Molecular Mechanisms of Action of Mood Stabilizers in Bipolar Disorder

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Research into the biological basis of bipolar disorder has been performed using two different but mutually complementing strategies: i) Identification of biological abnormalities associated with bipolar disorder; ii) Analysis of biological effects caused by antibipolar drugs such as lithium, valproate or carbamazepine. Results of both strategies have provided evidence for abnormalities in cellular signal transduction systems in both post-mortem brain and peripheral blood cells of bipolar patients (Warsh & Li 1996) and for a potential role of these systems as targets of lithium ions (Lenox et al 1998). However, it has been very difficult to decide which of the multitude of effects of lithium (and, much less extensively studied, of other mood stabilizers) on many neurobiological parameters including various components of the cellular signal transduction machinery are instrumental to the clinical actions and which are merely epiphenomena or responsible for the side-effects. A very similar problem encountered in the analysis of the mechanism of action of antidepressants was, tentatively, resolved by the observation that almost all known antidepressants inhibit the reuptake of serotonin and/or noradrenaline and that compounds newly synthesized for and limited in their action to this particular property (SSRI’s, SNRIs) indeed proved efficacious as antidepressants: It is thus now widely accepted that this reuptake inhibition is the initial causal event that triggers downstream adaptations in the function of neurotransmitter systems, which are instrumental to the antidepressant efficacy. Could a similar approach be successful also in the case of antibipolar drugs? All presently known mood stabilizers, with the exception of lithium, are anticonvulsants (valproate, carbamazepine and, the most recent member of the group, lamotrigine) and share, therefore, properties like inhibition of Na\(^+\), K\(^+\) and Ca\(^{2+}\)-channels that are most probably related to their anticonvulsant activity. Do they also exhibit actions in common with lithium ions that could qualify as potentially important in their mood stabilizing activity? In this article I will discuss effects of mood stabilizers on cellular signal transduction as a potential common target in their mechanism of action. I begin with a brief outline of these pathways (fig. 1).

**Cellular Signal Transduction Pathways**

The term „signal transduction“ denotes a process, which transmits the information impinging at receptors on the surface of the cell into the interior of the cell. The effects elicited there encompass both short-acting (e.g. changes in the phosphorylation of an ion channel) and long-lasting (e.g. changes of gene transcription) actions on cellular function. Particularly important components of the signal transduction system are the „G-proteins“, a family of guanylnucleotide-binding membrane associated proteins, which mediate the coupling of the hormone-activated receptors to „effector“-proteins. About 80% of all known hormones, neurotransmitters or neuromodulators act via G-proteins, which can couple to various intracellular effector proteins (for review see Birnbaumer 1990). G-proteins are heterotrimeric complexes located at the inner plasma membrane and consist of an α-subunit and the tightly associated βγ-subunits. The α-subunit binds guanyl nucleotides. Interaction with an activated receptor induces exchange of the bound GDP with GTP and dissociation of the heterotrimeric complex. The free α- and βγ-subunits subsequently activate various effectors. Activation is terminated by hydrolysis of GTP to GDP by the intrinsic GTPase activity of the α-subunit, which allows the reassociation of the heterotrimeric αβγ-complex. This latter process of signal termination is reinforced by a separate class of proteins (regulators of G-protein signaling, RGS), which enhance the GTPase activity of the α-subunit. G-proteins comprise a family of different proteins that can be subdivided in four major classes (G\(_s\), G\(_i\), G\(_q\), G\(_{12}\)), which show some limited specificity for effector proteins. Thus, e.g. G\(_s\) activates and G\(_i\) inhibits adenylylcy clase, while G\(_q\) activates phospholipase C.
Effector proteins encompass not only enzymes like adenylylcyclase or various phospholipases (C, D, A₂), which synthesize „second messenger“ molecules (see below) but also e.g. ion channels the opening of which is regulated by binding to a G-protein. „Second messenger“ are diffusible small molecules, which are either water soluble and thus easily diffuse into the cytoplasm (such as cyclic AMP, cyclic GMP or inositolpolypolyphosphates) or lipophilic compounds that remain associated to the cell membrane (such as diacylglycerol, DAG). „Second messenger“ often act via activation of protein kinases, which phosphorylate various target proteins thereby modifying their functional state. Thus, e.g. cyclic AMP activates protein kinase A (PKA), while DAG, together with Ca²⁺, activates protein kinase C (PKC). Targets for these phosphorylations are e.g. ion channels, proteins of the cytoskeleton, components of the signal transduction system like G-proteins or, in the case of long-lasting changes, transcription factors, which regulate gene transcription (for review see Karin 1994; Calkhoven and Ab 1996).

