point te krijgen.

Prof. Dr. Hengeveld, Prof. Dr. Drexhage en Prof. Dr. Kahn, leden van de kleine promotiecommissie. Hartelijk dank voor het zorgvuldig doorlezen van het manuscript.

Prof. Dr. Loonen. Beste Anton, jij bent zeer behulpzaam geweest bij het creëren van de nodige ruimte voor het onderzoek in het Delta Psychiatrisch Centrum.
Prof. Dr. Pepplinkhuizen. Beste Lolke, jouw enthousiasme en overvloedig toewerpen van ideeën, vooral in de eerste fase van mijn promotietraject, zijn zeer inspirerend geweest.

Een belangrijk deel van dit proefschrift is tot stand gekomen in het Delta Psychiatrisch Centrum. Veel patiënten zijn geïncludeerd vanuit de lithiumpolikliniek van het MFC Rotterdam-Zuid, waar ik jarenlang met veel plezier heb gewerkt. Alle medewerkers van het Delta Psychiatrisch Centrum, die op enigerlei wijze hebben bijgedragen aan dit proefschrift wil ik hartelijk danken.

Dit dankwoord geeft mij de gelegenheid Dr. Robert Agema te noemen, die mij geïnspireerd heeft en mij liet kennismaken met de psychiatrie. Ook wil ik graag noemen Prof. Dr. Max Kros, die mij heeft besmet met de gedrevenheid voor de neurowetenschappen en liet zien dat dat ook met de nodige humor gepaard kan gaan.

Mijn ouders, familie en vrienden dank ik voor hun liefde en steun. In het bijzonder wil ik nog danken Angelo Quinti, die bij nacht en ontij mij bijstond bij computerproblemen.

Tenslotte, het allergrootste woord van dank voor de Liefde van mijn leven. Lieve Cindy, jij bent mijn steun en toeverlaat. Zonder jou zou ik nooit in staat zijn dat te doen waar mijn hart ligt. Jij zorgt voor de fijne thuisbasis, waar we genieten met onze prachtige dochters, Maxime en Carmen.

Curriculum Vitae

Curriculum Vitae

Rocco Hoekstra is geboren op 29 juni 1968 te Vlaardingen. Hij behaalde het gymnasium-ß diploma in 1986 aan R.K.S.G. Spieringshoek te Schiedam. Van 1986 tot 1990 studeerde hij geneeskunde aan de Erasmus Universiteit Rotterdam. In januari 1993 behaalde hij het artsexamen.

Tijdens zijn studie maakte hij kennis met wetenschappelijk werk op de afdeling Neuropathologie van de Erasmus Universiteit Rotterdam van Prof. Dr. J.M. Kros. Daaruit vloeide een keuze-onderzoekstage in het Bellaria Ospedale in Bologna, Italië.

In 1993 was hij als arts-assistent werkzaam bij het toenmalige RNO te Rotterdam, op de afdeling acute en sociale psychiatrie (opleider R.B. Laport).

Van 1995 tot 1996 was hij werkzaam als arts-assistent op de afdeling neurologie van A.Z.R. Dijkzigt, het huidige Erasmus Medisch Centrum te Rotterdam.

Vanaf 1996 werd hij opgeleid tot psychiater in A.Z.R. Dijkzigt (opleider: Prof. Dr. W.J. Schudel). In 2000 volgde registratie in het specialistenregister.

Van 2000 tot 2006 was hij werkzaam in het MFC Rotterdam-Zuid, onderdeel van Delta Psychiatrisch Centrum, waar hij zich in het bijzonder toelegde op de diagnostiek en behandeling van de bipolaire stoornis. Sinds 2006 is hij behandeleindverantwoordelijk psychiater van het cluster MFC Spijkenisse, ook van Delta Psychiatrisch Centrum, een afdeling die zich richt op de behandeling van angst- stemmings- en persoonlijkheidsstoornissen.

Naast het onderhavige biologische onderzoek bij stemmingsstoornissen, heeft hij als aandachtsgebied het gebruik van nieuwe media, zoals het Internet, in de psychiatrische patiëntenzorg, waarover hij publiceerde en lezingen hield.

Tevens heeft hij verschillende beleidsfuncties bekleed. Op dit moment is hij onder andere lid van de commissie voorlichting van de Nederlandse Vereniging voor Psychiatrie en bestuurslid van de Stichting Lithiumpluswerkgroep.

Hij is getrouwd met Cindy en heeft 2 lieve dochters, Maxime en Carmen.

