Review Article

Intracellular Signalling Pathways and Mood Disorders

(signalling pathways / mood disorders / antidepressants / mood stabilizers)

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Abstract. Findings are summarized about basic intracellular signalling pathways influencing neurotransmission and involved in neurodegenerative or neuropsychiatric disorders. Psychotropic drugs used in the therapy of a series of mental disorders, mood disorders especially, show neurotrophic or neuroprotective effects after long-term treatment. Thus, beyond adenylate cyclase, guanylate cyclase and calcium system, attention has been paid to the tyrosine kinase pathway and Wnt pathway. New neurochemical hypotheses of mood disorders are disclosed; they were formulated on the basis of known effects of antidepressants or mood stabilizers on intracellular signal transduction, i.e. on the function, plasticity and survival of neurons. These hypotheses focus on the constituents of intracellular signalling pathways that could be studied as biological markers of mood disorders: transcription factor CREB, neurotrophin BDNF and its trkB receptor, anti-apoptotic factor Bcl2, pro-apoptotic enzyme GSK3, caspases, calcium, and a number of mitochondrial functions related to brain energy metabolism.

Introduction

Intracellular signalling cascades are the principal pathways in communication between the plasma membrane and control processes in different intracellular compartments. The understanding of new signalling pathways in neurons or investigation of new components and their connections with the already discovered ones can be considered as the basis to finding molecu-

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Abbreviations: AC – adenylate cyclase (adenylyl cyclase), AIF – apoptosis-inducing factor, Akt – protein kinase B (PKB), ANP – atrial natriuretic peptide, ANT – adenine nucleotide translocase, APC – adenomatous polyposis coli, BCL2 – B-cell CLL/lymphoma 2, ATP – adenosine-5'-triphosphate, BDNF – brain-derived neurotrophic factor, BMK1 – big-mitogen activated kinase 1 (ERK5), BNP – brain natriuretic peptide, CaM – calmodulin, CaMK – calcium and calmodulin-dependent protein kinase, CaMKK – kinase of CaMK, cAMP – cyclic adenosine monophosphate, CBP – CREB-binding protein, CK – casein kinase, cGMP – cyclic guanosine monophosphate, CNS – central nervous system, CRE – cAMP response element, CREB protein – cAMP response element-binding protein, cyt c – cytochrome c, DAG – diacylglycerol, $\Delta\psi_m$ – membrane potential of inner mitochondrial membrane, ECT – electroconvulsive therapy, ER – endoplasmic reticulum, ERK – extracellular signal-regulated kinase, GABA – γ-amino butyric acid, GC – guanylate cyclase (guanylyl cyclase), GPCR – G protein-coupled receptors, GRB2 – growth factor receptor-bound protein 2, GSK3 – glycogen synthase kinase 3, GTP – guanosine triphosphate, HDAC – histone deacetylase, IAP – inhibitor of apoptosis proteins, IP_3 – inositol 1,4,5-triphosphate, IP_3R – IP_3 receptor, JNK – c-Jun N-terminal kinase, LEF – lymphoid-enhancing factor, MAPK – mitogen-activated protein kinase, MAPKK – MAPK kinase (MAP2K, MEK), MAPKKK – kinase of MAPK kinase, mCU – mitochondrial Ca^{2+} uniporter, MEF2 – myocyte enhancer factor 2, MEK – mitogen-activated protein kinase kinase (MAP2K, MAPKK), mNCE – mitochondrial Na^+/Ca^{2+} exchanger, MOMP – mitochondrial outer membrane permeabilization, mtDNA – mitochondrial DNA, MPT – mitochondrial permeability transition, MPTP – mitochondrial permeability transition pore, NAA – N-acetyl aspartate, NCX – Na^+/Ca^{2+} exchanger, NF-κB – nuclear factor κB, NFAT – nuclear factor of activated T cells, NGF – nerve growth factor, NMDA – N-methyl-D-aspartic acid, NO – nitric oxide, NOS – nitric oxide synthase, nNOS – neuronal nitric oxide synthase, NT – neurotrophin, p75NTR – neurotrophic receptor p75, PDE – phosphodiesterase, PDPK1 – 3-phosphoinositide dependent protein kinase 1, PI3K – phosphoinositide 3-kinase, PIP_2 – phosphatidylinositol 4,5-biphosphate, PIP_3 – phosphatidylinositol 3,4,5-triphosphate, PKA – protein kinase A, cAMP-dependent protein kinase, PKB – protein kinase B (Akt), PKC – protein kinase C, PLC – phosphoinositide phospholipase C, phospholipase C, PMCA – plasma membrane Ca^{2+} ATPase, PYK2 – tyrosine kinase enriched with proline, ROC – receptor-operated channel, RSK – ribosomal protein S6 kinase, RyR – ryanodine receptor, SHC – sarcoma homologue and collagen homologue, SOS – son of sevenless protein, TCF – T-cell factor, TNF – tumour necrosis factor, trk – tropomyosin-related kinase, VDAC – voltage-dependent anion channel, VOC – voltage-operated channel, WntR – Wnt receptor.

lar-biological causes of the origin of neuropsychiatric diseases. Findings of intracellular signalling pathways are numerous and it is practically impossible to test every component of these pathways as potential biological marker in neuropsychiatric diseases. Therefore, the effects of psychotropic drugs are studied with the purpose to select those components that could be responsible for the therapeutic effects of tested drugs and consequently be related to the origin of the disease. The role of biochemical hypotheses on mental disorders is to suggest the relationship between symptoms of the disease, changes in signalling pathways and mechanisms of action of psychotropic drugs.

Neuropsychiatric diseases are caused by or associated with the disturbance of nervous signal transmission in the brain; changes in the signal transduction in chemical synapses seem to be the most important. At the cellular level, atrophy or neuronal death can be present in certain areas of the brain. At the molecular level, there is impaired neurotransmission mediated by neurotransmitters (mostly by acetylcholine, monoamines and amino acids), their receptors, transporters, and by intracellular processes related to the activation of receptors for neurotransmitters and growth factors.

One of the important tasks in neuropsychiatric research is to find out how neurodegenerative processes and mood (affective) disorders (Maes et al., 2009), or as the case may be schizophrenia (Fatemi and Folsom, 2009), are related. According to new developments, it is supposed that certain constitutive abnormalities of antiapoptotic nervous signalling pathways constitute the bases of clinical mood disorder manifestations in a number of patients. Signalling pathways included in the pathogenesis of depression are primarily activated mostly by serotonin, norepinephrine, dopamine, glutamate and γ-amino butyric acid (GABA), i.e. they are connected with the processes in adenylate cyclase and phosphoinositide systems and with changes of intracellular ion concentrations, especially with calcium.

Clinical effects of antidepressants are obviously caused by their ability to induce adaptive changes in neurotransmission, mainly serotonergic and noradrenergic. Changes in the availability of neurotransmitters and also in the density and sensitivity of their receptors and transporters are not sufficient to explain either the origin of the mood disorders or the mechanisms of action of antidepressants and mood stabilizers. It is supposed that intracellular processes leading to the activation of transcription factor cAMP response element-binding protein (CREB) and consequently elevated gene expression of brain-derived neurotrophic factor (BDNF) and its receptor tropomyosin-related kinase B (trkB) are responsible for final therapeutic effects (Duman et al., 1997). Results support the hypothesis that the treatment with antidepressants, mood stabilizers and even some antipsychotics leads to effects similar to neurotrophic. Due to feedbacks inside the neuron attention is devoted to pathways connected with receptors for monoamine neurotransmitters, receptors with inner calcium channel, and receptors for neurotrophins or Wnt glycoproteins.

Basic findings about signalling pathways included both in pathophysiology of the onset of mood disorders and in the mechanisms of action of administered psychotropic drugs are summarized in this work. Presented schemes of signal transduction are simplified with the purpose to show the components which play a key role from the viewpoint of biological psychiatry. A detailed description of the signalling pathways can be found in quoted literature or on web sites. Suggestions about the mode of action of diverse antidepressants and mood stabilizers follow. Finally, the mostly discussed current neurochemical hypotheses of mood disorders are mentioned.

1. Signalling pathways

The fate of each cell is influenced by a relatively small amount of signalling pathways, which leads to the majority of important intracellular physiological processes (Berridge, 2005). The regulation of complex signalling pathways has a determining role in different brain functions, such as mood, consciousness, taste, sexual excitement, sleep, weight, and others. They can also be affected by mental disorders. Therefore, signalling pathways are included in pathogenesis, pathophysiology and treatment of mental disorders. Their modulations influence gene expression and the function of cellular proteins, the change of synaptic functions, neuroplasticity and the response to neurotransmitters, neuropeptides, neurohormones, glucocorticoids and other bioactive molecules. Individual signalling systems interact mutually and form a complex intracellular system which enables neurons to compile signals from the different neurotransmitter systems and which is involved in the regulation of neuroplasticity and stress response.

Interconnections of various signalling pathways are mediated especially by protein kinases, proteases and by molecules regulating their activity. Reversible phosphorylation of proteins is probably the crucial molecular mechanism which is used to accomplish the biological response to extracellular signals in target neurons. The most important is phosphorylation of ion channels, receptors, enzymes involved in synthesis of neurotransmitters, proteins connected with synaptic vesicles (synapsines), cytoskeletal proteins, inhibitors of phosphatases, and transcription factors. Attention is paid to cAMP-dependent protein kinases (PKA), protein kinases C (PKC) activated by diacylglycerol (DAG), and protein kinases dependent on calcium and calmodulin (CaMK). Protein kinases B (PKB, Akt), glycogen synthase kinase 3 (GSK3) and components regulating programmed cell death, especially the family of Bcl2 proteins and various mitochondrial factors, are also intensively studied because of the known biochemical effects of long-term administration of antidepressants and mood stabilizers, and with regard to the supposed role of

the survival, plasticity and metabolism of neurons during mental and neurodegenerative diseases.