The process of G-protein dependent signal transduction described above results in a several thousand fold amplification of the original signal. Furthermore, since one type of receptor can couple to various different G-proteins and different G-proteins can converge to modulate the function of one single effector, these signaling pathways form a complex network that accounts for the remarkable versatility of neural pathways in the integration, fine-tuning and processing of diverse signals (Bhalla & Iyengar 1999; Weng et al 1999). Given this crucial role of signal transduction it is not surprising that these systems are also critically involved in phenomena such as neural plasticity and memory formation (Matzel et al 1998; Kandel 2001), kindling and behavioral sensitization (Ghaemi et al 1999; Manji & Lenox 2000; Gelowitz & Berger 2001) and are therefore candidates as potential targets in the mechanism of action of mood stabilizers (for a recent reviews see Manji et al 2001). Effects of lithium ions on signal transduction mechanisms have been a major focus of research during the last decade (for reviews see Manji 1992; Jope and Williams 1994; Manji et al 1995, 2001) while the actions of other mood stabilizers on these mechanisms have only very recently been explored.

**Effects of Mood Stabilizers on the Adenylylcyclase System**

Cyclic AMP, the prototypic second messenger, was first identified and described by Sutherland and Rall (1958) during their investigation of the noradrenaline or glucagon induced glycogenolytic activity of liver membranes, a nobel price-honoured milestone of biological research. Forn and Valdecasas (1971) were the first to describe an inhibitory effect of lithium ions at near therapeutic (2mM) concentrations on the noradrenaline-stimulated accumulation of cyclic AMP. Since then analogous effects were found in various other tissues including the brain (for review see Mork et al 1992; Manji et al 1995). Also activation of human adenylyl cyclase is inhibited by lithium at therapeutic concentrations, as shown by measurements of plasma cyclic AMP after stimulation with adrenaline (Ebstein et al 1976), investigations of peripheral cells (Murphy et al 1973; Lonati-Gallani et al 1989) and research with human brain tissue (for review see Manji et al 1995). It is now generally accepted that the two most important side-effects of lithium therapy, diabetes insipidus and hypothyroidism, are caused at least partially by the lithium-induced inhibition of the activation of adenylyl cyclase by vasopressine and thyrotropin respectively. Studies aimed at the mechanism of lithiums effects showed that the inhibitory action is exerted distal from the receptor, since also stimulation of adenylyl cyclase by GTP-analogues or Ca²⁺/calmodulin is acutely inhibited by lithium ions (for
review see Manji et al 1995; Mork et al 1992). As these acute effects of lithium are prevented by Mg$^{2+}$ ions, lithiums action appears to be due to a competition with Mg$^{2+}$ ions at the catalytic unit of adenyl cyclase. In contrast, the chronic inhibitory effects of lithium on the stimulation of adenyl cyclase via beta-adrenergic receptors are not influenced by Mg$^{2+}$ ions but reversed by GTP (Mork & Geissler 1989a,b). Thus these chronic effects are believed to be due to actions of lithium on G-proteins (discussed below).

Convincing evidence that lithium ions inhibit stimulated adenyl cyclase not only in vitro or ex vivo but also in the intact brain in vivo was obtained by the microdialysis technique (Masana et al 1992; Manji et al 2000).

Already early after the first description of the inhibitory effects of lithium on adenyl cyclase, Murphy and coworkers (1973) reported that also the attenuating action of noradrenaline on the activity of prostaglandin E$_1$-stimulated adenyl cyclase, is compromised by lithium ions. This inhibitory action of noradrenaline is now known to be mediated by $\alpha_2$-adrenergic receptors, which couple to the inhibitory G-protein G$_i$. More recent research has confirmed an inhibitory effect of lithium also on the inhibitory interaction of receptors with adenyl cyclase (for review see Manji et al 1995, 2000, 2001; Mork et al 1992) and revealed its mediation by an action of lithium on the G$_i$-protein (discussed below). This „inhibition of inhibition“ explains at least partially the increase of basal cyclic AMP in several regions of the brain that is observed after chronic lithium treatment. In addition chronic treatment with lithium salts induces an increase in the content of protein and mRNA of two adenyl cyclase subtypes, most likely due to an increase of gene transcription (discussed below).

The effects of the other mood stabilizers on adenyllylcyclase have not been studied as extensively as lithium. Carbamazepine inhibits basal and stimulated cyclic AMP accumulation both in vitro in neural cell cultures and in vivo in rodent brain and also decreased elevated cyclic AMP levels in cerebrospinal fluid of manic patients (for review see Manji et al 2000). While the acute effects of carbamazepine on cyclic AMP accumulation and on purified adenyllylcyclase activity (van Calker et al 1991; Cheng et al 1996) might be due to a direct interaction with the enzyme, the more chronic effects in vivo could, in principle, also be indirectly mediated by adenosine A$_1$-receptors, which inhibit cyclic AMP accumulation (van Calker et al 1978, 1979) and are upregulated by carbamazepine (Biber et al 1999) due to the specific antagonistic effect of carbamazepine at A$_1$-receptors (van Calker et al 1991). However, upregulation by carbamazepine potentiates predominantly the effects of A$_1$-receptor activation on phosphoinositol signaling (see below) and has little effect on cyclic AMP formation (Biber et al 1999).

