Depression, antidepressants, and peripheral blood components

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Abstract

The biological attributes of affective disorders and factors which are able to predict a response to treatment with antidepressants have not been identified sufficiently. A number of biochemical variables in peripheral blood constituents have been tested for this purpose, as a consequence of the lack of availability of human brain tissue. At first, the biological attributes of mental disorders were sought at the level of concentrations of neurotransmitters and their metabolites or precursors. Later on, attention shifted to receptor systems. Since the 1990s, intracellular processes influenced by an illness or its treatment with psychopharmaceuticals have been at the forefront of interest. Interest in biological predictors of treatment with antidepressants has reappeared in recent years, thanks to new laboratory techniques which make it possible to monitor cellular processes associated with the transmission of nerve signals in the brain. These processes can also be studied in plasma and blood elements, especially lymphocytes and platelets. The selection of the qualities to which attention is paid can be derived from today's most widely discussed biochemical hypotheses of affective disorders, especially the monoamine hypothesis and the molecular and cellular theory of depression. Mitochondrial enzymes can also play an important role in the pathophysiology of depression and the effects of antidepressants. In this paper, we sum up the cellular, neurochemical, neuroendocrine, genetic, and neuroimmunological qualities which can be measured in peripheral blood and which appear to be indicators of affective disorders, or parameters which make it possible to predict therapeutic responses to antidepressant administration.

INTRODUCTION. Affective disorders are a very common illness with a recurrent or chronic course. Not enough is known about the pathophysiology of depression (Fava & Kendler, 2000; Nestler et al. 2002). Without a doubt, genetic factors, as well as individual sensitivity to the depressogenic effects of unfavourable living situations, contribute to the risk of developing the illness. Treatment with antidepressants, electroconvulsive therapy, or psychotherapy is mostly efficient but some patients do not respond to it sufficiently (Fava & Rush, 2006). Currently there is no reliable biochemical, genetic, or other biological test which would make it possible to diagnose a depressive disorder and its subtypes, or which would make it possible to predict the success of pharmacotherapy. Research into this field focuses on the search for biological markers which can be measured in peripheral blood and indicate the affective disorders or effects of antidepressants. It also focuses on measuring *in vivo* changes and processes in the brain using non-invasive neurophysiological or structural and functional imaging methods.

REVIEW

Abbreviations

AC – adenylate cyclase
ACTH – adrenocorticotropic hormone
AIF – apoptosis-inducing factor
AMPT test – *α*-methyl-*p*-tyrosine test
APTD test – acute phenylalanine/tyrosine depletion test
ATD test – acute tryptophan depletion test
ATP – adenosine 5′-triphosphate
Bcl-2 protein – anti-apoptotic member of the Bcl-2 family proteins (Bcl-2 is acronym for B-cell lymphoma/leukemia-2)
BDNF – brain-derived neurotrophic factor
Ca^{2+}/CaM PK – calcium/calmodulin-dependent protein kinase
cAMP – cyclic adenosine monophosphate
CAR – constitutive androstane receptor
CNS – central nervous system
COMT – catechol-*O*-methyltransferase
CREB protein – cAMP response element-binding protein
CRF – corticotropin-releasing factor
CSF – cerebrospinal fluid
DA – dopamine
DAG – diacylglycerol
DST – dexamethasone suppression test
ECT – electroconvulsive therapy
G protein – guanine nucleotide-binding protein
G × E – gene-by-environment interaction
GSK-3β – [glycogen synthase]kinase-3β
HPA axis – hypothalamic-pituitary-adrenal axis
HPT axis – hypothalamic-pituitary-thyroid axis
5-HT – serotonin, 5-hydroxytryptamine
HVA – homovanillic acid
5-HIAA – 5-hydroxyindoleacetic acid
IDO – indoleamine 2,3-dioxygenase
IP – inositol monophosphate
IP_3 – inositol-1,4,5-triphosphate
K_M – apparent Michaelis constant
MAPK – mitogen-activated protein kinase
MARCKS – myristoylated alanine-rich protein kinase C substrate
MAO – monoamine oxidase
MHPG – 3-methoxy-4-hydroxyphenylglycol
MT receptor – melatonin receptor
MTP pores – mitochondrial permeability transition pores
NE – norepinephrine
NGF – nerve growth factor
NO – nitric oxide
PCPA test – *p*-chlorophenylalanine test
PET – positron emission tomography
PKA – type A protein kinase
PKC – type C protein kinase
PLC – type C phospholipase
PXR – pregnane X receptor
PUFA – polyunsaturated fatty acid
R-G_s, R-$G_{q/11}$ – G protein-coupled receptors
SPECT – single photon emission computed tomography
SERT – serotonin transporter
SSRI – selective serotonin reuptake inhibitor
T4 – thyroxine
TRF – thyrotropin-releasing factor
TrkB – high affinity catalytic receptor for several neurotrophins (incl. BDNF)
TSH – thyroid-stimulating hormone (thyrotropin)
TPH – tryptophan hydroxylase
V_{max} – apparent maximal velocity
VPA – valproate

1. MODEL SYSTEMS FOR MEASURING BRAIN BIOCHEMISTRY

Neuropsychiatric disorders are caused or accompanied by changes in the transmission of nerve signals in the brain, especially changes in the development and propagation of action potentials and their transduction via chemical synapses. At the molecular level, neurotransmitters and their receptors, neurotransmitter transporters, ion channels, enzymes involved in neurotransmitter synthesis and metabolism, and intracellular processes related to receptor activation are especially involved in these disorders. The basic methodological challenge of biological psychiatry is based on the fact that we do not know how to relate accurately and selectively the currently known biochemical phenomena in the central nervous system (CNS) to the symptoms of mental disorders or the mechanisms of their origin and treatment.

