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INTRACELLULAR SIGNALING SYSTEMS IN BIPOLAR AFFECTIVE DISORDER PATHOPHYSIOLOGY

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ABSTRACT:

Neurotrophic pathways, cellular plasticity and resilience abnormalities, both at a molecular and cellular level, were highlighted in bipolar affective disorder. The most studied aspects of bipolar disorder pathophysiology are the intracellular ones signaling pathway dysfunctions, such as: the protein G/cAMP pathway, phosphatidil-inositol pathway, alterations in calcium modulation, dysfunctions of the endoplasmic reticulum and mitochondria / Bcl-2 protein, neurotrophic signaling cascades (BDNF) and Wnt/GSK-3 β cascade and their interrelation with inflammation modulators and markers of oxidative stress. The therapeutic properties of mood stabilizers are presumed to be a result of restoring these pathways and the altered processes through diverse biochemical and molecular effects. The aim of this article is to review recent data about the roles of signaling systems and intracellular processes in the pathophysiology of bipolar affective disorder.

Keywords: bipolar disorder, neuroplasticity, cell resilience, apoptosis

REZUMAT:

În tulburarea afectivă bipolară, au fost puse în evidență, la nivel molecular și celular, anormalități ale căilor neurotrofice, ale plasticității și rezilienței celulare. Cele mai studiate aspecte ale fiziologiei tulburării afective bipolare sunt disfuncțiile căilor de semnalizare intracelulare: proteina G/cAMP, fosfatidil-inozitol, dereglări ale metabolismului calciului și disfuncții ale reticulului endoplasmatic și ale mitocondriilor / proteina Bcl-2, disfuncții ale cascadelor de semnalizare neurotrofice (BDNF), ale cascadei Wnt/GSK-3 β și ale interrelațiilor acestora cu modulatorii inflamației și markerii stresului oxidativ. Proprietățile terapeutice ale stabilizatorilor afectivi se presupune că rezultă din restaurarea funcțiilor acestor căi și ale proceselor alterate, prin diverse efecte biochimice și moleculare. Obiectivul acestui articol este de a trece în revistă datele mai recente cu privire la rolul unor sisteme de semnalizare și procese intracelulare în patofiziologia tulburării afective bipolare.

Cuvinte-cheie: tulburare afectivă bipolară, neuroplasticitate, reziliență celulară, apoptoză

INTRODUCTION

Bipolar affective disorder (BD) is a chronic mental disease characterized by recurrent mood episodes of the depressive or manic type, variations of the psycho-vegetative function, cognitive performance and general health and which is supposed to have a neurobiological substrate.

Numerous biochemical studies of the cerebrospinal fluid, of the endocrine response to pharmacological stimuli, neuroreceptors, neuropeptide and transporter protein studies have shown certain abnormalities in subjects with BD (1).

Using several neuroimaging techniques, it was discovered that some patients with BD had structural and functional abnormalities or even neuron loss in some areas of the brain, such as: the prefrontal and temporal cortex, the cerebellar cortex, basal ganglia and limbic system, areas which are known to regulate mood (2). There were also found enlargement of the cerebral ventricles (3) and increased or decreased volume of the amygdala (4, 5).

Post-mortem neuropathology studies confirmed the data obtained by the neuroimaging studies. They found a decreased number of neurons and glial cells, lower neuron density and plasticity in the aforementioned areas in patients with BD when compared with healthy subjects. They also found altered intracellular biochemistry (6, 7). Advanced post-mortem molecular biochemistry techniques facilitated the discovery of complex intracellular mechanism dysfunctions such as second messenger systems, gene expression regulation and neuroplasticity, which seem to have a critical role in the pathophysiology of BD (8-10). The term neuroplasticity refers to the ability of inducing and maintaining adaptative changes to internal and external stimuli for maintaining the physiological functionality of the central nervous system (CNS). These alterations refer to: the number of synapses, variations in neurotransmitter release, modulations of the intracellular signaling cascade, gene regulation, axonal and dendritic architecture modulation and, in some areas of the CNS, generating new neurons (11).