Effects of Mood Stabilizers on Inositol Phosphate Signaling

The great surge in the interest on the effects of lithium on the processes of intracellular signaling during the last decade was particularly stimulate by the discovery of the pivotal importance of the phosphatidylinositol (PI)-Ca$^{2+}$-„second messenger“-system for cellular signal transduction and the almost coincident recognition of the modulatory action of lithium ions on this system (for review see Berridge and Irvine 1989; Berridge et al 1989; Berridge 1993). PI’s are only minor components of the lipids in the cell membrane but play an important role in the process of receptor-activated signal transduction, particularly in the central nervous system. Neurotransmitters, including e.g.
noradrenaline, serotonin and acetylcholine, which are believed to be dysregulated in affective disorders, stimulate via particular receptor subtypes (e.g. M₁, M₃, M₅, α₁, 5-HT₂) and activation of G-proteins the hydrolysis of IP’s to two second messenger molecules, DAG and inositol-1,4,5-trisphosphate (IP₃), which, respectively, activate protein kinase C and the intracellular release of Ca²⁺. The latter also enter the cytoplasm from the outside via influx through receptor-operated ion channels. IP₃ is metabolized via several phosphorylation (IP₄) and dephosphorylation steps to myo-inositol, which is used together with CDP-DAG for the resynthesis of PI’s.

The Inositol Depletion Hypothesis

The last step in the metabolism of IP₃, the hydrolysis of inositolmonophosphate to myo-inositol, is inhibited by lithium ions in the therapeutic concentration range (Kᵢ=0.8 mM). In contrast, valproate and carbamazepine, the two other established mood stabilizer, do not inhibit inositolmonophosphatase (Vadnal & Parthasarathy 1995). The now famous „inositol depletion hypothesis“ (for review see Berridge et al 1989) postulates that the lithium-induced inhibition of inositolmonophosphatase leads to a depletion of the cell of myo-inositol and subsequently due to a compromised synthesis of PI’s to a reduction of receptor-stimulated formation of PI-dependent second messenger molecules. This hypothesis has received considerable attention, since for the first time it appeared to offer an explanation for the most enigmatic aspect of lithium’s activity, its almost specific therapeutic effects only on the pathologically altered mood of patients with affective disorders leaving almost unaffected the psychological functioning of normal subjects. According to the hypothesis, this unique feature is due firstly to the uncompetitive nature of lithium inhibitory action on inositolmonophosphatase, an unusual type of enzyme inhibition (Cornish-Bowden 1986), which is the more pronounced the more substrate for the enzyme is available and secondly to the fact that the more frequently the system is stimulated the more inositol phosphates accumulate at the expense of myo-inositol. It was concluded from these unusual properties that a pronounced inhibition by lithium of the PI-system would only occur under conditions, where the respective receptors and their signal transduction system are pathologically overactivated.

From these unique features that are postulated to govern lithium’s effects on PI-signaling it is conceivable, why it has not been possible to obtain a definite proof or falsification of the inositol depletion hypothesis from experiments with experimental animals, since these „normal“ animals are not expected to show a „pathological overactivation“ of the PI-system. The extent of „depletion“ of inositol in lithium treated rats amounts to maximally 35% and is limited to acute treatment with probably already toxic doses of lithium. Whether or not such a limited reduction in the inositol content could have functional consequences for PI-signaling is open to question. It has been argued, however, that a lithium-induced depletion of inositol might be limited to selected brain areas or even cells that might be particularly vulnerable to this effect due to restricted inositol supply and/or increased activity of the PI-system (for review see Gani et al 1993; Jope and Williams 1994). Indeed, we have recently shown that both the basal content of inositol and its uptake in vivo differs among various areas of the rat brain (Patishi et al 1996) and that a reduction in inositol levels after chronic lithium administration was limited to the hypothalamus, a brain region that might indeed be particularly activated during the stressful treatment with lithium (Lubrich et al 1997; Belmaker et al 1998). Thus, further investigations specifically targeted at the effects of lithium in animal models of depression such as the „learned helplessness“ paradigm or other stress-related paradigms might provide an opportunity to detect more meaningful lithium induced alterations in inositol content and PI-signaling. Further support for a more selective localized effect of
lithium on PI-signaling was obtained in experiments with cultured cells in vitro (Jenkinson et al 1993, 1994; Gray et al 1994; del Rio et al 1994; Batty et al 1998; Willars et al 1998). While earlier work with brain slices (for review see Jope and Williams 1994) and later investigations of neural cell cultures in vitro (Varney et al 1992, 1994; Batty and Downes 1994; Chen and Hertz 1996) had identified inhibitory effects of lithium on the agonist-stimulated release of inositol phosphates or Ca\(^{2+}\)-ions, measurements of IP\(_3\) in vivo (Whitworth et al 1990; Jope et al 1992; Gur et al 1996) did not provide evidence for an inhibition by lithium of PI-signaling. Furthermore, work by Hokin and his colleagues made clear that brain slices from species like rat and mice are particularly vulnerable for artificial depletion of inositol during the assay procedures and showed that in slices from primate brain and in neuronal cells of human origin lithium rather increases PI-signaling (Dixon et al 1992; Los et al 1995).