GSK3 is a serine/threonine kinase that phosphorylates transcription factors, enzymes and cytoskeletal elements. In human, it occurs in two isoforms, GSK3α and GSK3β; biological functions of the β isoform have been better analysed, it is widely present in the brain. First of all, the GSK3 role in the regulation of glycogen synthesis was recognized, and afterward other roles were found in different diseases, Alzheimer's disease, diabetes mellitus type 2, and various carcinomas (Peineau et al., 2008). The activity of GSK3 is regulated by phosphorylation; phosphorylation on tyrosine residues increases the enzyme efficiency, phosphorylation of the final serine has inhibitory effects. Furthermore, GSK3 can be inactivated by stimuli coming from different signalling pathways, e.g. Wnt pathway, phosphoinositide 3-kinase (PI3K) pathway, from PKA, PKC, or others. In the final effect GSK3 regulates a range of cellular signalling pathways, whereas there are probably segregated GSK3 stores used by different pathways. The main signalling pathways modulated by GSK3 are insulin pathway, pathway of neurotrophic factors and Wnt pathway. In neurons GSK3 phosphorylates a range of proteins associated with microtubules, which leads to their decreased ability to stabilize microtubules.

Different antidepressants, antipsychotics, amphetamines and growth factors have direct or indirect effects on GSK3 and GSK3-mediated signalling pathways. From the viewpoint of biological psychiatry, there is important finding that GSK3 is directly inhibited by lithium (by competition with Mg^{2+} ions) and indirectly inhibited by valproate; both of them are used in the treatment of bipolar disorder. GSK3 inhibition is anti-apoptotic because of the generally pro-apoptotic action of GSK3. The hypothesis is tested that lithium (and other medications) can, due to the GSK3 inhibition, induce cellular processes leading to changes in bioenergetics, neuroplasticity, neurogenesis, stability and survival of neurons (Gould and Manji, 2005).

The Bcl2 (acronym for B-cell CLL/lymphoma 2) protein family is involved in the regulation of apoptotic cell death and is formed of members with both anti-apoptotic (e.g. Bcl2, BclxL, Bclw) and pro-apoptotic (e.g. Bax, Bad) activities. Proteins of the Bcl2 family together with other factors control the permeability of mitochondrial membranes. Mitochondrial permeability transition (MPT) is an increase in the permeability of the mitochondrial membranes to molecules of less than 1.5 kD in molecular weight. MPT results from opening of mitochondrial permeability transition pores (MPTP). During pore formation, the role both of adenine nucleotide translocase (ANT) in the inner membrane and voltage-dependent anion channels (VDAC) in the outer membrane is supposed (Mattson and Kroemer, 2003). Activation of caspases leading to apoptosis is connected especially with mitochondrial outer membrane permeabilization (MOMP), when molecules closed between the outer and inner mitochondrial membrane are released into the cytoplasm (Green and Kroemer, 2004; Leber et al., 2007; Chipuk and Green, 2008). Mitochondrial proteins bind to inhibitor of apoptosis proteins (IAPs) and deactivate them, therefore allowing apoptosis to proceed. There also exists a caspase-independent apoptotic pathway that is mediated by mitochondrial apoptosis-inducing factor (AIF). In connection with the MOMP, the membrane potential of inner mitochondrial membrane ($\Delta\psi_m$) is lost. Therefore, the main changes in mitochondria during apoptosis are MOMP (regulated by members of Bcl2 family) and depolarization of inner membrane (caused sometimes by MPTP). Bcl2 itself is anti-apoptotic; it reduces apoptosis by sequestration of caspases, inhibition of release of mitochondrial apoptotic factors and by increasing calcium uptake by the mitochondria.

1.1 Adenylate cyclase pathway

The mechanism of activation of the adenylate cyclase system following the activation of G protein-coupled receptors (GPCRs) coupled to G_s is well known (Fig. 1). Catalytic subunits of activated PKA phosphorylate nearly all types of neuronal proteins, transcription factors included, e.g. transcription factor CREB (Taylor et al., 2005; Sands and Palmer, 2008). The activated CREB protein increases gene expression of BDNF and its trkB receptor, and therefore the neuronal plasticity is supported.

Inhibition of the adenylate cyclase system is probably achieved through the activation of G_i proteins; their subunits $G\alpha_i$ directly inhibit adenylate cyclase. However, it is possible that the activation of adenylate cyclase is also inhibited directly or indirectly by βγ complexes released from G_i (through the binding of free $G\alpha_s$ in the membrane). Both activation of certain inhibitory receptors and GPCR phosphorylation leading to coupling to $G\alpha_i$ instead to $G\alpha_s$ could be related to $G\alpha_i$ activation.

1.2 Guanylate cyclase pathway

Guanylate cyclase (GC, also known as guanylyl cyclase) catalyses production of cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP); the presence of Mg^{2+} is required. GCs occur both embedded in membranes and free in the cytosol. The membrane-bound form is activated by peptide hormones; the soluble form is a receptor for nitric oxide (NO), which can be produced in response to increased intracellular calcium levels. Soluble GC is most notably involved in vasodilatation. cGMP is a regulator of ion channel conductance, glycogenolysis and apoptosis; it also relaxes smooth muscles.

cGMP degradation to 5'-GMP by phosphodiesterases (PDEs) is analogous to cAMP degradation. cGMP is a secondary messenger in the phototransduction cascade; so, activation of PDE has an important role in visual phototransduction in the eye. Reduced levels of cytosolic cGMP cause closing of related sodium/calcium channels, which leads to visual information being sent to the brain. On the contrary, PDE inhibitors prevent the deg-

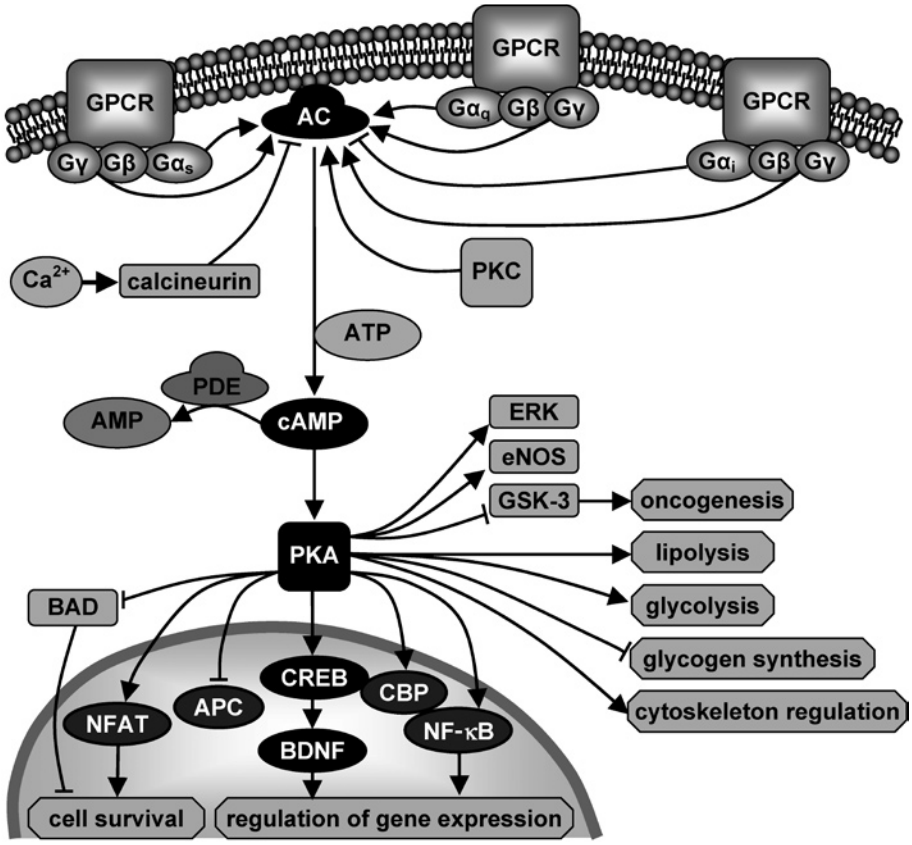

Fig. 1. Signal transduction in adenylate cyclase system
Neurotransmitter or other agonist binds to the specific binding site of G protein-coupled receptor (GPCR) and activates G proteins. The main G proteins regulating activation of adenylate cyclase (also known as adenylyl cyclase, AC) are G_s, G_q and G_i. Activated $G\alpha_s$ and $G\alpha_q$ subunits bind to AC and activate it directly; free βγ complexes activate certain AC subtypes as well. Activated AC catalyses conversion of ATP to cyclic adenosine monophosphate (cAMP); the presence of Mg^{2+} ions is required and the reaction is stopped by G protein inactivation. $G\alpha_i$ subunits decrease intracellular cAMP levels by inhibition of AC. Indirectly, AC activity can be modulated through activation of the phosphoinositide system, when protein kinases C (PKC) and calcineurin are activated. cAMP activates cAMP-dependent protein kinases (PKA); cAMP decomposition into 5'-AMP is catalysed by phosphodiesterase (PDE) in the presence of Mg^{2+}. Activated PKA phosphorylates neuronal proteins; it leads to cross-connections of various signalling pathways and to different physiological effects. Phosphorylation (activation) of transcription factor CREB and consequent expression of neurotrophin BDNF seems to be important for therapeutic efficiency of antidepressants (Duman et al., 1997). Further, phosphorylation of nuclear factor κB (NF-κB) is required for activation of transcription and interaction with CREB-binding protein (CBP). NF-κB obviously participates in survival of neurons (Hayden and Ghosh, 2004). PKA supports the neuronal survival by inhibition of pro-apoptotic factor Bad and by activation of nuclear factor of activated T cells (NFAT) protein (Wu et al., 2007). The importance of anaphase-promoting complex (APC) inactivation is also studied in the processes of axon growth, neuronal survival and synaptic functions (Kim and Bonni, 2007).
⟶ – activation; ⟞ – inhibition

radation of cGMP, thereby prolonging cGMP effects, e.g. inhibitors for PDE type 5, such as sildenafil (Viagra), tadalafil (Cialis) and vardenafil (Levitra), in this way increase the vasodilating effect of cGMP in *corpus cavernosum penis*.