While formulating and verifying the hypotheses regarding the molecular mechanisms which are related to the genesis or treatment of **affective disorders**, we especially follow on from observations of the neurochemical effects of **antidepressants** and other psychotropic drugs. Despite the relatively large quantity of findings from more than fifty years of studying the mechanisms of the effects of antidepressants, there are no biochemical, neuroendocrine, or genetic tests which would make it possible to diagnose the illness reliably, to classify their subtypes, or to predict the suitability and efficiency of pharmacotherapy. The causes of dissimilar individual responses to pharmacotherapy are also not sufficiently known. New findings from the fields of psychiatry, neurology, and neurochemistry, which are especially based on biochemical, molecular biology, genetic and imaging methods, have spurred on efforts to find procedures which will make it possible to determine the subtypes of the illnesses and predict the effects of psychotropic drugs.

For obvious ethical reasons, the effects of antidepressants on brain processes are mainly studied through model cellular systems and experimental animals. Positron emission tomography (PET) and single photon emission computed tomography (SPECT) have started to be used recently for the visualisation and *in vivo* measurement of the function of neurotransmitter systems in the brains of depressed patients. For instance, using suitable ligands, it is possible to study the synthesis of neurotransmitters and their receptors and transporters (D´haenen, 2001). Magnetic resonance spectroscopy makes it possible to study the chemistry of the brain *in vivo*. However, the potential of structural and functional imaging methods is not sufficient for monitoring the processes which take place at the cellular level. This explains the ongoing search for suitable parameters that are measurable in samples of cerebrospinal fluid (CSF), urine, saliva, and, especially, **peripheral blood**, which would reflect the changes in biochemical processes in the brain related to a depressive disorder or the effects of antidepressants. For a long time, we have dealt with the possibilities offered by **plasma, red blood cells** (erythrocytes), **white blood cells** (leukocytes), and **platelets**

(thrombocytes). Of course, neuroimmunological studies also investigate the functions of monocytes and individual types of lymphocytes or granulocytes.

2. EFFECTS OF ANTIDEPRESSANTS ON SYNAPTIC SIGNAL TRANSDUCTION

The biological attributes of affective disorders are sought on the basis of comparing the values of many biochemical parameters in healthy and ill persons, and especially on the basis of knowledge of the effects of antidepressants on synaptic signal transduction. Usually, antidepressants primarily act as inhibitors of serotonin (5-HT) or norepinephrine (NE) reuptake, or as inhibitors of monoamine neurotransmitter catabolism. Their administration causes an increase in synaptic concentrations of NE and 5-HT and results in receptor adaptation and changes in the activity of the components which are involved in intracellular signal transmission.

At first, attention was focused on the synthesis, metabolism, and membrane transport of monoamine neurotransmitters, especially 5-HT, NE, and dopamine (DA). Later, it shifted to changes in the density and sensitivity of neurotransmitter receptors, which correlate better with the delayed onset of the therapeutic effects of antidepressants (2–3 weeks). However, no receptor changes which would be common for all antidepressants have been found. Electrophysiological methods have confirmed that various classes of antidepressants and electroconvulsive therapy (ECT) facilitate serotonin neurotransmission in the brain with the use of different mechanisms (Dremencov et al. 2002, 2003). In addition, no significant progress has been made in the development of new, more effective drugs for affective disorder treatment. These facts led research to focus on the role of intracellular pathways in the pathophysiology and treatment of affective disorders.

The long-term administration of antidepressants and mood stabilizers is assumed to be associated with the increased synthesis of neurotrophic factors which inhibit or eliminate neurodegenerative processes caused by chronic stress or depression (Figure 1). Intracellular changes which activate the transcription factors which become activated in response to the increase in cAMP level (cAMP response element-binding proteins, **CREB proteins**) and subsequent gene expression of brain-derived neurotrophic factor (**BDNF**) and its receptors (Duman et al. 1997; Schloss & Henn, 2004). Therefore, the possibility that the factors involved in the atrophy of neurons and their survival can be the goals of antidepressive treatment is being investigated. CREB and BDNF are upregulated in response to various antidepressants, including noradrenergic, selective serotonin reuptake inhibitors (SSRI) and ECT (Bocchio-Chiavetto et al. 2006). Therefore, the results support the hypothesis that treatment with antidepressants leads to neurotrophic-like effects.

Therapeutic concentrations of mood stabilizers, e.g. lithium and valproate, also strongly activate neurotrophic signal cascade and other signal pathways and transcription factors. Currently, the activation of cytoprotective **Bcl-2** protein and [glycogen synthase]kinase-3β (**GSK-3β**) inhibition by lithium or the inhibitions of GSK-3β and histone deacetylase by valproate (Zarate et al. 2006; Dong et al. 2007) are the primary objects of discussion. The principal change in the current neurobiological approach to affective disorders follows on from the assumption that they are associated with neurochemical changes, as well as the disturbance of the structural plasticity and cellular resilience of brain cells.

3. BIOCHEMICAL HYPOTHESES OF AFFECTIVE DISORDERS

Concurrently with understanding the mechanisms of the action of antidepressants, biochemical hypotheses of affective disorders (Fišar, 1998) were generated, from neurotransmitter hypotheses to postreceptor ones. Available knowledge about the intracellular processes related to affective disorders and the long-term effects of antidepressants indicates that signal pathways which are primarily activated by monoamine neurotransmitters play a significant role. According to the classic **monoamine hypothesis** of depression, reduced concentrations of monoamine neurotransmitters (5-HT, NE, DA) in CNS represent the pathophysiological basis of depression. The shortcomings of this hypothesis involved unexplained causes and processes leading to a reduction in the availability of these neurotransmitters. An **advanced monoamine theory** (Meyer et al. 2006) has been proposed, according to which increased levels of monoamine oxidase type A (MAO-A) can be regarded as a general process which reduces brain monoamines (without a relation to certain symptoms of depression), while the regional density of monoamine transporters has a selective influence on individual monoamines (with a strong relation to certain symptoms).