The potential role of myo-inositol in the mechanism of action of lithium has been studied also in animal behavior paradigms. Thus, studies by Belmaker and colleagues provided evidence that effects of lithium administration on behavior (rearing) and the induction of seizures by a combination of pilocarpin and lithium could be prevented by myo-inositol (for review see Belmaker et al 1995), an indication of a role of inositol depletion in these effects.

Surprisingly, effects of lithium treatment on inositol levels in the human brain have been measured only recently (Moore et al 1999). Proton magnetic resonance spectroscopy (MRS) scans of bipolar patients were performed after a medication wash-out (minimum 2 weeks) at baseline and after 5 days and 4 weeks of lithium treatment. The results indicated a significant reduction of myo-inositol content in the frontal cortex already after 5 days of lithium administration, at a time when the patients clinical state was completely unchanged. Thus, while lithium indeed lowers myo-inositol content in the brain this action alone cannot explain the therapeutic effect of lithium but may be the initial trigger that initiates a cascade of events that ultimately account for the therapeutic effect.

In summary, work of the last decade has provided evidence that as predicted by the inositol depletion hypothesis lithium ions indeed modify inositol content and PI-signaling in neural tissue, but that the extent and even the direction of these effects apparently depend in a subtle manner from factors such as species, brain region, cell type and activation state of the cells and tissues. This provokes the question as to whether distinct neural circuits in the brain may be differentially influenced by lithium ions and to which extent these effects might vary with the particular activation state in depressive or manic mood. Thus, a major challenge of future work is to identify the factors that stipulate the sensitivity and direction of response of a cell or neural circuit to lithiums modulating effects on cellular signaling. One of these factors is the high affinity sodium/myo-inositol cotransporter (SMIT), which is discussed in more detail below.

**The High Affinity Sodium/Myo-inositol Cotransporter (SMIT)**

The inositol depletion hypothesis rests on the assumption that brain cells completely rely on inositol formed from inositolmonophosphate, either as a final product of the hydrolysis of inositolphospholipids or as an intermediate of the *de-novo*-synthesis of inositol from glucose-6-phosphate. However, recent evidence indicates that brain cells may also accumulate inositol from the extracellular space to a variable degree, which might stipulate the cellular sensitivity to the inositol depleting effect of lithium (for review see Gani et al. 1993). One potential source of the region-specific differences in inositol content and uptake (Patishi et al 1996) is the sodium/myo-inositol cotransporter
(SMIT), a high affinity myo-inositol transport system, which has been characterized in various cell types including those of neural origin (Wiesinger 1991; Lubrich et al 1999). The SMIT transports myo-inositol in exchange to sodium against a steep concentration gradient into the cell (for review see Gani et al 1993). Among neural cells SMIT appears to be particularly highly expressed in astrocytes (Glanville et al 1989; Lubrich and van Calker, unpublished) and its expression and regulation by osmolality and corticosteroids shows remarkable differences in astrocytes cultured from distinct brain regions (Lubrich et al 1999). Both the activity of SMIT and the expression of its mRNA in astrocytes and astrocytoma cells are downregulated after chronic treatment with therapeutical concentrations of lithium salts, an effect which develops slowly over a time period of 8 days, in remarkable agreement with the time course of lithiums clinical effects. Furthermore, downregulation of SMIT with a similar time course is also observed after treatment with valproate and carbamazepine, indicating that this effect might represent a common mechanism of action of all three antibipolar drugs (Lubrich and van Calker 1999; Wolfson et al 2000). This inhibition of inositol uptake by all three mood stabilizers might explain the recently published finding that not only lithium but also valproate after chronic application decreased the content of myo-inositol in the brain of rats (O’Donnell et al 2000) although valproate does not inhibit inositolmonophosphatase (Vadnal & Parthasarathy 1995). The effect of mood stabilizers on the uptake of inositol in astrocytoma cells was reported to depend on the concentration of inositol: At high extracellular concentrations (50 µM) of inositol the uptake was inhibited while at low (25 µM) concentrations the uptake was rather increased (Wolfson et al 2000). Thus, mood stabilizers may regulate both $K_m$ and $v_{max}$ of the transporter, perhaps via differential action on various variant forms. Indeed, two different $K_m$-values for inositol uptake (50 µM and 25 µM) were determined in astrocytes from different brain regions (Lubrich et al 2000). In addition, the sensitivity to downregulation of inositol uptake by the three antibipolar drugs is not uniform but shows differences among astrocytes from different brain areas. These regional differences both in the kinetic parameters of inositol uptake and in its regulation by hormones and antibipolar drugs might also be explained by the existence of various variant forms of the transporter. This highlights again the necessity to investigate the potential role of myo-inositol in the mechanism of action of lithium and other antibipolar drugs with particular consideration of localized effects.