There are at least seven plasma membrane GCs; they probably act as receptors; however, specific ligands have been identified for only three of them (GC-A, GC-B, and GC-C). GC-A is expressed in vascular smooth muscle, endothelial cells, adrenal gland, kidney, spleen, heart, and in peripheral nervous system. Atrial (ANP) and brain (BNP) natriuretic peptides are GC-A ligands. The principal function in the central nervous system (CNS) is modulation of sympathetic activity, inhibition of arginine vasopressin secretion, diminished salt appetite and water drinking, and effects on blood pressure/volume-regulating regions in the brain (Kuhn, 2003).

1.3 Phosphoinositide pathway

The phosphoinositide pathway includes activation of phosphoinositide phospholipases C (PLCs), which participate in phosphatidylinositol biphosphate (PIP_2) metabolism and lipid signalling pathways in a calcium-de-

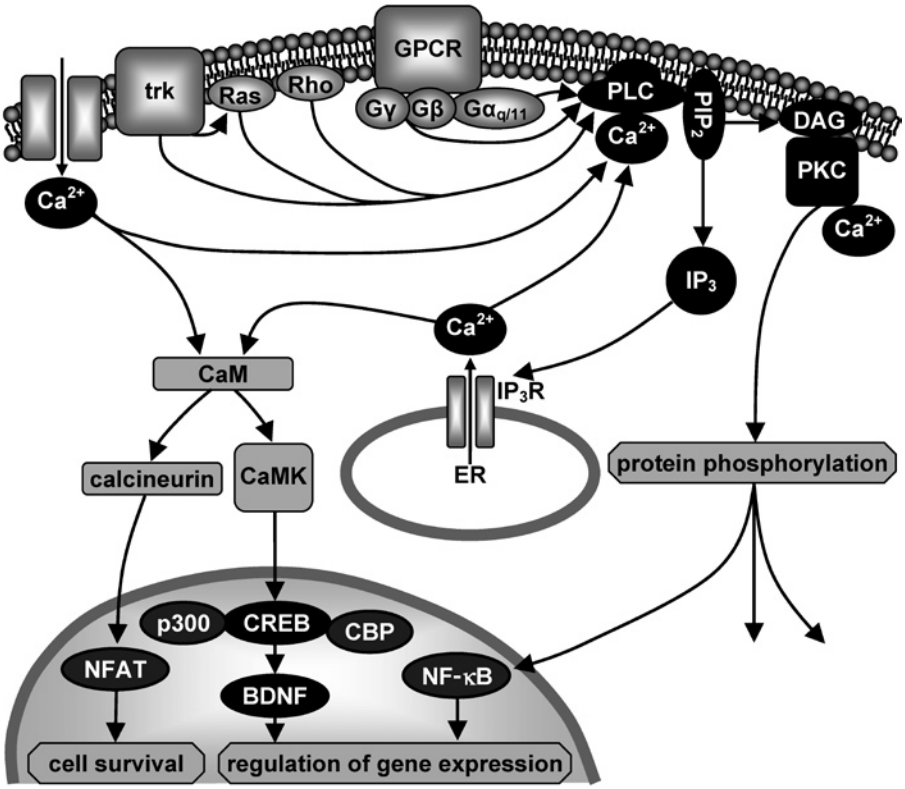

Fig. 2. Signal transduction in phosphoinositide system
Neurotransmitter or agonist binds to the receptor binding site and G proteins (mostly $G_{q/11}$, sometimes G_o or G_i) are activated. $G\alpha_{q/11}$ subunits activate (in the presence of Ca^{2+}) phosphoinositide phospholipase C (PLC), mostly PLCβ isoform. Gβγ subunits activate only isoenzymes PLCβ2 and PLCβ3. PLCγ subtype is activated by both receptor (trk) and non-receptor tyrosine kinases, PLCδ by elevated calcium levels, PLCε by Ras and Rho GTPases, PLCη by Gβγ subunits (Stewart et al., 2007). Activated PLCs catalyse hydrolysis of phoshatidylinositol 4,5-biphosphate (PIP_2) and give rise to the second messenger inositol triphosphate (IP_3) and diacylglycerol (DAG). DAG activates (in the presence of Ca^{2+}) protein kinases of type C (PKCs) and they phosphorylate many enzymes and other cellular proteins. IP_3 binds to intracellular receptors (IP_3R), leading to Ca^{2+} release from endoplasmic reticulum (ER). Ca^{2+} activates calmodulin (CaM), and Ca^{2+}/CaM-dependent protein kinases (CaMKs) are activated, which phosphorylate a wide range of neuronal proteins, including transcription factor CREB (with CBP and p300 as co-activators). Calcineurin, which dephosphorylates (activates) nuclear factor of activated T cells (NFAT) transcription factor, is also activated by CaM.

pendent manner. PLCs are localized mostly in plasma membranes; their functions in the cytoplasm and nucleus have not yet been clarified. The PLC family contains 13 isoenzymes divided into six subfamilies. PLCs catalyse production of diacylglycerol (DAG), inositol triphosphate (IP_3) and calcium (Fig. 2). DAG remains localized in the plasma membrane and enables activation of PKCs. PLC activation as well as PKC activation needs Ca^{2+} ions as cofactors.

Activated PKCs are translocated to the plasma membrane by membrane-bound receptor for activated PKCs and they stay in activated form for a relatively long time. Mechanisms of the termination of their function are not well known (Gould and Newton, 2008). The structure of all PKCs consists of a regulatory domain and a catalytic domain (the latter bounds adenosine-5'-triphosphate (ATP) and substrates); they occur in various isoforms (Saito and Shirai, 2002). The particular type of cell determines which proteins are phosphorylated by the individual isoform of PKC (Steinberg, 2008). Nervous tissue shows high PKC activities; there this enzyme system takes part in the regulation of ion channels, modulation of receptors, release of neurotransmitters, synaptic potentiation, synaptic depression, neuronal survival, etc. Changes of activities and concentrations of PKC isoforms have been described in neurodegenerative diseases both acute (ischaemia, trauma) and chronic (Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis) and in affective or psychotic disorders (Battaini, 2001; Pascale et al., 2007). A large range of biochemical data supports potential PKC involvement in pathophysiology of bipolar disorder and its treatment.

1.4 Calcium signalling pathway

Calcium operates as second or third messenger and its intracellular concentrations are increased both after the activation of receptors connected with phosphoinositide system (Fig. 2) and as the result of opening of voltage-dependent Ca^{2+} channels (N or P/Q type of chan-