Current hypotheses assume a connection between the onset of a depressive disorder and the disturbance of neurotransmission as a consequence of the disturbance of the mechanisms controlling the plasticity of neurons and their survival in certain brain areas. According to the **molecular and cellular theory of depression** (Duman et al. 1997; Duman, 2002), this damage can be caused by hypoxia, toxic substances, or the influence of stress, and is accompanied by reduced concentrations of the growth factor BDNF. The long-term administration of antidepressants then increases the expression of BDNF and its receptor TrkB by increasing the function of the serotonergic or noradrenergic system. Genetic vulnerability to depression can then be related to changes in monoaminergic neurotransmission, as well

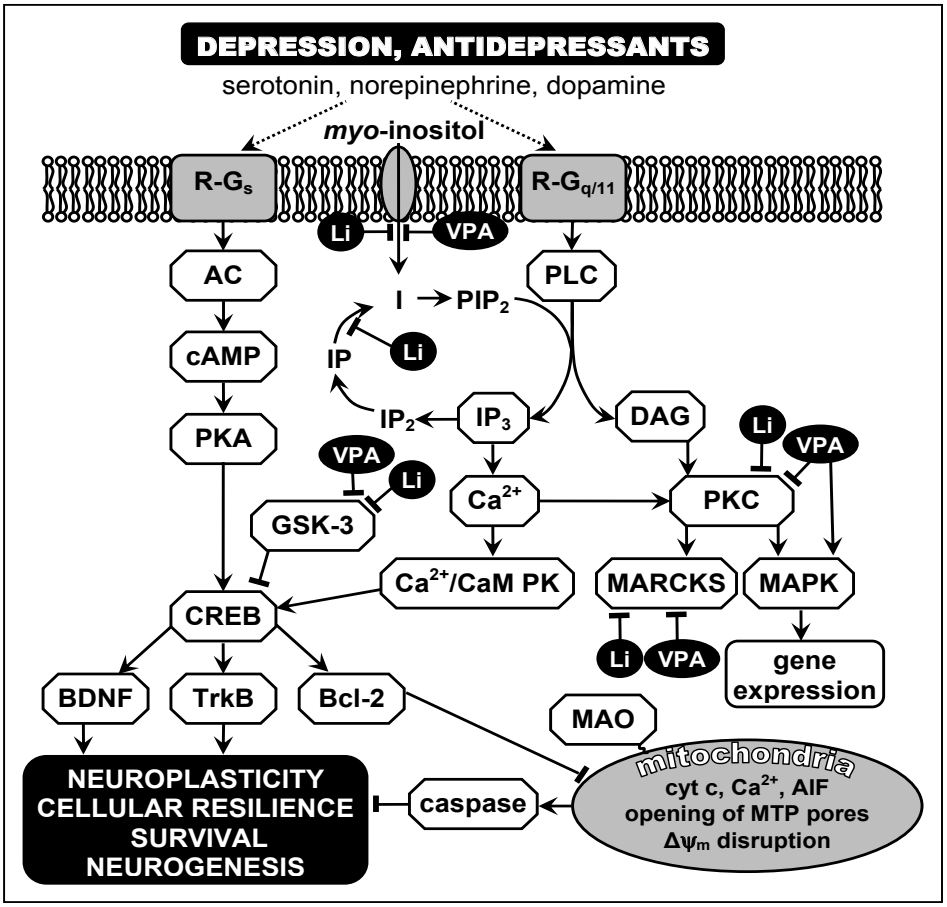

Figure 1. Neurotrophic and neuroprotective effects of antidepressants and mood stabilizers.

The administration of antidepressants stimulates an increase in synaptic concentrations of monoamine neurotransmitters, which consequently activates specific receptors and affects intracellular pathways. It is supposed that the common therapeutic effects of antidepressants are determined by intracellular processes leading to the activation of the cAMP response element-binding (CREB) proteins and a subsequent increase in the gene expression of brain-derived neurotrophic factor (BDNF) and its receptor (TrkB). Mood stabilizers, e.g. lithium (Li) and valproate (VPA), also influence signal transduction cascades that underlie the actions of neurotrophic factors, including the phosphatidylinositol-3 kinase pathway and the mitogen-activated protein kinase (MAPK) cascade. Lithium is a noncompetitive inhibitor of inositol monophosphatase (IP), which converts IP_3 to myo-inositol. Furthermore, VPA and lithium reduce the transport of myo-inositol into cells. Lithium inhibits [glycogen synthase]kinase-3β (GSK-3β) in the Wnt signalling pathway and upregulate antiapoptotic factor Bcl-2. Valproate activates the MAPK cascade and inhibits GSK-3β. These processes leads to neuroprotective effects and support for neuroplasticity, neurogenesis, and cellular resilience via the regulation of various signal pathways in a cell and via changes in the gene expression of the proteins involved in the mechanisms of apoptosis and synaptic plasticity. Bcl-2, a target of CREB, attenuates processes which lead to cellular death or neuronal atrophy by sequestering caspases, by preventing the release of mitochondrial apoptogenic factors such as calcium, cytochrome c (cyt c), and apoptosis-inducing factor (AIF) into the cytoplasm, by enhancing mitochondrial calcium uptake, by inhibiting the opening of mitochondrial permeability transition (MTP) pores and by inhibiting mitochondrial membrane potential $\Delta\psi_m$ disruption.

→: activation; ⊣: inhibition; R-G_s, R-$G_{q/11}$: G protein-coupled receptors; AC: adenylate cyclase; cAMP: cyclic adenosine monophosphate; PKA: type A protein kinase; TrkB: high affinity catalytic receptor for several neurotrophins; PLC: type C phospholipase; IP_3: inositol-1,4,5-triphosphate; DAG: diacylglycerol; PKC: type C protein kinase; MARCKS: myristoylated alanine-rich protein kinase C substrate; Ca^{2+}/CaM PK: calcium/calmodulin-dependent protein kinase; MAO: monoamine oxidase.

as the reduced production of growth factors which act during brain development (Wurtman, 2005).