The activity of SMIT is not only downregulated by antibipolar drugs but also by chronic treatment with myo-inositol at concentrations in the range that are achieved in CSF after oral administration of inositol (Lubrich et al 1999). Oral myo-inositol has therapeutic effects in depressive (Levine et al 1995), panic (Benjamin et al 1995) and obsessive-compulsive (Fux et al 1996) disorders that have been difficult to reconcile with inositol depletion as the postulated mechanism of lithiums action in affective disorder. However, since concentrations of inositol in the CSF and probably also in the extracellular space of the brain are about 400 µM and the $K_m$ values of the high affinity uptake system at least in astrocytes are in the range of 25-50 µM (Lubrich et al 1999) the system appears to work already under saturating conditions. Thus, an increase in extracellular inositol of 70 % as occurs in patients treated with oral inositol (Levine et al., 1993) is unlikely to raise significantly the uptake of inositol into the cells but might rather downregulate and consequently reduce the uptake and thus the intracellular content of inositol in accordance with the suggested mechanism of action of lithium.

Effects of Lithium Therapy on the PI-signaling in Humans
Since the PI-system mediates the effects of hormonal agonists not only in the CNS but also in peripheral cells, a potential inhibitory action of chronic lithium treatment on PI-
signaling as predicted by the inositol depletion hypothesis can be examined in peripheral blood cells of humans. Indeed we have observed in neutrophils of chronically lithium-treated patients that the agonist-stimulated intracellular release of inositolphosphates and Ca\(^{2+}\)-ions was compromised in comparison to untreated normal controls (Greil et al 1991; van Calker et al 1993). On the other hand both neutrophils and platelets of acutely depressive or manic patients show an increased sensitivity of the PI-system as compared to controls (Bohus et al 1996; for review see Warsh and Li 1996). This is, however, not a trait but rather a state marker of the disorder, since this abnormality of signaling is normalized again in remitted patients (Bothwell et al 1994; van Calker et al, to be published). On the other hand, studies using transformed lymphocytes from bipolar patients revealed similar abnormalities, suggesting that there might be also a trait-dependent component of this abnormality in Ca\(^{2+}\)-signaling in bipolar patients (Emanghoreishi et al 1997). An increased sensitivity of the PI-system is also suggested by the finding that protein kinase C activation (Friedman et al 1993) and PI-4,5-bisphosphate (Brown et al 1993) was increased in platelets of manic patients. Also the increased protein kinase C activation was found to be decreased after lithium treatment (Friedman et al 1993). In summary these results provide evidence for an increased sensitivity of the PI-system in peripheral cells of manic-depressive patients that is compensated or even overcompensated by treatment with lithium. A heightened state of activity of the PI-signaling system is also suggested by the finding of enhanced protein kinase C activity in post mortem brain of bipolar subjects (Wang and Friedman 1996).

**Effects of Mood Stabilizers on Proteinkinase Activities and Protein Phosphorylation**

As discussed above, the main effects of lithium on signal transduction are an increase in basal activity of adenylyl cyclase and a decrease of agonist-stimulated activity of this enzyme. Lithium and other mood stabilizers also exerts complex modulatory actions on PI-signaling, the extent and direction of which depend critically on various factors such as species, cell type and activational state. In cases of cellular depletion of inositol the subsequent decreased resynthesis of inositolphospholipids should result in an diminished consumption and thus increased accumulation of DAG and its metabolite CDP-DAG (for review see Fisher et al 1992). An increased cellular content of CDP-DAG is indeed now considered as a reliable indicator of inositol depletion (Stubbs and Agranoff 1993). Thus, lithium ions and, less well established, other mood stabilizing medications modulate the basal and agonist-stimulated concentrations of the second messenger molecules cyclic AMP, IP\(_3\), Ca\(^{2+}\) and DAG and should therefore also influence the activity of the protein kinases A, C, A\(_2\) and others that are regulated by these second messengers.