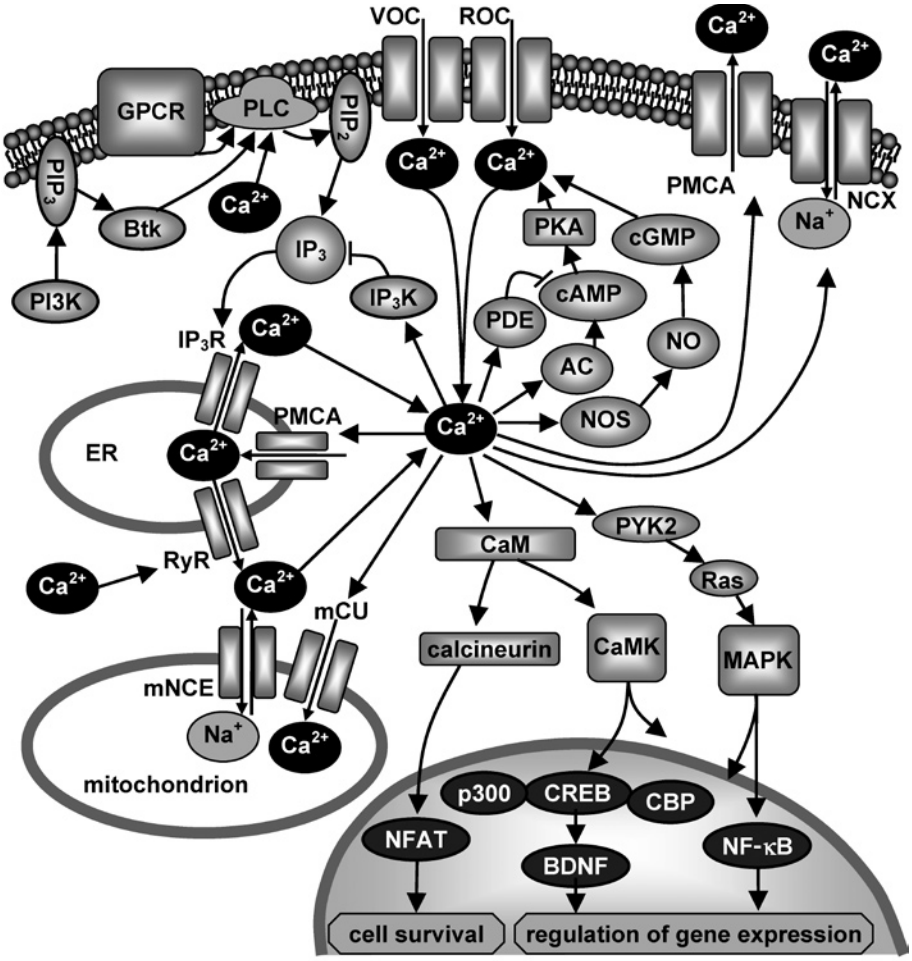

Fig. 3. Signal transduction related to calcium

Intracellular calcium concentrations increase after the opening of Ca^{2+} channels in the plasma membrane in response to membrane depolarization (VOC, voltage-operated channel) or in response to activation of ionotropic receptors (ROC, receptor-operated channel), or in response to activation of inositol triphosphate receptor (IP_3R) in the membrane of endoplasmic reticulum (ER). Concentrations of cytosolic calcium are decreased both by transport from the cell and by uptake into the mitochondria or endoplasmic reticulum; plasma membrane Ca^{2+}-ATPases (PMCA), sodium-calcium exchanger (NCX) or mitochondrial calcium transporter (mCU) are responsible for the depletion of cytosolic calcium. Following Ca^{2+} uptake into the mitochondria, Ca^{2+} is slowly released by mitochondrial sodium-calcium exchanger (mNCE). Ca^{2+} can also be released from ER, by Ca^{2+} activation of ryanodine receptor (RyR). Cytoplasmic calcium activates calmodulin (CaM), and consequently both calcineurin and Ca^{2+}- and CaM-dependent protein kinases (CaMK) are activated. Calcineurin (phosphatase) dephosphorylates nuclear factor of activated T cells (NFAT), which afterwards enters into the nucleus and completes NFAT transcription complexes; these complexes regulate expression of growth factors, cytokines and other molecules essential for morphogenesis, development and function of neurons and other cells. Stimulus activating the NFAT signalling pathway may originate from receptors with tyrosine kinase activity, ion channels, non-receptor tyrosine kinases, G protein-coupled receptors, and gap junctions (Wu et al., 2007). Other targets of calcium are adenylate cyclases (AC); some of them are activated, others are inhibited. Further, Ca^{2+} stimulates some of cAMP phosphodiesterases (PDEs), resulting in changes of cAMP concentration; cAMP concentrations affect calcium levels through the activation of Ca^{2+}-channels or pumps by protein kinase A (PKA). Activation of nitric oxide synthase (NOS) is an important role of Ca^{2+}. NOS enables NO production, activation of guanylate cyclase (GC) and cGMP production; cGMP influences activity of Ca^{2+} channels and pumps (feedback). Phosphoinositide-3-kinase (PI3K) interferes in the calcium pathways through the production of phosphatidylinositol 3,4,5-triphosphate (PIP_3), which activates non-receptor tyrosine kinase Btk, consequently activating phospholipase Cγ1 (PLC γ1), and IP_3 is produced. Increased Ca^{2+} concentrations also activate PLCδ, which increases IP_3 concentrations. Conversely, Ca^{2+} can decrease IP_3 production by the activation of IP_3-kinase (IP_3K). Calcium activates tyrosine kinases enriched with proline (PYK2), which activate small GTPases Ras and mitogen-activated protein kinases (MAPKs). Protein kinases phosphorylate cellular proteins, which results in the cellular response to elevated Ca^{2+} concentrations, including induction of gene expression.

→ – activation; ⊣ – inhibition

nels) or ligand-dependent Ca^{2+} channels (e.g. ionotropic glutamate or acetylcholine receptors). Further, Ca^{2+} levels can be modulated after the activation of other receptors. Therefore, neurotransmitter, hormone or growth factor as well as depolarization of the membrane in excitable cells are responsible for changes of intracellular calcium (Berridge et al., 2000). Very important for calcium signalling pathways is release of Ca^{2+} ions from intracellular stores in the endoplasmic reticulum induced by activation of IP_3 receptors (IP_3R) and potentially by ryanodine receptors (RyR); both of them are principal mediators of calcium-induced release of calcium (Fig. 3).

Intracellular calcium concentrations are regulated by transporters, which remove Ca^{2+} from the cell or store them in mitochondria and endoplasmic reticulum. The plasma membrane and endoplasmic reticulum contain Ca^{2+}-ATPases (PMCA) that actively pump calcium from the cytosol; the pump is powered by the hydrolysis of ATP. Passive facilitated diffusion of Ca^{2+} is enabled by sodium-calcium exchanger (Na^+/Ca^{2+} exchanger, NCX) which uses the energy that is stored in the electrochemical gradient of sodium. The exchanger is usually found in the plasma membranes, mitochondria and endoplasmic reticulum of excitable cells. The PMCA and NCX are together the main regulators of intracellular Ca^{2+} concentrations.

Mitochondria largely participate in reuptake of intracellular calcium. Ca^{2+} uptake by mitochondrial Ca^{2+} uniporter (mCU) is higher than uptake enabled by pumps and exchangers in the plasma membrane. Following the uptake, mitochondria slowly release Ca^{2+} by mitochondrial Na^+/Ca^{2+} exchanger (mNCE) (Kann and Kovács, 2007).

Increased plasma concentrations of Ca^{2+} lead to the activation of many enzymes. The first step in the activation of following cellular processes is binding of Ca^{2+} to Ca^{2+}-specific binding protein; e.g. calmodulin (CaM) has been well described. CaM can bind up to four calcium ions and can undergo posttranslational modifications. Further, CaMK and phosphatases are activated. The cascade of calcium and CaM-dependent protein kinases includes three kinases: kinase of CaM kinase (CaMKK) and CaM kinases CaMKI and CaMKIV, which are activated by CaMKK. They occur frequently in the brain and T lymphocytes. Nuclear CaMKIV regulates transcription through the phosphorylation of transcription factors, including CREB protein. Cross-connections of these protein kinases with other signalling pathways exist in the cytoplasm, e.g. with PKA, mitogen-activated protein kinases (MAPK), and PKB. Consequently, CaMK are connected to the processes related to neuroplasticity and neuronal protection against apoptosis (Soderling, 1999; Berridge et al., 2000; Myiamoto, 2006).

1.5 Wnt pathway

The Wnt signalling pathway activated by Wnt growth factors describes a complex of processes well known for their roles in embryogenesis and cancer, but also involved in normal physiological processes and in regulation of adult hippocampal neurogenesis (Lie et al., 2005; Clevers, 2006; Fuerer et al., 2008). Wnt signals are transmitted at least by three intracellular pathways; the most investigated one is the canonical Wnt signalling pathway, which primarily modulates cellular processes during the cell development. Recently, this pathway has been linked to therapeutic effects of lithium administered in bipolar disorders, probably through the activation of transcription factors by β-catenin (Fig. 4). The Wnt signalling pathway has also been postulated to have a role in Alzheimer's disease; activation of Wnt receptor (WntR) inhibits GSK3β, enzyme which activates apoptosis and phosphorylation of tau protein.

1.6 Tyrosine kinase pathway

The tyrosine kinase pathway is activated by growth factors; this pathway is not directly activated by neurotransmitters; however, it is connected to the signalling pathways mentioned above (Fig. 5). Growth factors are usually proteins or steroid hormones which typically act as signalling molecules between cells. They are capable of stimulating cell growth, proliferation and differentiation. Growth factors that promote the survival, development and function of neurons are known as neurotrophic factors. Neurotrophic factors act by preventing the neuron from initiating programmed cell death (apoptosis); they induce differentiation of progenitor cells to form neurons. The term "neurotrophin" is reserved for four structurally related neurotrophic factors – nerve growth factor (NGF), BDNF, neurotrophin 3 (NT3), and neurotrophin 4 (NT4). Each of the four neurotrophins activates one or more of three trk receptors with intracellular tyrosine kinase activity called trkA, trkB and trkC. Furthermore, each neurotrophin can still bind (with low affinity) neurotrophic receptor p75 (p75NTR), homologous to tumour necrosis factor (TNF) and without any tyrosine kinase activity.

Neurotrophins activate various MAPK signalling pathways. MAPKs regulate cellular activities such as gene expression, mitosis, differentiation, and cell survival/apoptosis in response to extracellular stimuli, stress included. To date, six groups of MAPKs have been characterized: extracellular signal-regulated kinases (ERK1, ERK2; also known as classical MAPKs), p38 isoforms, c-Jun N-terminal kinases (JNKs; also known as stress-activated protein kinases), ERK5 (also known as big-mitogen activated kinase 1, BMK1), ERK3/4, and ERK7/8. Each of these MAPK pathways contains a three-level kinase cascade composed of kinase of MAPK kinase (MAPKKK), MAPK kinase (MAPKK) and MAPK (Wada and Penninger, 2004). Activated MAPKs phosphorylate specific substrates on their serine or threonine residues.

ERK is the most frequently studied MAPK cascade, it is activated in response to growth factors, and regulates cell differentiation and cell proliferation. It has an important role especially in neurodegenerative processes because of its importance for anti-apoptotic signals. However, ERK is also a predominant inducer of non-

