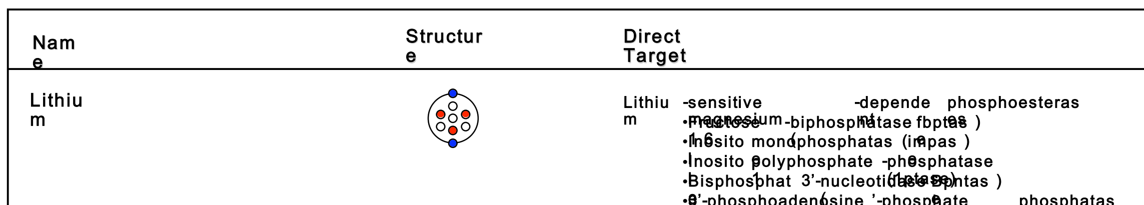
Appendix A14.21

Regulation of Gene Expression Changes by Mood Stabilizers

Although a number of acute, in vitro effects of mood stabilizers have been identified, the clinical effects in the treatment of bipolar disorder are only seen after chronic administration, thereby precluding any simple mechanistic interpretations based on its acute biochemical effects. Patterns of effects requiring such prolonged administration of the drug suggest that the therapeutic effects involve the strategic regulation of gene expression in critical neuronal circuits. In this context, it is noteworthy that substantial progress has recently been made in identification of genes responsive to trans-synaptic stimulation, as well as in determining the processes that convert often/occasionally ephemeral second messenger-mediated events into long-term cellular phenotypic alterations. These developments have been particularly important with respect to our attempts to understand the mechanisms by which short-lived events (e.g. stressors) can have profound, long-term (perhaps lifelong) behavioral consequences. More importantly for the present discussion, these findings help to unravel the processes by which seemingly “simple” molecules including monovalent cations (e.g. lithium) and fatty acids (e.g. valproic acid) may produce a long-term stabilization of mood in individuals vulnerable to bipolar disorder. However, several factors impede our attempts to fully understand the molecular and cellular mechanisms of action of mood stabilizers. For instance, a suitable experimental model of bipolar disorder is currently not available. Thus, many studies are of necessity conducted on “normal” rodents. This is done with the view that any targets identified may have functions conserved by evolution, lending therapeutic relevance to the human treatments. In this context, the animal models of drug dependence have been very instrumental in accelerating the pace of research on their molecular mechanisms.

Another inherent problem in the identification of therapeutically relevant target genes for the actions of mood stabilizers is the relative paucity of easily detectable phenotypic changes induced by these agents. This makes the task of ascribing functional significance to the multiple treatment-induced changes at the genomic level quite daunting. Moreover, the genetic basis of mood as a quantitative trait is still tentative; therefore, we cannot focus on a group of already known genes. Finally, there is a real lack of knowledge concerning the underlying etiology and pathophysiology of what is likely a group of complex, heterogeneous disorders that show overlap of symptom clusters, and are subsumed under the rubric of “manic depressive illness”--and even the bipolar subtype is clinically heterogeneous.

Appendix Table A14.21a



Name	Structure	Direct Target
Lithium		Lithium-sensitive magnesium-dependent phosphoesterases • Fructose 1,6-biphosphatase (fbptase) • Inositol monophosphatase (impase) • Inositol polyphosphate 1-phosphatase (1ptase) • Bisphosphate 3'-nucleotidase (bpntase) • 3'-phosphoadenosine 5'-phosphate phosphatase

Appendix Table A14.21 Pathways Regulated by Both Lithium and VPA

Pathway	Effect
Wnt signaling	up
ERK-MAP kinase and bcl-2	up
Cyclic AMP	stimulated down
Phosphoinositol/Protein kinase C (PKC)	PKC and MARCKS down
Arachidonic acid turnover	down

Nevertheless, despite these significant obstacles, there is currently considerable excitement about the progress that is being made, using two fundamental strategies to identify changes in gene expression that may have therapeutic relevance in the long-term treatment of mood disorders:

- Firstly, investigators have been focusing on the known primary biochemical targets for the actions of mood stabilizers (e.g. inositol monophosphatases), and subsequently identifying alterations in downstream signaling cascades, transcription factors, and ultimately the expression of genes known to be regulated by these primary biochemical targets.

- Second, several technological advances are allowing more "black box" screening approaches to be increasingly utilized; these approaches attempt to focus directly on changes in gene expression produced by the administration of mood stabilizers in therapeutically meaningful paradigms, without necessarily focusing upon the "initiating biochemical events" (i.e. the medication's primary biochemical target). Using screening methods like subtractive hybridization, microarrays, and mRNA differential display, this strategy usually attempts to simultaneously identify treatment-induced changes in multiple genes often numbering in the thousands without any a priori focus on specific

"candidate genes". However, as we will discuss in greater detail , these methodologies can, if necessary, be biased towards the detection of certain classes of candidate genes.