Several studies have reported that lithium can modulate protein kinase A-mediated protein phosphorylation (Casebolt and Jope 1991; Guitard and Nestler 1992; Jensen and Mork 1997; Zanardi et al 1997; Mori et al 1996, 1998). Chronic lithium treatment increases in the frontal cortex of the rat the level of DARPP-32 (32 KDa dopamine and cyclic AMP regulated phosphoprotein), a protein phosphatase inhibitor. DARPP-32 is itself activated by phosphorylation, e.g. by dopamine via D\(_1\)-receptors and several other hormones and neurotransmitters (Guitard and Nestler 1992). Alterations in DARPP-32 could modify the phosphorylation state of several other proteins. The specific proteins regulated by DARPP-32-phosphorylation have not been identified, although Na\(^+\)-K\(^+\)-ATPase represents a possible target. A potential role of this enzyme in the mechanism of action of lithium has long been discussed (El-Mallakh and Li 1993; El-Mallakh and Wyatt 1995; for review of the older literature see Wood and Goodwin 1987).
Alterations of PKC activity by lithium have been conjectured already early after the formulation of the inositol depletion hypothesis (Drummond 1987), since a depletion of inositol should result in a decreased consumption for PI-resynthesis and thus increased accumulation of DAG, the activator of PKC. Indeed, a modulation by chronic lithium of PKC activity is now considered to play a key role in the mechanism of lithium's therapeutic effects (for review see Manji and Lenox 1994; Jope and Williams 1994; Manji et al 1995; Manji et al 1996b; Lenox et al 1996, 1998). PKC activity represents the action of a family of closely related subspecies, which are highly enriched in the brain. It regulates many pre- and postsynaptic aspects of neurotransmission including long-term alterations in gene expression and neuronal plasticity (for review see Dekker and Parker 1994; Shearman et al 1998; Ben Ari et al 1992). Persistent activation of PKC is often followed by its rapid proteolytic degradation and downregulation of enzyme activity. This explains, why in several cell systems lithium after acute or subchronic treatment induces an increase, while chronic treatment results in a decrease of PKC or PKC-mediated processes (for review see Manji and Lenox 1994). This lithium-induced decrease in PKC activity appears to be restricted to specific isoenzymes of PKC such as α and ε, which may have a particular role in the regulation of neurotransmitter release (Manji et al 1996a,b; Li and Jope 1995; Manji & Lenox 1999). The modulation by lithium of PKC isoenzymes in the brain is apparently not uniform but specific for particular hippocampal structures. Furthermore, the proteolytic degradation of PKC may lead to the formation of a constitutively active fragment („PKM“) that could be involved in the regulation of gene expression (Manji and Lenox 1994). Thus, the alterations in gene expression observed after chronic lithium treatment (see below) could at least in part be mediated by effects on PKC. The modulation by lithium of PKC activity could also explain its actions on neurotransmitter systems, since PKC regulates the release of several neurotransmitters, such as serotonin and noradrenaline (Wang and Friedman 1989), which is also influenced by lithium (Sharp et al 1991). Lithiums ability to potentiate in rats the seizures evoked by stimulation of muscarinic receptors, might also involve modulation of PKC (Manji and Lenox 1994; Jope and Williams 1994).

The modulation by lithium of PKC activity should result in altered phosphorylation of PKC substrates. Indeed, a major substrate of PKC in the brain, myristoylated alanine-rich C-kinase substrate (MARCKS) is downregulated by both PKC activation and chronic treatment with lithium at therapeutically relevant concentrations. This effect of lithium is particularly compelling since MARCKS appears to play an important role in the restructuring of cytoskeletal elements that are involved in the long-term alteration of processes such as signal transduction and neurotransmitter release (for review see Lenox et al 1996, 1998). Since MARCKS is involved in the regulation of PI-signaling (Glaser et al 1996) its alteration by chronic lithium might also be responsible for some of the effects of lithium on signal transduction via this system. The potential importance of these effects of antidepressive drugs on PKC becomes also evident from results of the post-mortem studies mentioned earlier (see above), which suggested an increased PKC-mediated phosphorylation and elevated levels of PKC isoenzymes in the brains of patients with bipolar disorder as compared to controls (Wang and Friedman 1996).

The precise molecular mechanisms by which lithium modulates PKC isoenzymes are presently unclear. However, both the effects of lithium on the ε (and perhaps α) isoform of PKC (Manji et al 1996a) and the apparently PKC mediated downregulation of MARCKS (Watson and Lenox 1996) by chronic lithium are reversed or prevented by coadministration of myo-inositol in agreement with the principal role of inositol depletion
in these effects (for review see Lenox et al 1998). Valproate at therapeutic concentrations has similar effects on PKC isoenzymes (Chen et al 1994) and MARCKS protein expression (Lenox et al 1996; Manji & Lenox 1999; Watson et al 1998) as lithium. Both drugs also inhibit at chronic application the high affinity inositol uptake (see above) with a strikingly similar triphasic time course (Lubrich and van Calker 1999), which can be partially mimicked by PKC inhibitors (Lubrich et al, unpublished). Thus, both lithium and valproate may act by way of modulation of PKC activity, which feeds back on regulation of myo-inositol homeostasis and phosphoinositide signaling and also modifies proteins such as MARCKS that are involved in restructuring of cytoskeleton and consequently neuronal architecture.