Arguments that support the idea of some dysfunctions in neurotransmission in BD pathophysiology have also been brought forward by pharmacological studies. The immediate effect of antidepressant therapy by increasing the neurotransmitter level (serotonin, norepinephrine) in the synapse is well-known. However, the clinical improvement becomes obvious only after chronic administration (weeks/months), which might suggest that there might be other events in the intracellular signaling pathways that are responsible for the therapeutic effect (11, 12).

The high complexity level of the signaling networks might be one of the important mechanisms through which the neurons acquire the necessary flexibility to generate numerous responses, and the dysfunction of some of these networks may lead to aberrant information processing in the synapse and critical circuits. More and more data published in the last decades suggest that the abnormalities in the neuronal plasticity cascade level and apoptosis control are important events regarding BD pathophysiology and are the targets of the most efficient treatments.

The aim of this article is to review the most recent data regarding the role of the most studied intracellular signaling systems in the pathophysiology of BD, such as: cAMP-protein kinase A pathway, phosphatidylinositol pathway (PIP₂), the metabolism of intracellular calcium, the Bcl-2 protein, neurotrophic-GSK3 β factors and the Wnt signaling cascade.

PROTEIN G-cAMP SIGNALING PATHWAY

Transmitting information from the synapse to the cell nucleus of the postsynaptic cell is a complex process, mediated by messengers such as cAMP and phosphatidylinositol, the final aim being the modulation of certain protein families which act as gene transcription factors and then bind to DNA-specific loci and regulate the expression of many genes which modulate cellular functions such as cell proliferation and apoptosis (programmed cellular death). Most neurotransmitters and neuroproteins bind specific GPCR (G Protein Coupled Receptors) in the neuronal membrane and activate Protein G (PG) and these, in effect, activate the effector system. G proteins may have a stimulatory effect (Gs) or may act as an inhibitor (Gi). G proteins modulate through specific

receptors the ion flux by regulating the ion channels and the activity of effector enzymes (1, 13). Young LT *et al.* (14) and Dowlatshahi *et al.* (15) were one of the first authors who reported increased PG levels in the frontal, temporal and occipital cortex of BD patients (post-mortem studies). The studies carried out on peripheral blood elements have reported increased PG in mononuclear leucocytes of manic patients and decreased levels in depressive patients (16, 17) and increased PG levels in the platelets of bipolar patients (18, 19). This data suggests a possible implication of PG in BD pathophysiology, although it is not yet very clear if there is a direct dysfunction of PG activity or if it represents a secondary manifestation of another's signaling pathway dysfunction.

One of the effector proteins regulated by PG is adenylate cyclase (AC), an enzyme which catalyzes the formation of cAMP (cyclic adenosine monophosphate) from ATP. cAMP is an important second messenger, one of its functions being to activate another enzyme, Protein-Kinase A (PKA), which phosphorylates the CREB (cAMP Response Element Binding Protein) transcription factor. CREB is then translocated in the nucleus where it binds to CRE (cAMP Response Element) and mediates the transcription of neurotrophic factors BDNF (Brain-Derived Neurotrophic Factor) and Bcl-2 (Beta Cell Lymphoma-2), among other targets (8). Several post-mortem studies reported an increased cAMP, AC and PKA activity in BD patients, especially in the temporal, parietal and occipital cortex (20, 21). Dysfunctions of the cAMP signaling pathway have also been reported by studies carried out on platelets (22) and by pharmacological studies as well as on animal models (22, 23).

Chronic usage of lithium decreases AC activity and has a different effect depending on the state of PG receptors. Thus, in basal conditions, when the tonic inhibition of Gi is predominant, lithium increases the production of cAMP, whilst when AC is activated by the Gs receptor complex, the build-up of cAMP drops (1). The therapeutic effect of lithium in both mania and depression can be explained by this bimodal action mechanism (1). Valproate also inhibits PKA, which thus inhibits cAMP build-up (22).