Both of these strategies require an initial reductive step, which attempts to isolate the specific genes and proteins that are the targets of mood stabilizing agents. Also included, ideally, is a subsequent integrative step that attempts to establish the relationship between the molecular/cellular changes and certain facets of the therapeutic response.

Effects of Mood Stabilizers on Immediate Early Genes

Several independent laboratories have now demonstrated that both lithium and valproate (VPA) exert complex, isozyme-specific effects on the PKC signaling cascade. Not surprisingly, considerable research has recently attempted to identify changes in the activity of transcription factors known to be regulated (at least in part) by the PKC signaling pathway--in particular the AP-1 family of transcription factors. In the CNS, the genes that are regulated by AP-1 include those for various neuropeptides, neurotrophins, receptors, transcription factors, enzymes involved in neurotransmitter synthesis, and proteins that bind to cytoskeletal elements.

Recent studies have demonstrated that lithium (and to a lesser extent VPA), produces, at therapeutically relevant concentrations, complex alterations in basal and/or stimulated DNA-binding of 12-o-tetradecanoyl-phorbol 13-acetate (TPA) response element (TRE) to activator protein 1 (AP-1) transcription factors. These alterations are produced not only in human SH-SY5Y cells in vitro, but also in rodent brain following chronic, in vivo administration. Corresponding to an increase in basal AP-1 DNA binding activity, lithium and VPA have been shown to increase the expression of a luciferase reporter gene driven by an SV40 promoter that contains TREs in a time- and concentration-dependent fashion. Mutations in the TRE sites of the reporter gene promoter markedly attenuate lithium's effects. In order to ascribe potential therapeutic relevance to the changes in AP-1 regulated gene expression, it is necessary to demonstrate that they occur in the CNS in vivo. It is well established that the expression of tyrosine hydroxylase (TH) is mediated largely by the AP-1 family of transcription factors. The effects of acute and chronic lithium on the levels of TH have therefore been investigated in brain areas that have been implicated in the pathophysiology of mood disorders. It has been found that chronic lithium significantly increases the levels of TH in all three areas examined: frontal cortex, hippocampus, and striatum. Recent research has also revealed important roles for the different nitric oxide synthases (NOS) in mediating various aspects of CNS function; the expression of endothelial NOS (eNOS) is known to be regulated by AP-1 sites. Thus, the effects of chronic lithium and VPA on eNOS levels in the rat frontal cortex have been investigated; chronic lithium

or VPA has been found to produces a 2-3 fold increase in the levels of eNOS. Importantly, independent laboratories have also recently demonstrated lithium-induced increases in the levels of proteins whose genes are known to be regulated by AP-1 sites. These results clearly show that, in addition to increasing basal AP-1 DNA binding activity and the expression of the luciferase reporter gene in vitro, chronic lithium increases the levels of several endogenous proteins whose genes are known to be regulated by AP-1 sites, in rat brain ex vivo. Together, these data suggest that lithium and VPA, via their effects on the AP-1 family of transcription factors, may bring about strategic changes in gene expression in critical neuronal circuits, effects that may ultimately underlie their efficacy in the treatment of bipolar disorder.

However, while many specific genes which are the targets of long term lithium and/or VPA action have indeed been identified, it has been estimated that ~ 10,000 - 15,000 genes may be expressed in a given cell at any time. Clearly, additional, novel methodologies are required to study the complex pattern of gene expression changes induced by chronic drug treatments. In recent years, new methodologies have evolved to identify the differential expression of multiple genes (e.g. in pathological vs. normal tissue, or in control vs. treated tissue); a series of mRNA differential display and microarray studies have been undertaken to identify genes regulated by structurally highly dissimilar mood stabilizers (Zhou et al., 2002; Chen G et al., 1999, 2001; Chen B et al., 2001; Hua et al., 2001a,b; Detera-Wadleigh 2001; Wang et al., 1996; 2001; Manji et al., 2002).

Complementary proteomic studies have also been investigating altered protein expression patterns. Several novel and hitherto completely unexpected targets have been identified. Indeed, using a "knowledge-based" analysis, many of the genes can be categorized as (1) those exerting trophic effects and regulating cell survival; (2) those regulating critical cytoskeletal proteins; (3) those regulating cellular signaling; (4) those regulating metabolic events. and cell death. Among these are transcription factors, an mRNA binding protein, Bcl2-associated athanogene (which regulates the glucocorticoid receptor and MAP kinases), and several members of the Rho signaling cascade. These studies have demonstrated the utility of identifying both gene cluster categories as well as individual genes which may represent therapeutically relevant targets for the actions of mood stabilizing agents. An analysis of the temporal and spatial pattern of gene expression alterations in the brain may provide important clues about the circuits involved in mood stabilization, and have the potential to lead to the development of novel therapeutics. The concerted use of genomic and proteomic strategies to refine these complex diseases into mechanism-based subcategories may ultimately allow for the matching of particular target-based therapies to particular markers in subgroups of patients.

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