Evidence has accumulated recently, that one major aspect of lithiums mechanism of action could be its effect on cytoskeleton dynamic, since lithium reduces the phosphorylation of cytoskeleton associated proteins (Burstein et al 1985; Bennet et al 1991) and may influence cytoskeletal rearrangements not only by its effects on PKC but also via inhibition of glycogen synthase kinase 3β (GSK3β). This enzyme was originally identified as regulator of glycogen synthesis and is now known to exert also important actions in the central nervous system via regulation of cytoskeletal processes. It phosphorylates microtubule associated proteins (MAPs), such as tau and MAP-1B, important components of the cytoskeletal network. Inhibition of GSK3β by lithium leads to reduced phosphorylation of tau (Klein and Melton 1996; Stambolic et al 1996; Hong et al 1997) and MAP-1B (Lucas et al 1998; Garcia-Perez et al 1998). Most importantly, an inhibitory effect on GKS3β (and GSK3α) was also shown for valproate at therapeutic concentrations (Chen et al 1999). Recent evidence indicates, that inhibition by lithium and valproate of GSK3 might contribute to the effects of these mood stabilizers on CREB DNA binding activity (Grimes & Jope 2001) and also to their anti-apoptotic effects (King et al 2001)(discussed below). Thus inhibition of GSK3 represents another potential common target in the mechanism of action of mood stabilizers.

**Effects of Mood Stabilizers on G-Proteins**

As discussed above, the effects of mood stabilizers on the adenylyl cyclase system and its actions on phosphoinositol signaling suggest the involvement of mechanisms beyond alterations of the catalytic subunit of adenylyl cyclase or depletion of intracellular inositol respectively. One additional mechanism by which mood stabilizer could modify the activity of these signal transduction systems is the alteration of activity of the heterotrimeric G-proteins, crucial molecular switches that amplify the signals impinging on the outside of a cell and relay them to the subsequent effector systems. Accordingly, early studies attracted much attention, which appeared to show a direct inhibitory influence of lithium on the coupling of various receptors to G-proteins (Avissar et al 1988; Drummond 1988). However, the robustness of these findings is now questionable (Ellis and Lenox 1991; Mork et al 1992; Manji et al 1995). An influence of lithium on G-protein function can be assessed by agents, which directly activate or inactivate G-proteins via specific mechanisms that bypass the receptors. Such agents are cholera toxin and pertussis toxin, which via ADP-ribosylation activate or inhibit, respectively, the Gs-protein, which stimulates and the Gi-protein, which inhibits adenylyl cyclase. Using this approach it was shown by in-vivo experiments with microdialysis technique that chronic lithium treatment inhibits the function of Gi thereby increasing the basal level of cyclic AMP in the brain of rats (Masana et al 1992; Manji et al 2000). Experiments with humans chronically treated with lithium also revealed an increase of basal cyclic AMP, and also
of stimulated cyclic AMP accumulation, when the stimulation was performed in a way that bypassed the receptors (for review see Manji et al 1995). Also these effects were explained by an lithium induced inactivation of the inhibitory \( G_i \). On the other hand, chronic lithium treatment apparently also inhibits the function of \( G_s \), since the inhibition by chronic lithium of the receptor-mediated activation of adenylyl cyclase is counteracted by GTP (for review see Mork et al 1992; Jope and Williams 1994). The acute inhibitory effect of lithium on adenylyl cyclase, in contrast, are apparently mediated by competition with Mg\(^{2+}\) ions at the catalytic subunit (see above). The biochemical mechanisms of lithiums action on the function of \( G_i \) and \( G_s \) are presently unclear. The inhibition of \( G_i \) is apparently due to a lithium-induced stabilization of the undissoziated, inactive heterotrimeric \( \alpha \beta \gamma \) state of the \( G_i \)-protein. Only this form is subject to ADP-ribosylation by pertussis toxin, which was indeed increased in platelets from humans under chronic lithium therapy and in rats chronically treated with lithium (for review see Manji et al 1995, 2000).