PHOSPHATIDYLINOSITOL PATHWAY (PIP₂)

Activated G proteins stimulate Phospholipase C (PLC) which then hydrolyzes a membrane phospholipid called Phosphatidylinositol (PIP₂), which forms two important second messengers: diacylglycerol (DAG) and inositoltriphosphate (IP₃). DAG activates Protein-Kinase C (PKC), an important enzyme in the PIP₂ signaling pathway and which has a role in regulating neuronal excitability, neurotransmitter release, gene expression and synaptic plasticity (1). Studies carried out on hippocampal cell cultures showed that PKC phosphorylates MARCKS (Myristoylated Alanine-Rich C-Kinase Substrate), which is a protein implicated in regulating neuroplastic events and which alters the conformation of the cytoskeleton actin filaments (24).

Post-mortem studies have found elevated PKC activity in the frontal cortex of BD patients, whilst studies conducted on the peripheral blood have reported and increased PKC activity in the platelets of bipolar patients (25, 26). Studies carried out on the genome of bipolar patients have shown alterations of the eta DAG-Kinase (DGKH), which encodes the kinase which inhibits DAG by metabolizing it to phosphatidic acid and thus activating the PKC pathway (27, 28). A dysfunction or mutation of the DGKH level can result in excessive PKC activity, which can lead to neuronal and dendritic atrophy (29). The existence of an increased PKC activity in bipolar patients is supported by pharmacological studies who found that chronic administration of lithium inhibits PKC activity indirectly and restores grey matter in the prefrontal cortex in bipolar patients. The same inhibitory effect of lithium on PKC activity has been proven in other studies on animal models (30, 31).

The other second messenger (IP₃) initiates another cascade of events, by acting upon a specific receptor which is located on the endoplasmic reticulum (ER). Receptor activation is followed by Ca²⁺ release. Calcium release has as effect the transfer of PKC in the membrane, where it is activated by DAG (1). cAMP and PIP₂ signaling pathways enhance each other, and calcium activates AC, thus increasing cAMP. cAMP facilitates binding to IP₃ receptors (32). PKA, PKC and other calcium-dependent kinases activate (directly or indirectly) several transcription factors such as: CREB, CREM (cAMP Response Element Modulator), ATF1 (Activating Transcription Factor 1), CFos and Cjun (1).

ABNORMALITIES IN THE METABOLISM OF INTRACELLULAR CALCIUM

Neurons have a highly developed Ca^{2+} signaling system, which is responsible for numerous neuronal functions. Through the action which calcium ions have upon cellular proteins such as MARCKS, calmodulin and PKA, PKC, CaM Kinase (calmodulin kinase), neurotransmitter synthesis and release, neuronal excitability, energetic metabolism, remodeling of the cytoskeleton, synaptic plasticity, cell survival and apoptosis are modulated. Several studies evaluated calcium levels in peripheral blood cells (lymphocytes, platelets, neutrophils) in bipolar patients (33, 34) and reported high basal and post-stimulation calcium levels. Emamghoreishi *et al.* (2000) (35) found in the lymphoblasts with an increased basal level of calcium a decreased production of cAMP after stimulating beta-adrenergic receptors with isoproterenol. They also found high levels of AC. By corroborating this data with increased PG activity, the authors suggested that the dysfunction of a signaling pathway may induce adaptative alteration of another signaling pathway. High levels of intracellular calcium in bipolar patients have been interpreted by some authors as determinants of an increased vulnerability to injury or impairment of cellular resilience (the ability of cells to adapt to injury and maintain their form and function) (36, 37). The abnormalities of the intracellular calcium metabolism is considered to be the result of some dysfunctions at ER and mitochondrial level (36, 38, 39).