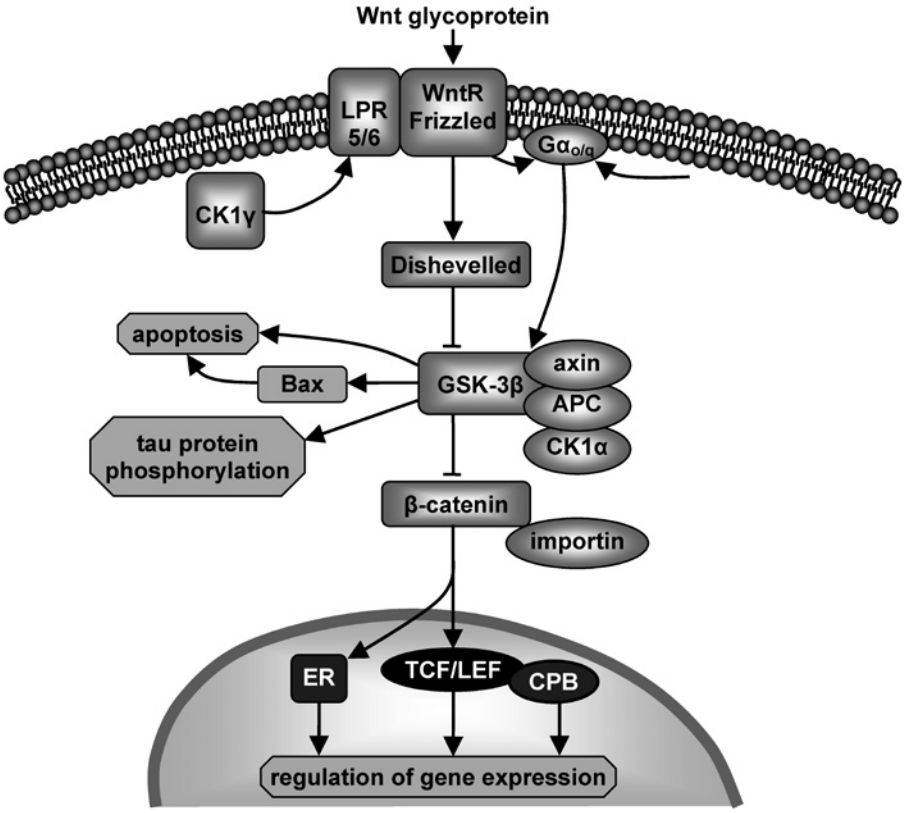

Fig. 4. Canonical Wnt pathway
The canonical Wnt pathway describes a series of events occurring when Wnt glycoproteins bind to transmembrane receptor of the Frizzled family (WntR); membrane protein LRP 5/6 phosphorylated by casein kinase 1γ (CK1γ) participates in the binding. Signal is transferred to the cytoplasmic protein Dishevelled that increases phosphorylation of glycogen synthase kinase 3β (GSK3β). Therefore, the complex of proteins that includes GSK3β, axin, adenomatous polyposis coli (APC) protein and casein kinase 1α (CK1α) is inhibited, because phosphorylation of both axin and β-catenin (mediated by GSK3β) is decreased, and non-phosphorylated axin is degraded (Lee et al., 2003). It is suggested that heterotrimeric G proteins participate in the disturbance of interaction of GSK3β with axin; some of these G proteins are probably associated with WntR (Liu et al., 2005). If the GSK3β/axin complex is not inhibited, β-catenin is phosphorylated by CK1α and GSK3β and β-catenin is degraded. If β-catenin is not degraded, it accumulates in the cytoplasm and is transported by importin into the nucleus. The main target of β-catenin in the nucleus is transcription factor T-cell factor (TCF) from the lymphoid-enhancing factor (LEF) family; connection of TCF and β-catenin leads to activation of the transcription factor. TCF, β-catenin and other transcription cofactors, such as CREB-binding protein (CBP) and others, activate transcription of the target genes. β-catenin activated by the Wnt pathway also interacts with oestrogen receptor (ER) and other nuclear receptors. Inhibition of GSK3β also links the Wnt pathway with apoptosis (e.g. through the activation of pro-apoptotic factor Bax from the Bcl2 family) and Alzheimer's disease (through tau protein phosphorylation).
→ – activation; ⊣ – inhibition

apoptotic neuronal death (Subramaniam and Unsicker, 2006). Key elements of the ERK cascade are ERK1 and ERK2. The ERK cascade is mediated by different receptors, GPCR included. The general activation scheme includes activation of receptor with internal tyrosine kinase activity by neurotrophins (Fig. 5) (Wada and Penninger, 2004).

Trk receptor activation on the cell body leads to ERK1/2 and ERK5 activation within the cell body. At trk activation in axon terminal, both ERK1/2 and ERK5 are locally activated within the axon terminal and ERK5 is activated in the cell body because activated trk receptors are internalized into endosomes that are transported to the cell body, where the receptors selectively activate ERK5. Therefore, the ERK5 signalling pathway is necessary for CREB phosphorylation following the neurotrophic receptor activation on the distal parts of axon, whereas both ERK1/2 and ERK5 take part in CREB activation following stimulation by neurotrophins in the areas of cell bodies (Watson et al., 2001).

2. Effects of antidepressants and mood stabilizers on signalling pathways

2.1 Antidepressants

At the level of chemical synapses, antidepressants usually act as serotonin or norepinephrine reuptake in-

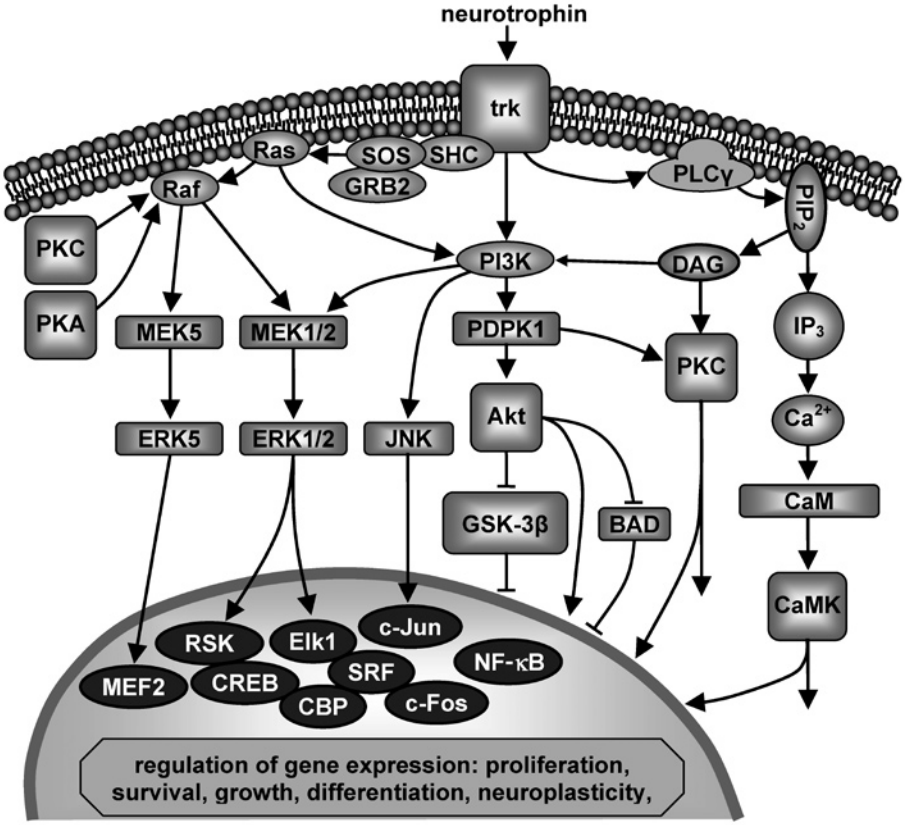

Fig. 5. Neurotrophic signalling pathways

Binding of neurotrophin to its trk receptor leads to various consequent processes:

1. Receptor autophosphorylation leads to phosphorylation of SHC-adaptor protein, on which the complex of growth factor receptor bound protein 2 (GRB2) and son of sevenless protein (SOS) is attached to the membrane. SOS activates small G protein Ras and it attaches Raf kinase to the membrane; activated Raf phosphorylates mitogen-activated protein kinase kinases MEK1, MEK2 or MEK5, and activated MEKs phosphorylate extracellular signal-regulated kinases (ERKs). G protein-coupled receptors are also involved in the ERK activation, namely by the activation of protein kinases PKA or PKC that activate Raf; consequently, ERKs are activated. Activated ERK1 and ERK2 are transported into the nucleus, where they phosphorylate nuclear targets, especially transcription factor Elk1 and ribosomal protein S6 kinase (RSK). RSK has a principal role in the regulation of gene transcription because of its phosphorylation of a range of various factors. Phosphorylated Elk1 interacts with serum response factor (SRF) transcription factor, and transcription is initiated after the binding of a relevant response element in the area of c-Fos promoter. Phosphorylated RSK enables CREB phosphorylation; it binds to the CREB-binding protein (CBP) and to the SRF/Elk1 complex, which leads to c-Fos transcription. As a result, there is regulation of immune and inflammatory processes, and control of cell growth and apoptosis. ERK5 is phosphorylated and activated by kinase of MAP kinase 5 (MEK5). In contrast to ERK1 and ERK2, ERK5 is activated only by neurotrophins. ERK5 is included in cell survival through the myocyte enhancer factor 2 (MEF2) substrate (Wada and Penninger, 2004).

2. Phosphorylation of phospholipase Cγ (PLCγ) by trk receptor enables catalysis of phosphatidylinositol 4,5-biphosphate (PIP_2) cleavage to diacylglycerol (DAG) and inositol triphosphate (IP_3). DAG can activate phosphoinositide-3-kinase (PI3K) or various protein kinases C (PKCs). IP_3 releases calcium from intracellular stores and it activates calmodulin (CaM) and Ca^{2+}/CaM-dependent protein kinases (CaMKs).

3. Phosphatidylinositol 3,4,5-triphosphate (PIP_3) is formed after the stimulation of PI3K heterodimers, which activates 3-phosphoinositide-dependent protein kinase 1 (PDPK1) and therefore, protein kinase Akt (also known as PKB) is activated. Akt supports survival and differentiation of neurons both by stimulation of transcription factors and by inhibition of pro-apoptotic factor Bad or glycogen synthase kinase 3β (GSK3β). PI3Ks also activate PKCζ or they can indirectly activate protein kinases c-Jun N-terminal kinases (JNK), which are responsible for c-Jun phosphorylation and initiation of apoptosis (Cowan and Storey, 2003).

→ – activation; ⊣ – inhibition

hibitors, as inhibitors of the degradation of monoamine neurotransmitters, or as agonists or antagonists of their receptors. Therefore, the administration of antidepressants induces increased concentrations of norepinephrine and serotonin in the synaptic cleft. Consequently, increased activation or inhibition occurs in the processes

of intracellular signalling pathways, mainly in the adenylate cyclase or phosphoinositide pathway (Fišar et al., 2009).