An interesting bioenergetic and neurochemical model of bipolar illness can be found in the **hypothesis of mitochondrial dysfunction in bipolar disorder** (Stork & Renshaw, 2005) that involves impaired oxidative phosphorylation, a resultant shift toward glycolytic energy production, a decrease in total energy production or substrate availability, and altered phospholipid metabolism. So, the energy production of the synapses during a depressive disorder and its treatment with antidepressants is strongly influenced and the mitochondria may be the modulators responsible for the efficiency or non-efficiency of different antidepressants. Besides the fact that the mitochondria are generators of chemical energy for cells, they also carry MAO on their surface and so they influence the intracellular metabolism of many neurotransmitters (including 5-HT, NE and DA) and antidepressants.

At the same time, we must not ignore the role of membrane lipids in the proper functioning of the neuronal membranes, because the existence of membrane "rafts", the presence of arachidonic acid, docosahexaenoic acid, and cholesterol, and the activity of phospholipases seem to be important for signal transduction (Stillwell et al. 2005). Depressive disorders are associated with the depletion of ω-3 polyunsaturated fatty acids (PUFAs) and an elevated ratio of ω-6/ ω-3 PUFAs (Edwards et al. 1998; Peet et al. 1998; Sublette et al. 2006). **Membrane hypotheses** assume that changes in membrane lipids can induce changes in lipid-protein interactions and, as a consequence, also in various neurotransmitter systems which are thought to be related to the pathophysiology of depression. It can be speculated that the distribution of antidepressants between plasma and membranes can be used as one of the parameters for studying interindividual differences in response to pharmacotherapy (Fišar et al. 2006). The lipid bilayer has a marked influence on the transmembrane transport of molecules, for instance on the functioning of the serotonin transporter (SERT) in blood platelets and other cells (Scanlon et al. 2001) or on the transport of tryptophan, tyrosine (Bovier et al. 1988), and triiodothyronine (Kališová-Stárková et al. 2006) to erythrocytes. Changes in the cellular metabolism of depressed persons are indicated by reduced activity of various types of ATPases (Rybakowski & Lehmann 1994), especially Na^+K^+-ATPase (Goldstein et al. 2006b), which is modulated by interactions with membrane phosphatidylserine and PUFAs.

4. BIOLOGICAL MARKERS OF AFFECTIVE DISORDERS AND EFFECTIVENESS OF ANTIDEPRESSANT TREATMENT

There has been considerable interest in identifying biochemical markers indicative of a genetic predisposition to affective disorders ("trait markers"), as well as biochemical markers of acute depressive episode ("state markers") and predictors of the outcome of antidepressant treatment (Balon, 1989; Joyce & Paykel 1989). Biological predictors of drug response in depression are not yet sufficiently established to be of routine clinical use.

The interactions among nervous, endocrine, and immune systems have been a focus that provides a better understanding both for physiological homeostasis (Zhuang et al. 2006) and pathophysiological processes in depression. Peripheral blood constituents have been tested for parameters leading to neurochemical processes in the brain, as well as neuroendocrine, neuroimmunological, and genetic parameters (see Table 1). Significant findings have also been brought about by *in vivo* measurements of brain neurochemistry (using magnetic resonance spectroscopy), functional brain anatomy (using SPECT, PET, or functional magnetic resonance imaging), and neurophysiological measurements (electroencephalography, magnetoencephalography, polysomnography, auditory evoked potentials, and others), which can be used as noninvasive indicators of central monoaminergic function (Soares & Mann, 1997; Bob et al. 2006; Bob et al. 2007a,b; Norra, 2007; Pogarell et al. 2007). However, this article does not discuss neurophysiological approaches and their possible combinations with imaging methods, because their methodology does not include direct measurements in peripheral blood constituents.

4.1. NEUROENDOCRINE TESTS

In affective disorders, there are changes in the activity of the hypothalamic-pituitary-adrenal (HPA) or the hypothalamic-pituitary-thyroid (HPT) axis. Other hypothalamic peptidergic systems, e.g. the suprachiasmatic nucleus, supraoptic nucleus, and paraventicular nucleus, are also involved in the symptoms of major depression (Swaab et al. 2005). The interactions of these peptidergic systems with monoaminergic systems in the brain play a role also. The greatest attention is paid to the corticotropin-releasing factor (CRF), and consequently to the process in the HPA axis. In more than 50% of depressed patients, the slightly increased function of the HPA axis is, for instance, indicated by an increased concentration of CRF in CSF. The increased activity of the HPA axis in a severe depressive episode also manifests itself by a less marked reduction in plasma concentrations of **adrenocorticotropic hormone** (ACTH) and **cortisol** after the administration of dexamethasone. However, the activation of the HPA axis is a characteristic sign of chronic stress and many neuropsychiatric disorders (Stratakis & Chroustos, 1995; Höschl & Hajek, 2001). The **dexamethasone suppression test** (DST) was designed as a specific challenge test for diagnosing severe depressive episodes and is based on the fact that the administration of dexamethasone suppresses cortisol for 24 to 48 hours in healthy persons. However, it has been shown that DST is very sensitive to stress and its sensitivity in major depression is insufficient (approximately 44%) (Arana et al. 1985; Rush et al. 1996). It has been stated that the usefulness of a free-standing DST test for predicting the response to antidepressants is problematic (Balon, 1989). However, in combination with other parameters, the test may contribute to an estimation of the response to the short-term administration of antidepressants and may help distinguish several subtypes of depressive disorders (Carroll, 1985; Arana et al. 1985; Gitlin & Gerner, 1986; Rush et al. 1996; Duval et al. 2005).