The mitochondria is an intracellular organelle with an important role in energy production via oxidative phosphorylation, regulating intracellular calcium levels and mediating apoptosis. Konradi C *et al.* (40) have shown dysfunctional mitochondrial processes in a post-mortem study on bipolar patients. The authors found that the nuclear mRNA, which codes mitochondrial proteins, was decreased in bipolar patients, comparing to schizophrenia patients and normal subjects. This data suggests the implication of gene expression which regulates oxidative phosphorylation in the mitochondrial membrane and the ATP-dependent process of proteasome degradation. Mitochondrial dysfunction in BD probably represents the deterioration of oxidative phosphorylation as a result of a shift towards energy production through glycolysis, a decline of total energy and/or substrate available energy and altered phospholipid metabolism (41). Intracellular calcium metabolism abnormalities have been associated with dysfunctions at ER level, as well as mitochondrial activity, both cellular organelles interacting directly with Bcl-2 protein, which has a key role in regulating intracellular calcium signaling (36, 38, 39).

Bcl-2 protein has anti-apoptotic and neuro-protective features, it regulates diverse neurobiological processes, such as neurogenesis, morphogenesis and synaptic plasticity (40, 42-44). Kim *et al.* (2010) (45) found low levels of Bcl-2 protein and messenger mRNA in the frontal cortex of BD patients (post-mortem study). Paraclinical models have shown that after lithium treatment (4 weeks), there was an increase in Bcl-2 activity in the hippocampus and the dentate gyrus (46). Other authors reported an increase in Bcl-2 levels after administering lithium in cell cultures from the frontal cortex, hippocampus and corpus striatum, both in rodents and in-vitro human cells (47). On a cellular level, Bcl-2 controls calcium dynamics through direct interaction on cytosolic targets (ER and mitochondria) (48, 49). At ER level, Bcl-2 interacts with inositol-1,4,5-triphosphate receptors (InsP3R) and the calcium channels, thus decreasing calcium release from the ER or indirectly by stimulating the influx of calcium in the ER. It also stabilizes the integrity of the mitochondrial membrane, limiting the release of C cytochrome, which activates the apoptotic pathway. Recent data suggest that Bcl-2 suppresses InsP3R-mediated calcium release (50). This data supports the idea that Bcl-2 expression can be involved in intracellular calcium metabolism abnormalities found in subjects with BD. This hypothesis is supported by the observation that chronic lithium treatment attenuates calcium mobilization in lymphoblasts of bipolar patients (51), effect associated with inositol depletion (52) and heightened Bcl-2 and ER-chaperone activity (52, 53). Machado-Vieira *et al.* (2010) (54) examined (in cultured lymphoblasts) the potential role of Bcl-2SNP (single nucleotide polymorphism) rs956572 in calcium dynamics in human carriers of A and G alleles in patients with bipolar disorder. The study indicated that the direct interaction Bcl-2/InsP3R reduces the permeability of ER's calcium channels, thus lowering the cytosolic level of the ionic calcium. Studies have

also demonstrated that in BD patients with abnormal gene expression (Bcl-2 in AA variant) it contributes – potentially – to dysfunctional calcium homeostasis through ER-inositol-1,4,5-triphosphate-dependent mechanisms. Taking into consideration the fact that other studies have found a similar genotype (GG variant) (55-57) that also influences calcium levels, the conclusion is that rs956572 polymorphism cannot be considered specific for BD, but according to the author (54), Bcl-2SNP may play a role in BD pathophysiology. Alterations in the expression of certain mitochondrial/ER genes and plasticity, regulated by calcium and Bcl-2 have proven to affect cellular viability significantly. Lithium seems to have a neuroprotective role.

NEUROTROPHIC SIGNALING CASCADES

Neurotrophic factors (BDNF, neurotrophins 4 and 5, Bcl-2 proteins) maintain cell survival by ensuring nourishment intake and by the inhibitory effects which they have upon some intracellular enzymes and molecules which mediate apoptosis.