Time-dependent therapeutic effects of antidepressants could be related to changes in gene expression in the brain. This hypothesis is supported by the findings that chronic administration of different antidepressants or electroconvulsive therapy (ECT) causes up-regulation of mRNA for CREB in the hippocampus, increases binding of CREB to cAMP response element (CRE) and elevates concentrations of neurotrophin BDNF and its trkB receptor (Nibuya et al., 1996; Bocchio-Chiavetto et al., 2006). The transcription activity of CREB is induced mostly by CREB phosphorylation; therefore, the influence of antidepressants on this phosphorylation is being investigated (Thome et al., 2000).

Attention is focused especially on the CREB activation via adenylate cyclase pathway, i.e. through increased PKA activity. Other signalling pathways also take part in the regulation of gene expression, e.g. MAPK cascade, CaMKs and others that can also be affected by administration of antidepressants (Tardito et al., 2006). Furthermore, it was found that expression of CREB targets might be region-specific and CREB activity has very different roles, sometimes beneficial, sometimes detrimental, depending on the brain region involved (Newton et al., 2002; Carlezon et al., 2005; Blendy, 2006).

The results support the hypothesis that treatment by antidepressant leads to effects similar to neurotrophic. One of the target genes of pharmacotherapy is the *BDNF* gene. BDNF supports processes implicated in neuronal plasticity and in renewal or improvement of neuronal connectivity. The renewal of synaptic connections and signalling pathways enables the normal function of neurotransmitters. It is suggested that antidepressants can eliminate stress effects on the hippocampus and in this way improve the symptoms of depression. Due to the complexity of intracellular processes it is relatively difficult to establish this hypothesis.

Chronic administration of antidepressants also increases neurogenesis in hippocampal structures, i.e. it increases proliferation and survival of new neurons. Reverse effects of stress and antidepressant on the hippocampal neurogenesis indicate that changes in hippocampal neurogenesis can be significant in clinical syndrome of depression, although it is a complex disorder that targets more than one region of the brain. Coupling of hippocampal neurogenesis to pathophysiology of depression requires further research to be confirmed (Santarelli et al., 2003; Gass and Riva, 2007).

The sites of action of novel neurostimulation techniques with antidepressant potential are elicited from structural and functional brain abnormalities in patients with major depressive disorder. Structural abnormalities in the brains of patients with major depressive disorder have been observed in the cortical and subcortical regions. Structural neuroimaging studies indicate that individuals with recurrent major depressive episodes may have relatively small hippocampi even during periods of clinical remission. Patients may also present with volumetric abnormalities in other subcortical brain regions, including the amygdala and ventral striatum, and in cortical regions, including the anterior cingulate cortex, orbitofrontal cortex and prefrontal cortex (aan het Rot et al., 2009). Growing body of research implicates the ventromedial and dorsolateral sectors of prefrontal cortex as key neural substrates underlying depression (Koenigs and Grafoman, 2009). Structural brain changes in patients with major depressive disorder have been attributed to abnormal function of the hypothalamic-pituitary-adrenal axis. Transcranial magnetic stimulation of the dorsolateral prefrontal cortex and electrical deep brain stimulation of the subgenual cingulate have been shown to have antidepressant effects in some patients. Vagus nerve stimulation might have antidepressant properties via its effects on the *locus coeruleus*.

2.2 Mood stabilizers

Lithium, valproate, carbamazepine, lamotrigine and several atypical antipsychotics (olanzapine, quetiapine, aripiprazole) belong to used or tested mood stabilizers. These mood stabilizers have a large variety of effects at the molecular level and it has not been unambiguously determined which of them are related to therapeutic effects. The main targets of mood stabilizers are the neurotrophin BDNF, ERK pathway, and pathways modulated by GSK3 or BCL2 (Gould and Manji, 2005; Einat and Manji, 2006; Shaltiel et al., 2007).

The same biochemical target, affected both by lithium and valproate, is the signalling pathway involving PKC. It has been known for a long time that lithium is a non-competitive inhibitor of inositol monophosphatase. Furthermore, lithium and valproate reduce transport of myo-inositol into the cells; this leads to reduced PKC activity. Lithium also increases cell resilience by decreasing N-methyl-D-aspartic acid (NMDA) excitotoxicity. Increased concentrations of N-acetyl aspartate (NAA, marker of neuronal viability and functionality) in grey matter after chronic lithium administration support its strong neuroprotective and neurotrophic effects in humans. Lithium also increases hippocampal neurogenesis.

Lithium and valproate increase BCL2 concentrations (Chen et al., 1999) and inhibit GSK3 activity (lithium directly, valproate indirectly). Valproate activates the MAPK signalling pathway, inhibits histone deacetylase (HDAC) (Phiel et al., 2001; Zarate et al., 2006; Kostrouchová et al., 2007) and regulates stress proteins of the endoplasmic reticulum (Bown et al., 2002). Activation of the MAPK pathway is crucial for neurotrophic effects of valproic acid. GSK3 inhibition leads to neuroprotective effects and supports neuroplasticity, neurogenesis and cell resilience through the regulation of different signalling pathways and through changes in gene expression of proteins participating in apoptosis and synaptic plasticity. Bcl2 factor reduces processes linked to cell death or cell atrophy through the sequestration of

caspases, inhibition of release of mitochondrial apoptotic factors, such as calcium, cytochrome c (cyt c) or AIF (Bachmann et al., 2005). Through the effects on BCL2 and p53 (pro-apoptotic protein), lithium affects mitochondria by stabilization of membrane integrity and prevention of MPTP opening; i.e. by regulating the key process in cell death leading to at least temporary loss of $\Delta\psi_m$, input of water into the matrix and equilibration of ion concentrations.

3. Neurochemistry of mood disorders

Recent findings about intracellular processes associated with mood disorders and long-term effects of antidepressants demonstrate an important role of signalling pathways primarily regulated by monoamine neurotransmitters; this was settled as the basis of many biochemical hypotheses (Fišar, 1998; Fišar et al., 2009).

3.1 Neurotrophic hypotheses

The neurotrophic hypothesis of depression (Duman et al., 1997; Duman, 2002; Einat and Manji, 2006; Zarate et al., 2006) supposed that vulnerability to depression can arise as a result of neuronal damage, e.g. after chronic stress, long-term increased levels of glucocorticoids, hypoglycaemia, ischaemia, certain viral infections, effects of neurotoxins, etc. The therapeutic effects of antidepressants consist in increased function of the noradrenergic or serotonergic system. This leads to increased activity of transcription factor CREB, higher expression of neurotrophin BDNF and its receptor trkB, and consequently to increased neuronal plasticity and resumption of cellular functions.

According to the neurogenic hypothesis (Jacobs, 2002), depression may develop due to the decreased neurogenesis in the hippocampus and antidepressants take effect through the stimulation of neurogenesis. The hypothesis of cellular plasticity (Kempermann and Kronenberg, 2003) relates the neurotrophic and the neurogenic hypothesis in the statement that depression can be generally caused by damaged cellular plasticity leading to inadequate relations between structure and function. Molecular mechanisms leading to a disturbance of neuroplasticity are not known. The bioenergetic and neurochemical model of bipolar disorder attempts to identify these mechanisms and focuses attention on mitochondrial dysfunctions (Kato and Kato, 2000; Stork and Renshaw, 2005).

3.2 Mitochondrial hypotheses

Mitochondrial dysfunctions (decreased ATP production, oxidative stress, and induction of apoptosis) occur in the early stages of different neurodegenerative diseases, often associated with mood disorders.

The role of mitochondrial dysfunction during bipolar disorder is supported both by observation of the changes of metabolic energy and by effects of mood stabilizers (lithium and valproate) on mitochondrial functions. mtDNA mutations in the brain, associations of mtDNA polymorphisms and bipolar disorder and changes in gene expression related to mitochondria in the brain were observed (Kato, 2008). The mitochondrial dysfunction hypothesis of bipolar disorder is based on these observations. According to this hypothesis, mtDNA polymorphisms/mutations or mtRNA deletions caused by nuclear gene mutations can cause mitochondrial dysregulation of calcium leading to symptoms of bipolar disorder (Kato and Kato, 2000; Kato, 2007, 2008). The mitochondrial hypothesis corresponds to the abovementioned neurotrophic hypothesis because of an important role of the calcium signalling pathway in synaptic plasticity regulation.

Metabolic changes in the brain were observed in bipolar disorder by magnetic resonance spectroscopy. The results suggest that mitochondrial dysfunctions include impaired oxidative phosphorylation, final shift to glycolytic production of energy, general decrease of energy (decreased ATP production), changed concentrations of phosphomonoesters and changed lipid metabolism (Stork and Renshaw, 2005).

3.3 The role of nitric oxide system

The human hippocampus contains a high density of NMDA receptors and neurons expressing neuronal nitric oxide synthase (nNOS), suggesting that the NMDA-NO transduction pathway can be involved in the pathogenesis of affective disorders and in the mechanism of action of antidepressant drugs (Paul and Skolnick, 2003). It is well established that nNOS-derived NO inhibits neurogenesis (Zhu et al., 2006); moreover, several studies have indicated that nNOS inhibitors have antidepressant-like properties (Jefferys and Funder, 1996; Joca and Guimarães, 2006).

NO appears to play an important role in normal brain function and may have significant implications for the treatment of stress-related psychiatric disorders (Chiavegatto and Nelson, 2003). NO can modulate neuronal excitability and neurotransmitter release (Snyder and Ferris, 2000). Several studies have suggested the link between nNOS and 5-HT (Tagliaferro et al., 2003; Joca et al., 2007). It seems that nNOS over-expression in the hippocampus is essential for chronic stress-induced depression, and inhibiting nNOS signalling in the brain may represent a novel approach to the treatment of depression (Zhou et al. 2007). Furthermore, several reports have demonstrated that increased plasma NO levels were associated with suicide attempts, especially in depressive patients (Kim et al., 2006; Lee et al., 2006). Depressed patients show increased NO production (Suzuki et al. 2001) and enhanced hippocampal expression of the nNOS (Oliveira et al., 2008). On the other hand, it has been reported that the plasma levels of nitrates and nitrites in depressed patients were significantly lower than those in healthy controls, and were increased after treatment with milnacipran (Ikenouchi-Sugita et al., 2009).