It is assumed that the thyroid gland and thyroid hormones play an important role in the etiopathogenesis of major depression. Some authors recommend augmentation of antidepressant therapy with the co-administration of thyroid hormones (especially T3) in pharmacoresistant depression. Depression is not characterised by an apparent thyroid dysfunction, but subgroups of

Table 1. Potential biomarkers of affective disorders or predictors of effective treatment with antidepressants which can be measured in blood

plasma/serum	cortisol (basal concentration; DST test)
	TSH (basal concentration; TRF stimulating test)
	T4, prolactin, ACTH, melatonin
	cytokines
	serotonin, norepinephrine, dopamine
	tryptophan (basal concentration; ATD test, PCPA test)
	tyrosine (basal concentration; APTD test, AMPT test)
	5-HIAA, MHPG, normetanephrine
	concentrations of antidepressants
	BDNF
	ω-3 and ω-6 PUFA
	neopterin, tetrahydrobiopterin
	homocysteine, folic acid (folate)
platelets	SERT (transport kinetics, imipramine or paroxetine binding)
	serotonin, NO
	MAO-B
	5-HT_{2A}, α_2-adrenoceptors
	BDNF, GSK-3
	specific enzymatic defects in the mitochondrial respiratory complexes, oxygen kinetics, mutations and polymorphisms of mtDNA
lymphocytes/ leucocytes	gene polymorphisms and mutations: SERT, MAO, BDNF, TPH, COMT, cytochrome P450 enzymes, glycoprotein P, nuclear receptors PXR and CAR, neuronal NO synthase, etc.
	G proteins, β-arrestin1
	IDO
	5-HT_{1A}, β-adrenoceptors
	norepinephrine transporter
	SERT (transport kinetics; imipramine or paroxetine binding)
	CREB, BDNF, NGF, Bcl-2, GSK-3, NO
	specific enzymatic defects in the mitochondrial respiratory complexes
	pyruvate dehydrogenase, fumarate hydratase
erythrocytes	ATPase
	membrane lipids (ω-3 and ω-6 PUFA), cholesterol
	membrane transport of tryptophan, tyrosine, L-triiodothyronine
	calcium
	folic acid (folate)

DST: dexamethasone suppression test; TSH: thyroid-stimulating hormone; TRF: thyrotropin-releasing factor; T4: thyroxine; ACTH: adrenocorticotropic hormone; ATD test: acute tryptophan depletion test; PCPA test: *p*-chlorophenylalanine test; APTD test: acute phenylalanine/tyrosine depletion test; AMPT test: *α*-methyl-*p*-tyrosine test; 5-HIAA: 5-hydroxyindoleacetic acid; MHPG: 3-methoxy-4-hydroxyphenylglycol; BDNF: brain-derived neurotrophic factor; PUFA: polyunsaturated fatty acid; SERT: serotonin transporter; NO: nitric oxide; MAO: monoamine oxidase; 5-HT: serotonin; GSK-3: [glycogen synthase]kinase-3; TPH: tryptophan hydroxylase; COMT: catechol-*O*-methyltransferase; PXR: pregnane X receptor; CAR: constitutive androstane receptor; IDO: indoleamine 2,3-dioxygenase; CREB: cAMP response element-binding protein; NGF: nerve growth factor; Bcl-2: anti-apoptotic member of the Bcl-2 family proteins

depressed persons may display slight thyroid abnormalities or the activation of autoimmune processes. However, there are no conclusive data on the role of thyroid hormones in depression (Vandoolaeghe et al. 1997; Sullivan et al. 1997; Fountoulakis et al. 2006). Variations in the function of the HPT axis have been studied using a test with thyrotropin-releasing factor (TRF), in which some clinically tested depressed persons show a reduced response of **thyroid-stimulating hormone** (thyrotropin, TSH) to the administration of TRF (Arana et al. 1990). This test was also tested for predicting the response to antidepressants, but had no significant success. Recent recommendations have involved the measurement of the basal level of TSH instead of the TRF stimulating test. It is assumed that a lower basal TSH and TRF-induced TSH response can be partly attributed to increased levels of **thyroxine** (T4) (Maes et al. 1989).

For a long time, attention has been paid to those hypotheses which assume that the pathogenesis of affective disorders involves the disturbance of particular biological rhythms. **Melatonin** is widely known as a neurohormone affecting circadian rhythms and exerting multiple physiological effects (Di Bella & Gualano 2006). Measurements of melatonin in saliva, plasma or platelets, or its metabolites in urine, indicate significant changes in melatonin concentrations and circadian changes in persons during a depressive episode, especially in seasonal affective disorder. Like bright light therapy and antidepressant treatment, the administration of melatonin can also bring about clinical benefits (Lewy et al. 2006; Srinivasan et al. 2006). Antidepressive effects have been demonstrated for agomelatine, an agonist of the melatonin receptors MT_1 and MT_2 and an antagonist of 5-HT_{2C} receptors (Pjrek et al. 2007).

4.2. NEUROIMMUNOLOGICAL APPROACHES

Neurotransmitters, neuromodulators, peptide hormones, cytokines and their receptors provide a link between the nervous, the endocrine, and the immune systems. There is abundant evidence that major depression is accompanied by an immune activation, e.g. an increase in pro-inflammatory cytokines, such as interleukin 1, interleukin 6, interferon-γ, and tumor necrosis factor-α. Activation of the inflammatory response system, increased oxidative and nitrosative stress, and autoimmunity are pathophysiological mechanisms accompanying depressive disorders. Activation of the immune system involves phagocyting cells (monocytes, neutrophiles), the activation of T cells, the proliferation of B cells, changes in levels of acute phase proteins, lowered levels of negative acute phase reactants, a higher titre of antibodies (antinuclear, antiphospholipid), increased secretion of prostaglandin, disorders in exopeptidase enzymes, and increased production of several interleukins and their receptors in peripheral blood

(Maes, 1995; Maes 1999; Maes et al. 2007a). Most antidepressants reduce the production of pro-inflammatory cytokines. It is unclear whether changes in the immune system are the aetiological agent of depressive disorder or vice versa (Kitzlerová & Anders, 2007).