List of publications

List of publications

papers

- A comparison between the diagnostic value of measurement of gonadotropins, α-subunit and chromogranin-A and their responses to TRH in clinically non-functioning, α-subunit secreting and gonadotroph pituitary adenomas. F.R.E. Nobels, D.J. Kwekkeboom, W. Coopmans, R. Hoekstra, W.W. de Herder, R. Bouillon, S.W.J. Lamberts. Journal of Clinical Endocrinology and Metabolism 1993; 77 (3): 784-789.
- Het grijze gebied tussen gedwongen en vrijwillige opnames. Een verkenning vanuit de dagelijkse praktijk van de acute psychiatrie. R. Hoekstra, P.K.J. Ronhaar, R.B. Laport. Tijdschrift voor Psychiatrie 1996; 38 (10): 713-722.
- Vervolgbehandeling met elektroconvulsietherapie bij medicatieresistente depressie. M. Gieteling, R. Hoekstra, WW. van den Broek, J.A. Bruijn. Nederlands Tijdschrift voor Geneeskunde 1998; 142 (38): 2110-2.
- Cybertherapie? R. Hoekstra, B.J.M. van de Wetering. Tijdschrift voor Psychiatrie 1999; 41(5): 293-297.
- Effect of electroconvulsive therapy on biopterin and large neutral amino acids in severe, medication-resistant depression. R. Hoekstra, W.W. van den Broek, D. Fekkes, J.A. Bruijn, P.G.H. Mulder, L. Pepplinkhuizen. Psychiatry Research 2001; 103: 115-123.
- Pteridines and affective disorders. R. Hoekstra and D. Fekkes. Acta Neuropsychiatrica 2002; 14:120-126.
- The effect of light therapy on biopterin, neopterin and tryptophan in patients with seasonal affective disorder. R. Hoekstra, D. Fekkes, B.J.M. van de Wetering, L. Pepplinkhuizen, W.M.A. Verhoeven. Psychiatry Research 2003; 120: 37-42
- E-mailcontact op een polikliniek psychiatrie. R. Hoekstra. Tijdschrift voor Psychiatrie 2003; 45 (7): 449-453.
- Bipolar mania and plasma amino acids: increased levels of glycine. R. Hoekstra, D. Fekkes, A.J.M. Loonen, L. Pepplinkhuizen, S. Tuinier, W.M.A. Verhoeven. European Neuropsychopharmacology 2006; 16: 71-77.
- Nitric oxide and neopterin in bipolar affective disorder. R. Hoekstra, D. Fekkes, L. Pepplinkhuizen, A.J.M. Loonen, S. Tuinier, W.M.A. Verhoeven. Neuropsychobiology 2006; 54: 75-81.

abstracts

- BOPZ en de criteria voor dwangopnames. P.K.J. Ronhaar, R. Hoekstra, R.B. Laport. Samenvattingen Voorjaarscongres van de Nederlandse Vereniging voor Psychiatrie 1994: 90.
- Internet en een polikliniek psychiatrie, nieuwe kansen? R. Hoekstra, T. Mirck, H. v.d. Knaap,

E. v.d. Most. Samenvattingen Voorjaarscongres van de Nederlandse Vereniging voor Psychiatrie 2002: 146.

- BIRD: Bipolar disorder Retrospective Data. Switching antipsychotics in patients with bipolar disorder: reasons and outcome. B. Lepage, R. Hoekstra, H. Morre. European Neuropsychopharmacology 2003, 13 (suppl. 4): 288-289.
- Biopterine en de bipolaire manie. R. Hoekstra, D. Fekkes, A.J.M. Loonen, W.M.A. Verhoeven. Samenvattingen Voorjaarscongres van de Nederlandse Vereniging voor Psychiatrie 2004: 206.
- Biopterin and amino acids in bipolar mania. R. Hoekstra, D. Fekkes, A.J.M. Loonen, W.M.A. Verhoeven. European Psychiatry 2004, 19 (suppl. 1): 202.
- Improved efficacy and tolerability following direct switch from conventional depot neuroleptics to risperidone long-acting injectable. R. Hoekstra, R. Teijero, A. Lex, V. Curtis. International Journal of Neuropsychopharmacology 2004; 7 (S2).
- Direct treatment change to risperidone long-acting injectable led to improved efficacy and safety in the dutch subgroup of the stormi trial. W.J. Arnoldussen, R. Teijeiro, R. Hoekstra, H.H.W. Janssen, C.T.M. Melman. International Journal of Neuropsychopharmacology 2004; 7 (S2).
- Glutamate and glycine in bipolar mania. R. Hoekstra, D. Fekkes, A.J.M. Loonen, L. Pepplinkhuizen, W.M.A. Verhoeven. European Psychiatry 2005; 20 (suppl. 1).
- Glutamaat en glycine bij bipolaire stoornissen. R. Hoekstra, D. Fekkes, A.J.M. Loonen, L. Pepplinkhuizen, W.M.A. Verhoeven. Samenvattingen Voorjaarscongres van de Nederlandse Vereniging voor Psychiatrie 2005: 234-235.
- Glycine in bipolar mania. R. Hoekstra, D. Fekkes, A.J.M. Loonen, S. Tuinier, W.M.A. Verhoeven. The World Journal of Biological Psychiatry 2005; 6 (suppl. 1): 185.
- Pterins and nitric oxide in bipolar mania. R. Hoekstra, D. Fekkes, A.J.M. Loonen, W.M.A. Verhoeven. European Neuropsychopharmacology 2005; 15 (suppl. 3): 403.
- Citrulline-arginine ratio in affective disorders. European Psychiatry 2006; 21 (suppl. 1): 195-196.
- Stikstofoxide en stemmingsstoornissen. Samenvattingen Voorjaarscongres van de Nederlandse Vereniging voor Psychiatrie 2006: 220.
- Treatment with ECT is associated with an increase of nitric oxide. R. Hoekstra, D. Fekkes, A.J.M. Loonen, S. Tuinier, W.M.A. Verhoeven. European Psychiatry 2007; 22: S174.
- Pterins and nitric oxide in the pathophysiology of affective disorders. R. Hoekstra, D. Fekkes, A.J.M. Loonen, S. Tuinier, W.M.A. Verhoeven. Nordic Journal of Psychiatry 2007: 18

overig

- Manisch-depressieve stoornis. Juni 2005. Website aan de hand van het boek 'Als je geest een vuurpijl is' van Hans Kamp, in de serie Spreekuur Thuis.