BDNF is the most studied neurotrophic factor and because of its function it is considered a plausible candidate in BD pathophysiology. The cellular action of BDNF is mediated by receptors with high affinity for tyrosine kinase (TrK- β) (1). When BDNF binds to TrK- β receptors, it initiates their dimerization and self-phosphorylation, followed by the formation of tyrosine residues, which will activate the Ras/MAPK (Mytogen Activated Protein Kinase), PI3-Kinase (phosphoinositide-3-kinase) and PLC signaling pathways. The phosphorylated tyrosine in association with adaptor proteins activates the small G protein (Ras), which activates the subsequent phosphorylation of Raf kinases (serine-threonine kinase), MAPK, ERC (Extracellular Receptor Coupled Kinase) and RSK (Ribosomal S6 Kinase) (1). The MAPK-RSK pathway influences cell survival in two ways: by activating the transcription factor CREB (located in the nucleus) and the transcription of the genes which induce the synthesis of important neurotrophins such as BDNF and Bcl-2 is facilitated and secondly it inhibits the BAD protein (Bcl-2 Associated Death Promotor) which has pro-apoptotic function (58). The TrK- β -IP3 Kinase signaling pathway is activated either via Ras or by activation of adaptor proteins and has a direct target: Akt. Through dephosphorylation, Akt suppresses BAD and the pro-apoptotic enzymes Caspase-9 and GSK-3 β (Glycogen Synthase Kinase-3 β). Studies which assessed plasmatic and serum BDNF levels reported lower values when compared to normal subjects, both in bipolar patients (59) and in patients with recurrent depressive disorder (RDD) (60-62). Thus, BDNF may be implicated as a bio-marker in BD pathophysiology (63).

GSK-3 β (Glycogen Synthase Kinase-3 β)

GSK-3 β is a serine-threonine type kinase, with an important role in the glucose metabolism, in modulating intracellular signaling pathways, cellular multiplication, gene expression and apoptosis (64). Among the roles of the enzyme, we must add the modulation of proteins associated to the microtubules of the cytoskeleton, such as “tau” proteins, MAP-1 β and MAP-2. The phosphorylation of these proteins by GSK-3 β determines the microtubules to lose the conformation or to destabilize (64, 65, 66). GSK-3 β facilitates apoptosis through the inhibitory effect which it has upon the proteins that promote cell survival, such as CREB and Bcl-2 (67, 68). Regulating GSK-3 β levels is done by PKA, PKC, Act proteins and Bcl-2, which decrease GSK-3 β activity and by calcium and glutamate, which increase its activity (68, 69, 70). Studies on animal models have reported that augmenting GSK-3 β activity in cerebral tissue lead to both depressive and manic behavior, which were then controlled by administering inhibitors of this enzyme (71, 72). Pharmacological studies carried out on cerebral tissue of animal origin and human lymphocytes have shown that lithium, valproate, antidepressants, atypical antipsychotics have an inhibitory effect on GSK-3 β (71-73). Polter *et al.* (2004) (74) found a significantly reduced platelet GSK-3 β expression in BD patients when compared to RDD patients and healthy subjects. It still remains to be proven if GSK-3 β or other GSK-3 β -regulated signaling molecules dysfunction are involved in the pathophysiology of BD (74).