A change in central neurotransmission is a significant mechanism by which **cytokines** modulate behaviour. According to the macrophage theory of depression (Smith 1991), the symptoms of depression could be caused by hypersecretion of cytokines. At the same time, the induction of depression by cytokines can be determined by their influence on the serotonergic, noradrenergic, glutamatergic, and HPA systems (Wichers & Maes, 2002). For instance, pro-inflammatory cytokines activate **indoleamine 2,3-dioxygenase** (IDO), an enzyme which degrades serotonin and tryptophan, a phenomenon which plays a role in the pathophysiology of depression (Müller & Schwarz, 2007; Maes et al. 2007b).

Immune system cells can be used as appropriate model cells because they have transporters and receptors for neurotransmitters (for instance, β-adrenergic, acetylcholine, 5-HT_1, dopamine 1 and 2, and purine receptors) and antidepressants influence the transduction of cellular signals in immune system cells (Kovářů & Kovářů, 2005). Numerous findings indicate the involvement of the function of G proteins and regulators of coupled receptors in the pathophysiology and treatment of affective disorders. The elevated function of G_s and G_i proteins detected in patients during a manic episode was normalised by the administration of lithium, and their reduced function during a depressive episode was corrected by antidepressants and ECT. Measuring **G proteins** or **β-arrestin1** (which causes receptor-G protein uncoupling) in peripheral blood cells could help characterise the illness and predict clinical response to antidepressant treatments (Avissar & Schreiber, 2006).

Eicosanoids are important lipid mediators in CNS made by the oxygenation of twenty-carbon essential fatty acids, and they participate in numerous homeostatic biological functions and immune system control. This is why the concentrations of **essential fatty acids** in plasma or membranes are also monitored, because the ratio between ω-3 and ω-6 PUFAs influences many cellular functions which are related to the production of endocannabinoids (Fišar, 2006) or eicosanoids (Funk, 2001). ω-3 PUFAs are frequently used to treat depression; however, docosahexaenoic acid (22:6, n-3) induces an immune response and should be avoided in the treatment of depression (Maes et al. 2007c).

Pterins, released by macrophages, are important factors which link the immune and the nervous systems. **Neopterin** production is associated with increased degradation of tryptophan (a serotonin precursor). **Tetrahydrobiopterin** is an essential cofactor of hydroxylases of phenylalanine, tyrosine, and tryptophan (enzymes limiting the speed of biosynthesis of 5-HT, NA and DA), plasmalogen oxygenase, and nitric oxide synthases (Werner-Felmayer et al. 2002). Increased plasma concentrations of neopterin (Maes et al. 1994; Widner et al. 2002) and a reduced concentration of biopterins (van Amsterdam & Opperhuizen, 1999) were observed in patients with depressive disorders.

4.3. GENETICS AND PHARMACOGENETICS

Genetic factors contributing to the etiology of affective disorders have been studied for a long time; however, no specific genes or changes in DNA which evoke these mental disorders have been identified yet. Vulnerability to depression is only partly genetic, with non-genetic factors also being important. Nevertheless, a number of candidate genes, especially for bipolar disorder, have been found and tested (MacQueen et al. 2005; Kato, 2007a). The substantial influence of the external environment on the occurrence of an illness must be taken into consideration in molecular genetic studies of depressive disorders. A new developmental model of affective disorders is based on the role of the interactions of certain genes and the surrounding environment (hypothesis of a gene-by-environment interaction, G × E). The studies especially focus on the role of functional polymorphisms in genes encoding SERT, MAO-A, BDNF, tryptophan hydroxylase (TPH), and catechol-*O*-methyltransferase (COMT), as well as many other candidate genes (Kato, 2007a; Caspi et al. 2002; Caspi et al. 2003; Moffit et al. 2005; McClung, 2007).

COMT is an enzyme participating in the metabolism of catecholamine neurotransmitters in the brain (norepinephrine, dopamine) and it influences the functioning of the frontal lobe. The COMT gene is monitored in studies of psychoses, bipolar disorders, and many other illnesses. However, studies of the variability of the COMT gene have not confirmed any significant role for it in mental disorders (Craddock et al. 2006; Hosák, 2007).

In searching for genetic parameters related to the incidence of affective disorders and the clinical effects of the long-term administration of antidepressants, the greatest attention is paid to gene products involved in the synthesis, metabolism, and action of 5-HT, especially **SERT**, which provides for the reuptake of extracellular 5-HT (Lesch, 2001). The specific genetic locus for serotonin uptake involves the polymorphic area, and the gene for the serotonin transporter exists as a short ("s") and long ("l") allele; the short allele has been consistently associated with a lower transcription efficiency of the promoter in comparison with the long allele. A stronger effect of stressful life events on depressive symptoms was observed among the genotypes "s/s" or "s/l" in comparison to "l/l" subjects (Wurtman, 2005). At the same time, variations in the **TPH** gene, i.e. the enzyme which controls serotonin synthesis in the brain,

probably predispose individuals to affective disorders, especially suicidal behaviour (Li & He, 2006).