Taken together, these findings suggest that activation of NOS in the hippocampus may be involved in the

pathogenesis of affective disorders, possibly triggered by effects of stress on the hypothalamic-pituitary-adrenal axis and mediated by impaired serotonin function.

3.4 Biological markers

Biological marker (biomarker) is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. In medicine, biomarker is an indicator of a particular disease state or a particular state of an organism.

Identification of biomarkers of mood disorders and factors capable of predicting the response to treatment with antidepressants has not brought sufficient results (Balon, 1989; Joyce and Paykel, 1989; Fišar and Raboch, 2008). In accordance with current neurochemical hypotheses of mood disorders, biological markers have been primarily found at the level of neurotransmitter concentrations, their metabolites or precursors. Subsequently, attention was shifted to the receptor systems, and since the 1990's, intracellular processes have become of primary interest. The chance to find sensitive and specific biological predictors of antidepressant treatment has been increased because of introduction of new molecular biology methods. These methods enable better observation of cellular processes connected with the transduction of nervous signals in the brain. The choice of parameters, which should be studied as prospective biological markers of mood disorders, have been derived first of all from new findings of signalling pathways involved in neurotransmission and from the abovementioned neurochemical hypotheses of mood disorders. From the viewpoint of intracellular processes, energetic metabolism, activities of PKC, CREB, BDNF, Bcl2, GSK3, caspases or calcium could play a principal role in finding biological markers of mood disorders. According to the complexity and connectivity of signalling pathways involved in the aethiopathogenesis of mood disorders, the number of selected parameters is not final.

Conclusion

The understanding of signalling pathways and their interconnections at the intracellular level is necessary to understand normal and damaged functions of the CNS. The exact neurobiological processes included in mood disorders have not yet been sufficiently explored; the main attention has been focused on changes in monoaminergic neurotransmitter systems and neuroplasticity. It is suggested that the changes in cognitive functions, learning, memory and the emotions during depression are caused by damaged neuroplasticity in the hippocampus, amygdala and cerebral cortex. Changes in effects of neurotransmitters, hormones and growth factors probably cause changes of neuroplasticity; processes leading to changed neuroplasticity can be predicted by mitochondrial dysfunctions in bipolar disorder.

Long-term administration of antidepressants leads to the effects similar to neurotrophic, as seen mainly through the activation of transcription factors and increased gene expression of neurotrophins. Mood stabilizers, such as lithium and valproate, strongly activate also neurotrophic signalling cascades and other signalling pathways. Intracellular processes between receptor activation and increased gene expression of neurotrophins and other molecules have not been sufficiently clarified. The variability in these intracellular processes probably participates in inter-individual differences of the response to treatment with antidepressants or in drug resistance.

On the basis of clinical analyses and findings about signalling pathway components affected by administration of psychotropic drugs, studies of the following components seem to be prospective: neurotransmitter receptors and transporters (especially for serotonin and norepinephrine), monoamine oxidases, adenylate cyclase and phosphoinositide cascade and MAPK pathway, mitochondrial dysfunctions, reactive oxygen species, nitric oxide and cytoplasmic calcium. The role of membrane potential and membrane lipids in the proper function of neuronal membranes should not be forgotten.

References

aan het Rot, M., Mathew, S. J., Charney, D. S. (2009) Neurobiological mechanisms in major depressive disorder. *CMAJ* **180**, 305-313.

Bachmann, R. F., Schloesser, R. J., Gould, T. D., Manji, H. K. (2005) Mood stabilizers target cellular plasticity and resilience cascades: implications for the development of novel therapeutics. *Mol. Neurobiol.* **32**, 173-202.

Balon, R. (1989) Biological predictors of antidepressant treatment outcome. *Clin. Neuropharmacol.* **12**, 195-214.

Battaini, F. (2001) Protein kinase C isoforms as therapeutic targets in nervous system disease states. *Pharmacol. Res.* **44**, 353-361.

Berridge, M. J., Lipp, P., Bootman, M. D. (2000) The versatility and universality of calcium signalling. *Nat. Rev. Mol. Cell Biol.* **1**, 11-21.

Berridge, M. J. (2005) Unlocking the secrets of cell signaling. *Annu. Rev. Physiol.* **67**, 1-21.

Blendy, J. A. (2006) The role of CREB in depression and antidepressant treatment. *Biol. Psychiatry* **59**, 1144-1150.

Bocchio-Chiavetto, L., Zanardini, R., Bortolomasi, M., Abate, M., Segala, M., Giacopuzzi, M., Riva, M. A., Marchina, E., Pasqualetti, P., Perez, J., Gennarelli, M. (2006) Electroconvulsive Therapy (ECT) increases serum Brain Derived Neurotrophic Factor (BDNF) in drug resistant depressed patients. *Eur. Neuropsychopharmacol.* **16**, 620-624.

Bown, C. D., Wang, J. F., Chen, B., Young, L. T. (2002) Regulation of ER stress proteins by valproate: therapeutic implications. *Bipolar Disord.* **4**, 145-151.

Carlezon, W. A. Jr., Duman, R. S., Nestler, E. J. (2005) The many faces of CREB. *Trends Neurosci.* **28**, 436-445.

Chen, G., Zeng, W.-Z., Yuan, P.-X., Huang, L.-D., Jiang, Y.-M., Zhao, Z.-H., Manji, H. K. (1999) The mood-stabilizing

agents lithium and valproate robustly increase the levels of the neuroprotective protein Bcl2 in the CNS. *J. Neurochem.* **72**, 879-882.

Chiavegatto S, Nelson, R. J. (2003) Interaction of nitric oxide and serotonin in aggressive behavior. *Horm. Behav.* **44**, 233-241.

Chipuk, J. E., Green, D. R. (2008) How do Bcl2 proteins induce mitochondrial outer membrane permeabilization? *Trends Cell Biol.* **18**, 157-164.

Clevers, H. (2006) Wnt/β-catenin signaling in development and disease. *Cell* **127**, 469-480.

Cowan, K. J., Storey, K. B. (2003) Mitogen-activated protein kinases: new signaling pathways functioning in cellular responses to environmental stress. *J. Exp. Biol.* **206**, 1107-1115.

Duman, R. S., Heninger, G. R., Nestler, E. J. (1997) A molecular and cellular theory of depression. *Arch. Gen. Psychiatry* **54**, 597-606.

Duman, R. S. (2002) Synaptic plasticity and mood disorders. *Mol. Psychiatry* **7**, S29-S34.

Einat, H., Manji, H. K. (2006) Cellular plasticity cascades: genes-to-behavior pathways in animal models of bipolar disorder. *Biol. Psychiatry* **59**, 1160-1171.

Fatemi, S. H., Folsom, T. D. (2009) The neurodevelopmental hypothesis of schizophrenia, revisited. *Schizophr. Bull.* **35**, 528-548.

Fišar, Z. (1998) *Biochemical Hypotheses of Affective Disorders*. Galén, Prague. (in Czech)

Fišar, Z., Raboch, J. (2008) Depression, antidepressants, and peripheral blood components. *Neuroendocrinol. Lett.* **29**, 17-28.

Fišar, Z., Jirák, R., Papežová, H., Bob, P. (2009) *Selected Chapters in Biological Psychiatry*. 2nd ed., Grada, Prague. (in Czech)

Fuerer, C., Nusse, R., Ten Berge, D. (2008) Wnt signalling in development and disease. *EMBO Rep.* **9**, 134-138.

Gass, P., Riva, M. A. (2007) CREB, neurogenesis and depression. *Bioessays* **29**, 957-961.

Gould, C. M., Newton, A. C. (2008) The life and death of protein kinase C. *Curr. Drug Targets* **9**, 614-625.

Gould, T. D., Manji, H. K. (2005) Glycogen synthase kinase-3: a putative molecular target for lithium mimetic drugs. *Neuropsychopharmacology* **30**, 1223-1237.

Green, D. R., Kroemer, G. (2004) The pathophysiology of mitochondrial cell death. *Science* **305**, 626-629.

Hayden, M. S., Ghosh, S. (2004) Signaling to NF-κB. *Genes Dev.* **18**, 2195-2224.

Ikenouchi-Sugita, A., Yoshimura, R., Hori, H., Umene-Nakano, W., Ueda, N., Nakamura, J. (2009) Effects of antidepressants on plasma metabolites of nitric oxide in major depressive disorder: Comparison between milnacipran and paroxetine. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **33**, 1451-1453.

Jacobs, B. L. (2002) Adult brain neurogenesis and depression. *Brain Behav. Immun.* **16**, 602-609.

Jefferys, D., Funder, J. (1996) Nitric oxide modulates retention of immobility in the forced swimming test in rats. *Eur. J. Pharmacol.* **295**, 131-135.

Joca, S. R. L., Guimarães, F. S. (2006) Inhibition of neuronal nitric oxide synthase in the rat hippocampus induces antidepressant-like effects. *Psychopharmacology (Berl.)* **185**, 298-305.

Joca, S. R. L., Ferreira, F. R., Guimarães, F. S. (2007) Modulation of stress consequences by hippocampal monoaminergic, glutamatergic and nitrergic neurotransmitter systems. *Stress* **10**, 227-249.

Joyce, P. R., Paykel, E. S. (1989) Predictors of drug response in depression. *Arch. Gen. Psychiatry* **46**, 89-99.