Wnt SIGNALING CASCADE

The Wnt protein class (*Drosophila wingless*) activates signaling pathways which control developmental processes and in adults influence neuroplasticity, cell survival and neurogenesis in some areas of the brain (75). The gene transcription via β -catenin is modulated on this pathway (76). Wnt pathway anomalies have been implicated in the pathophysiology of chronic diseases like cancer and more recently, in recurrent severe mood disorders such as BD (77, 78). Wnt proteins bind to a Protein G-type cross membrane receptor (Frizzled), which activates the cytoplasmic proteins Dvl or Dsh (Dishevelled), which inhibit the GSK-3 β protein. By inhibiting GSK-3 β , β -catenin is no longer being degraded and thus it accumulates in the cytosol, and migrates towards the nucleus, where it stimulates the transcription factors Tcf (T-cell Transporter Factor) and Lef (Lymphoid Enhancer Binding Protein). These factors activate the transcription of the target genes, which have different actions, such as: apoptosis prevention, stimulating the neuron dimension growth, cell development, oxidative stress and inflammation (1, 79). A dysfunction of the Wnt signaling pathway in BD is supported by experimental animal studies, pharmacological and genetic studies. Thus, inactivating the Wnt gene in mice had as effect the poor development of large brain portions, whilst the stimulation of the Wnt gene lead to increased neurogenesis in the hippocampus (79). Matigian *et al.* (2007) (80) reported differences in Wnt expression in monozygotic twins discordant for BD. One of these was Tcf-7 (β -catenin activated transcription factor). Zandi *et al.* (2008) (77) tested the association between candidate genes in the Wnt pathway and the susceptibility for BD in a familial study. The authors found an association between BD and the PPARG gene, which is located on the 6p21 chromosome. PPARG genes encode PPAR receptors (Peroxisome Proliferator Activated Receptor), which mediate varied biochemical cellular processes, including peroxisomal function, lipid synthesis and oxidation, cell proliferation and inflammation (81). If PPARG influences susceptibility for BD, this is realized through neurodevelopmental processes. The PPARG gene is a target of the Wnt- β -catenin pathway and the only one implicated in the mechanism of action of the medication in BD (77). The Wnt pathway interacts both with neurotrophic factors (BDNF) as well as with mediators of oxidative stress and inflammation. These interactions are involved in keeping the balance between cell survival and apoptosis (82). BDNF needs the presence of β -catenin in order to exert its effects (83). The dysfunction of the Wnt pathway may decrease BDNF levels, which has been reported by many studies that assessed the peripheral level of this factor (84-86). The interrelation of the Wnt system with cytokines and the oxidative markers are complexes with a self-potential capability and have a pro-apoptotic effect (87, 88). In patients with BD, some studies have reported high peripheral levels of inflammation mediators (interleukins and tumoral necrosis factors) (89-92), while other studies reported elevated peripheral levels of oxidative markers (Theobarbituric reactive substance and nitric oxide) (93-95). These findings indicate the existence of a neurotrophic and anti-apoptotic effect of the Wnt-BDNF interrelation and a possible pro-apoptotic and anti-apoptotic modulated effect by interrelation with inflammatory mediators and oxidative markers considering the self-potential between them, with the presence of complex feed-back circuits between them (96). The existence of a potential imbalance in these interrelations is presumed to be involved in the pathophysiology of BD.

CONCLUSIONS

In the last few decades, the role of the dysfunctions in neuroplasticity, cell resilience, survival and apoptosis in the pathophysiology of BD has been one of the focus-points of researchers worldwide. The existence of certain anomalies in the intracellular signaling pathways in bipolar patients, such as heightened activity in signaling pathways, G Protein-cAMP, PIP₂, calcium metabolism (a result of ER and mitochondrial dysfunction), which can promote cellular atrophy/apoptosis, have already been proven. Decreased peripheral levels of neurotrophic factors (BDNF and Bcl-2), dysfunctions of the Wnt-GSK-3 β pathway and their interrelations with inflammatory modulators and markers of oxidative stress have also been described. Dysfunctions of the existing complex interrelations between these intracellular systems and mechanisms may unbalance the delicate equilibrium

between cell survival and apoptosis and explain brain volume changes in certain areas, highlighted by neuroimaging exploration. Mood stabilizers act on many of these intracellular systems and trigger intracellular cascades which can alter protein synthesis, produce reparative effects in synaptic plasticity and restore neurotransmission. Optimal long-term treatment is an attempt to prevent cellular dysfunctions and disorder progression. In order for this to succeed, new pharmacological strategies which increase cellular plasticity are needed.

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