Pharmacogenetic approaches have tried to identify genetic factors related to the efficacy and side effects of psychotropic drugs, especially the variability of enzymes, transporters, receptors, and nuclear regulators of transcription (Ishikawa et al. 2004; Malhotra et al. 2004). These approaches provide new possibilities for detecting new biological predictors of the response to psychopharmaceuticals. Attention is paid to pharmacogenetic attributes which influence the pharmacokinetics and pharmacodynamics of antidepressants, especially the activity and polymorphisms of **cytochrome P450** drug metabolizing enzymes (Kirchheiner & Seeringer, 2007), **P-glycoprotein**, which transports many structurally unrelated compounds from cells and participates in the regulation of the distribution and availability of drugs (Thuerauf & Fromm, 2006), the nuclear receptors **PXR** or **CAR**, which identify extrinsic toxic substances and regulate the expression of the proteins which are involved in their detoxification and elimination (Stanley et al. 2006), and, last but not least, polymorphisms of neurotransmitter receptors and transporters, which are also being investigated.

4.4. NEUROCHEMISTRY

Neurochemical approaches to affective disorders follow on from the assumption that their occurrence is caused by the disturbance of signal transmission and processing in CNS, namely in the areas of chemical synapses. On the basis of the mechanisms of the effects of psychoactive substances, one can conclude that the disorder occurs especially during nerve signal transduction, i.e. when there is a change in the propagating action potential to processes which lead to the release of neurotransmitters to the synaptic cleft, with subsequent activation of pre- or post-synaptic receptors and appropriate post-receptor changes. Then the search for neurochemical markers follows on from the assumption that the disturbance of transduction processes in the brain is reflected in measurable changes in identical or similar processes and substances in peripheral blood. The interest in neurochemical markers and predictors of treatment with antidepressants has been renewed in recent years thanks to new laboratory techniques which make it possible to monitor neurotransmission-related intracellular processes. The intracellular processes can also be studied in blood elements, especially in lymphocytes and platelets.

Changes in the availability of neurotransmitters, especially **5-HT**, **NE,** and **DA**, are regarded as a significant factor accompanying the occurrence and treatment of affective disorders. Considering how difficult it is to directly measure neurochemical processes in the brain, attention is paid to changes in concentrations of breakdown products of serotonin (5-hydroxyindoleacetic acid, **5-HIAA**), norepinephrine (3-methoxy-4-hydroxyphenylglycol, **MHPG**), and dopamine (homovanillic acid, HVA) in CSF, plasma, or urine (Caldecott-Hazard et al. 1991; Goldstein et al. 2006a). Increased plasma levels of norepinephrine were observed in some persons with unipolar depression, especially in melancholic patients. These patients secrete relatively higher quantities of norepinephrine and normetanephrine (the main extraneuronal metabolite of NE) as compared to the total synthesis of catecholamines (Potter & Manji, 1994). MHPG is a principal metabolite of brain norepinephrine and was usually measured in urine as a possible predictor of the response to treatment with antidepressants. However, MHPG concentrations do not provide sufficiently reliable information, even when combined with other markers (Balon, 1989). The assumption that lower serotonergic activity in the brains of depressed patients will demonstrate itself by a reduction in 5-HIAA concentrations in CSF has not been confirmed either (Reddy et al. 1992). The results have shown that lower values of 5-HIAA concentrations in CSF and low levels of serum cholesterol are more related to violent suicidal behaviour and violent impulsive behaviour than to depression (Kunz et al. 1995; Vevera et al. 2003).

Serotonin is synthesised in the brain from **tryptophan**, while norepinephrine and dopamine are synthesised from **tyrosine**. Plasma concentrations of these precursors can be related to the efficiency and speed of the clinical response to the administration of various antidepressants (Møller et al. 1983; Møller et al. 1985). For instance, the transport (facilitated diffusion) of tryptophan and tyrosine to erythrocytes was investigated as a marker of depression (Bovier et al. 1988). The importance of precursors of monoamine neurotransmitters has been shown in monoamine depletion studies. Acute tryptophan depletion (ATD test) or of *p*-chlorophenylalanine (PCPA test) deplete 5-HT. A reduction of NE and DA concentrations occurs after acute phenylalanine/tyrosine depletion (APTD test) or because of α-methyl-*p*-tyrosine (AMPT test), an inhibitor of tyrosine hydroxylase. Challenge studies using the monoamine depletion proved a slight worsening of mood in persons with a family history of a major depressive disorder. The effect of tryptophan depletion was most marked among patients with a major depressive disorder in remission who used serotonergic antidepressants (Ruhé et al. 2007). It can be explained by the fact that the acute lowering of synaptic serotonin levels leads to a reduction in BDNF values, which then leads to a rapid reduction in the release of many neurotransmitters, which induces a depressive relapse in these patients.

COMT and **MAO** are the main enzymes which participate in the metabolism of monoamine neurotransmitters. From the point of view of the effects of antidepressants, most attention has been focused on the activity of MAO, because the inhibitors of this enzyme were one of the first antidepressants ever to have been discovered (Youdim & Bakhle, 2006). MAO is localised on the outer membrane of mitochondria and exists in

two types, A and B. MAO-A prevails in the brain (80%). It is also present in leukocytes and platelets, while only the MAO-B subtype occurs in platelets. MAO-B activity in platelets can be related to the function of the central serotonergic system (Eriksson et al. 2006) and it is studied as a possible predictor of the response to the treatment of depression. These studies are aggravated by the fact that MAO is sensitive to many antidepressants but also to nicotine and many personality attributes. The interest in MAO as a biological marker of depression or predictor of the response to treatment with antidepressants has reappeared recently thanks to the abovementioned advanced monoamine theory (Meyer et al. 2006) and the hypothesis regarding mitochondrial dysfunction (Stork & Renshaw, 2005).