Kann, O., Kovács, R. (2007) Mitochondria and neuronal activity. *Am. J. Physiol. Cell Physiol.* **292**, C641-C657.

Kato, T., Kato, N. (2000) Mitochondrial dysfunction in bipolar disorder. *Bipolar Disord.* **2**, 180-190.

Kato, T. (2007) Mitochondrial dysfunction as the molecular basis of bipolar disorder: therapeutic implications. *CNS Drugs* **21**, 1-11.

Kato, T. (2008) Role of mitochondrial DNA in calcium signaling abnormality in bipolar disorder. *Cell Calcium* **44**, 92-102.

Kempermann, G., Kronenberg, G. (2003) Depressed new neurons – adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. *Biol. Psychiatry* **54**, 499-503.

Kim, A. H., Bonni, A. (2007) Thinking within the D box: initial identification of Cdh1-APC substrates in the nervous system. *Mol. Cell. Neurosci.* **34**, 281-287.

Kim, Y.-K., Paik, J.-W., Lee, S.-W., Yoon, D., Han, C., Lee, B.-H. (2006) Increased plasma nitric oxide level associated with suicide attempt in depressive patients. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **30**, 1091-1096.

Koenigs, M., Grafoman, J. (2009) The functional neuroanatomy of depression: distinct roles for ventromedial and dorsolateral prefrontal cortex. *Behav. Brain Res.* **201**, 239-243.

Kostrouchová, M., Kostrouch, Z., Kostrouchová, M. (2007) Valproic acid, a molecular lead to multiple regulatory pathways. *Folia Biol. (Praha)* **53**, 37-49.

Kuhn, M. (2003) Structure, regulation, and function of mammalian membrane guanylyl cyclase receptors, with a focus on guanylyl cyclase-A. *Circ. Res.* **93**, 700-709.

Leber, B., Lin, J., Andrews, D. W. (2007) Embedded together: the life and death consequences of interaction of the Bcl2 family with membranes. *Apoptosis* **12**, 897-911.

Lee, B.-H., Lee, S.-W., Yoon, D., Lee, H.-J., Yang, J.-C., Shim, S.-H., Kim, D.-H., Ryu, S.-H., Han, C., Kim, Y.-K. (2006) Increased plasma nitric oxide metabolites in suicide attempters. *Neuropsychobiology* **53**, 127-132.

Lee, E., Salic, A., Krüger, R., Heinrich, R., Kirschner, M. W. (2003) The roles of APC and Axin derived from experimental and theoretical analysis of the Wnt pathway. *PLoS Biol.* **1**, E10.

Lie, D. C., Colamarino, S. A., Song, H. J., Désiré, L., Mira, H., Consiglio, A., Lein, E. S., Jessberger, S., Lansford, H., Dearie, A. R., Gage, F. H. (2005) Wnt signalling regulates adult hippocampal neurogenesis. *Nature* **437**, 1370-1375.

Liu, X., Rubin, J. S., Kimmel, A. R. (2005) Rapid, Wnt-induced changes in GSK3β associations that regulate β-catenin stabilization are mediated by Gα proteins. *Curr. Biol.* **15**, 1989-1997.

Maes, M., Yirmyia, R., Noraberg, J., Brene, S., Hibbeln, J., Perini, G., Kubera, M., Bob, P. Lerer, B., Maj, M. (2009) The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. *Metab. Brain Dis.* **24**, 27-53.

Mattson, M. P., Kroemer, G. (2003) Mitochondria in cell death: novel targets for neuroprotection and cardioprotection. *Trends Mol. Med.* **9**, 196-205.

Miyamoto, E. (2006) Molecular mechanism of neuronal plasticity: induction and maintenance of long-term potentiation in the hippocampus. *J. Pharmacol. Sci.* **100**, 433-442.

Newton, S. S., Thome, J., Wallace, T. L., Shirayama, Y., Schlesinger, L., Sakai, N., Chen, J., Neve, R., Nestler, E. J., Duman, R. S. (2002) Inhibition of cAMP response element-binding protein or dynorphin in the nucleus accumbens produces an antidepressant-like effect. *J. Neurosci.* **22**, 10883-10890.

Nibuya, M., Nestler, E. J., Duman, R. S. (1996) Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *J. Neurosci.* **16**, 2365-2372.

Oliveira, R. M. W., Guimarães, F. S., Deakin, J. F. W. (2008) Expression of neuronal nitric oxide synthase in the hippocampal formation in affective disorders. *Braz. J. Med. Biol. Res.* **41**, 333-341.

Pascale, A., Amadio, M., Govoni, S., Battaini, F. (2007) The aging brain, a key target for the future: the protein kinase C involvement. *Pharmacol. Res.* **55**, 560-569.

Paul, I. A., Skolnick, P. (2003) Glutamate and depression. Clinical and preclinical studies. *Ann. N. Y. Acad. Sci.* **1003**, 250-272.

Peineau, S., Bradley, C., Taghibiglou, C., Doherty, A., Bortolotto, Z. A., Wang, Y. T., Collingridge, G. L. (2008) The role of GSK3 in synaptic plasticity. *Br. J. Pharmacol.* **153**, S428-S437.

Phiel, C. J., Zhang, F., Huang, E. Y., Guenther, M. G., Lazar, M. A., Klein, P. S. (2001) Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. *J. Biol. Chem.* **276**, 36734-36741.

Saito, N., Shirai, Y. (2002) Protein kinase Cγ (PKCγ): function of neuron specific isotype. *J. Biochem.* **132**, 683-687.

Sands, W. A., Palmer, T. M. (2008) Regulating gene transcription in response to cyclic AMP elevation. *Cell. Signal.* **20**, 460-466.

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C., Hen, R. (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **301**, 805-809.

Shaltiel, G., Chen, G., Manji, H. K. (2007) Neurotrophic signaling cascades in the pathophysiology and treatment of bipolar disorder. *Curr. Opin. Pharmacol.* **7**, 22-26.

Snyder, S. H., Ferris, C. D. (2000) Novel neurotransmitters and their neuropsychiatric relevance. *Am. J. Psychiatry* **157**, 1738-1751.

Soderling, T. R. (1999) The Ca-calmodulin-dependent protein kinase cascade. *Trends Biochem. Sci.* **24**, 232-236.

Steinberg, S. F. (2008) Structural basis of protein kinase C isoform function. *Physiol. Rev.* **88**, 1341-1378.

Stewart, A. J., Morgan, K., Farquharson, C., Millar, R. P. (2007) Phospholipase C-eta enzymes as putative protein kinase C and Ca^{2+} signalling components in neuronal and neuroendocrine tissues. *Neuroendocrinology* **86**, 243-248.

Stork, C., Renshaw, P. F. (2005) Mitochondrial dysfunction in bipolar disorder: evidence from magnetic resonance spectroscopy research. *Mol. Psychiatry* **10**, 900-919.

Subramaniam, S., Unsicker, K. (2006) Extracellular signal-regulated kinase as an inducer of non-apoptotic neuronal death. *Neuroscience* **138**, 1055-1065.

Suzuki, E., Yagi, G., Nakaki, T., Kanba, S., Asai, M. (2001) Elevated plasma nitrate levels in depressive states. *J. Affect. Disord.* **63**, 221-224.

Tagliaferro, P., Ramos, A. J., Lopez-Costa, J. J., López, E. M., Brusco, A. (2003) Changes in the postnatal development on nitric oxide system induced by serotonin depletion. *Dev. Brain Res.* **146**, 39-49.

Tardito, D., Perez, J., Tiraboschi, E., Musazzi, L., Racagni, G., Popoli, M. (2006) Signaling pathways regulating gene expression, neuroplasticity, and neurotrophic mechanisms in the action of antidepressants: a critical overview. *Pharmacol. Rev.* **58**, 115-134.

Taylor, S. S., Kim, C., Vigil, D., Haste, N. M., Yang, J., Wu, J., Anand, G. S. (2005) Dynamics of signaling by PKA. *Biochim. Biophys. Acta* **1754**, 25-37.

Thome, J., Sakai, N., Shin, K., Steffen, C., Zhang, Y. J., Impey, S., Storm, D., Duman, R. S. (2000) cAMP response element-mediated gene transcription is upregulated by chronic antidepressant treatment. *J. Neurosci.* **20**, 4030-4036.

Wada, T., Penninger, J. M. (2004) Mitogen-activated protein kinases in apoptosis regulation. *Oncogene* **23**, 2838-2849.

Watson, F. L., Heerssen, H. M., Bhattacharyya, A., Klesse, L., Lin, M. Z., Segal, R. A. (2001) Neurotrophins use the Erk5 pathway to mediate a retrograde survival response. *Nat. Neurosci.* **4**, 981-988.

Wu, H., Peisley, A., Graef, I. A., Crabtree, G. R. (2007) NFAT signaling and the invention of vertebrates. *Trends Cell Biol.* **17**, 251-260.

Zarate, C. A. Jr., Singh, J., Manji, H. K. (2006) Cellular plasticity cascades: targets for the development of novel therapeutics for bipolar disorder. *Biol. Psychiatry* **59**, 1006-1020.

Zhou, Q.-G., Hu, Y., Hua, Y., Hu, M., Luo, C.-X., Han, X., Zhu, X.-J., Wang, B., Xu, J.-S., Zhu, D.-Y. (2007) Neuronal nitric oxide synthase contributes to chronic stress-induced depression by suppressing hippocampal neurogenesis. *J. Neurochem.* **103**, 1843-1854.

Zhu, X. J., Hua, Y., Jiang, J., Zhou, Q. G., Luo, C. X., Han, X., Lu, Y. M., Zhu, D. Y. (2006) Neuronal nitric oxide synthase-derived nitric oxide inhibits neurogenesis in the adult dentate gyrus by down-regulating cyclic AMP response element binding protein phosphorylation. *Neuroscience* **141**, 827-836.