The metabolism of monoamines in the brain is linked to **folic acid** (folate) and *S*-adenosyl methionine through the biopterin pathway (Thöny et al. 2000). In some cases, low concentrations of folate in the serum and red blood cells of patients with major depression were reported (Gilbody et al. 2007), while a higher initial concentration of folate predicted a greater improvement in depressive symptoms after the administration of antidepressants (Alpert et al. 2003) and the administration of folate had potentiating effects on treatment with antidepressants (D'Anci & Rosenberg, 2004). Total plasma **homocysteine** is a more sensitive indicator of a functional folate or vitamin B12 deficiency (Bottiglieri, 2005). The synthesis of methionine from homocysteine requires a supply of methyl groups from methyl folate and also vitamin B12 as a cofactor. Accordingly, a functional deficiency of one vitamin or the other results in an increased concentration of homocysteine. Methionine is the immediate precursor of *S*-adenosyl methionine, a donor of methyl groups in the brain.

SERT, a regulator of intracellular serotonin uptake and reuptake, plays a critical role in maintaining serotonergic processes in the brain (Haase et al. 2001). It has been shown that not only serotonin reuptake inhibitors but also many other antidepressants with different primary pharmacological effects caused adaptive changes in the kinetics of serotonin transfer via membranes when administered on a long-term basis (Fišar et al. 2005). The properties of SERT can be studied in platelets, as well as in lymphocytes. It is generally assumed that the serotonergic system in platelets reflects the central presynaptic serotonergic system (Lesch et al. 1993; Rausch et al. 2005). Peripheral parameters of serotonergic transmission, such as SERT activity, the 5-HT_{2A} receptor density, or concentrations of 5-HT in plasma and platelets, are used for studies into the mechanism of the effects of antidepressants and for the prediction of clinical responses to their administration (Hrdina et al. 1997; Franke et al. 2003; Maurer-Spurej et al. 2004). SERT in the platelets of depressed patients is studied in two manners: 1. parameters of the binding of imipramine or parotexine to plasma membranes; 2. parameters of SERT kinetics (apparent maximal velocity V_{max} and apparent Michaelis constant K_M). However, the results have been inconsistent. Current research is attempting to find out whether differences in initial SERT activity (before the start of pharmacotherapy) can be related to the severity of depression determined on the basis of a standard clinical assessment scales, or a different response to the administration of SSRI. Our results support the assumption that more severe symptoms of depression are accompanied by smaller values of V_{max} (Fišar et al. 2008).

4.5. CELLULAR BIOLOGY

The accumulated evidence has suggested that brain ATPase activity may be involved in the etiology of mood disorders. In particular, Na^+K^+-ATPase activity was studied, because it is the major determinant of cytoplasmic sodium concentration and it plays an important role in regulating the cell volume and the cytoplasmic pH and calcium levels. Abnormalities in the **Na^+K^+-ATPase** activity were found in erythrocytes of bipolar patients (Rybakowski & Lehmann, 1994; Looney and El-Mallakh 1997, Goldstein et al. 2006b).

From the point of view of the processes which take place inside a cell, it seems worthwhile to study the adenylate cyclase pathway, the phosphoinositide pathway, and the cascades of mitogen activated protein kinases (Manji et al. 2000), as well as to study the **mitochondrial dysfunction** (Stork & Renshaw, 2005; Kato, 2007b), **reactive oxygen species** (Xia et al. 1999), **nitric oxide** as a pro-apoptotic as well as anti-apoptotic modulator (McLeod et al. 2001; Choi et al. 2002), **cytoplasmatic calcium, calmoduline** (Kamei et al. 1998; Xia & Storm, 2005), and **membrane potential** of blood cells (Thiruvengadam & Chandrasekaran, 2007).

Energetic metabolism and concentrations of CREB, BDNF, nerve growth factor (NGF), Bcl-2 and GSK-3β could play a pivotal role in the pathophysiology of affective disorders; e.g., low BDNF levels were found in antidepressant-naive patients with a major depressive disorder (Shimizu et el. 2003, Hashimoto et al. 2004). Upregulation of CREB and BDNF occurs in response to the long-term administration of various antidepressants, including norepinephrine reuptake inhibitors and SSRI, and after ECT. It is assumed that CREB and BDNF are common postreceptor targets of antidepressants and so their administration leads to neurotrophic-like effects. Mood stabilizers, e.g. lithium and valproate, also strongly activate the neurotrophic signal cascade and other signal pathways and transcription factors (Einat & Manji, 2006; Zarate et al. 2006). Neurotrophic factor BDNF is stored in human platelets (Fujimura et al. 2002); low serum and plasma BDNF concentrations, which have been reported in depressed patients compared with control subjects, result from lowered platelet BDNF release (Karege et al. 2005).

5. PERSPECTIVES FOR FURTHER STUDIES

Various biological parameters have been monitored on a long-term basis in depressed patients in order to characterise the illness or predict the response to the administration of antidepressants. At first, this involved monoamine neurotransmitters and their precursors, metabolites, and transporters; then ion changes, neuroendocrine systems, and biological rhythms were studied. However, the observed abnormalities are not consistent, probably because of interindividual differences and the heterogeneity of the illness, which is hard to quantify from a clinical point of view.

The effects of antidepressants on biochemical processes in the brain and peripheral blood are usually greater than the influence of the illness itself, and so it is necessary to obtain baseline values of measured clinical and biochemical parameters, i.e. values from patients with a depressive disorder prior to the initiation of pharmacotherapy. By comparing data obtained before treatment, during it, and while the patient is in remission, it is possible to distinguish between the changes caused by the illness and the changes caused by antidepressants.

A principal change in the current neurobiological approach to affective disorders is based on the fact that it is assumed that they are connected with neurochemical changes, as well as with disturbances of synaptic plasticity and cellular resilience. Therefore, biological markers are now being sought in the area of intracellular processes which are related to nerve signal transduction and lead to changes in gene expression under the influence of stress, depressive disorder, or the long-term administration of antidepressants. It is likely that the dissimilarities in these intracellular processes are related to interindividual differences in their response to treatment with antidepressants and appropriate pharmacoresistance. Attention is especially focused on pharmacogenetics, neuroendocrine tests, serotonin transporter properties, monoamine oxidase, mitochondrial functions, and growth factors.

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