The reason for this stabilization by chronic lithium of the heterotrimeric form of \( G_i \) is presently unclear. We have, however, very recently identified in neutrophils of patients chronically treated with lithium an upregulation of the mRNAs of one member of the regulators of G-protein signaling (RGS-3) (Spleiss et al 1999), which might be related to this effect. RGS-3 acts as a GTPase-activating protein, which inhibits the function of \( G_i \) by decreasing its GTP-dependent dissociation into \( \alpha \) and \( \beta \gamma \) subunits and thus stabilizing the undissoziated heterotrimeric form, i.e. the very effect of lithium thought to be instrumental to inhibition of \( G_i \) functioning. Thus, as already may have conjectured from their limitation to chronic treatment, these lithium-induced effects on G-proteins appear to be due to an influence of lithium on gene transcription of factors that postranscriptionally modify G-protein function. However, also the gene transcription of G-proteins themselves may be modified by chronic lithium treatment. Chronic lithium application appears to downregulate the mRNAs of a number of G-protein \( \alpha \)-subunits in rat brain including \( \alpha_{i(1,2)} \) and \( \alpha_s \), while varying results were reported for the respective proteins (for review see Manji et al 1995, Lenox et al 1998). More recent studies pointed to more localized effects of chronic lithium on several different G-protein \( \alpha \)-subunits in various regions of the rat brain (McGowan et al 1996; Dwivedi et al 1997; Jakobsen et al 1998).

We have recently shown that in neutrophils of bipolar patients under chronic lithium therapy the content of \( \alpha_i \) mRNA is increased as compared to untreated patients or controls, while lithium treated unipolar depressive patients show no alterations of \( \alpha_i \) mRNA (Spleiss et al 1998). In addition, also the amount of \( \alpha_s \)-mRNA (Spleiss et al 1998) and \( \alpha_s \)-protein (our unpublished results) is increased in neutrophils of bipolar but not unipolar patients. This increase is, however, also observed in untreated bipolar patients and thus independent of chronic treatment with lithium. Similarly, enhanced levels of \( \alpha_s \)-protein were also found in post-mortem brain samples (for review see Warsh and Li 1996) and in platelets of bipolar patients (Mitchell et al 1997). Since it is independent of the psychopathological state and was not found in unipolar patients, this enhanced level of \( \alpha_s \)-mRNA may turn out as a potential trait-marker of bipolar illness. However, although these results suggest a potential pathophysiological role of \( \alpha_s \) in bipolar disorder, no abnormalities in the gene for the \( \alpha_s \)-subunit could be identified in bipolar patients (Ram et al 1997). Thus, the ultimate genetic alterations leading to an enhanced vulnerability for bipolar disorder may be rather found in the genes for transcription factors that regulate \( \alpha_s \)-gene transcription. Furthermore, since only bipolar patients that show
abnormal $\alpha_i$-expression but not unipolar patients are sensitive to the lithium-induced upregulation of $\alpha_i$ mRNA (see above), this limitation of the effect of lithium to bipolar patients raises the intriguing possibility that only abnormal signal transduction may be affected by lithium and that the upregulation of $\alpha_{i2}$ -mRNA might be related to the clinical efficacy of lithium in bipolar disorder.

**Effects of Mood Stabilizers on Gene Expression**

As already mentioned above the activation by second messengers of protein kinases can lead to phosphorylation of nuclear transcription factors that regulate gene expression (for review see Karin 1994). Therefore, in agreement with the effects of mood stabilizers on second messenger systems, they also affects the expression of a number of genes, most likely at least in part also secondary to modulation of PKC and/or GSK3 activity (for review see Manji et al 1995, 2001). The effects of chronic lithium treatment on various components of signal transduction systems such as G-proteins and adenylyl cyclase subtypes have been already mentioned above. Of particular interest are the actions of mood stabilizers on so called „immediate early genes“, members of the c-fos and c-jun families, which encode proteins that form the constituents of a family of transcription factors called AP-1 (activator protein 1). These genes are of pivotal importance for long-term changes in neuronal function (for review see Silva and Giese 1994). Genes regulated by AP-1 include e.g. neurotrophins, neuropeptides, neurotransmitter synthesizing enzymes and other transcription factors (Hughes & Dragunow 1995). It is now well established that lithium regulates AP-1 binding activity and function (Yuan et al 1998, 1999). Also this effect it shared by valproate (Cheng et al 1999b). There is, however, a disconcerting complexity of many apparently contradictory findings in this area of research, with reports indicating both increases and decreases as well as no changes evoked by lithium in various different in vitro and in vivo models. Jope (1999) has recently attempted to integrate these data in his model of a „bimodal mechanism of action of lithium“, which conjectures as the critical action of lithium a balancing of positive and negative regulators of signaling processes, thereby stabilizing signaling activities within an optimal range. The mechanisms by which lithium and valproate regulate AP-1 have not been elucidated but a role of both PKC isoenzymes and GSK3 (see above) can be envisaged (Manji et al 2001). The regulation by lithium of transcription factors is obviously not restricted to AP-1, since it also modulates two other such factors, cyclic AMP responsive element binding protein (CREB) and NF(nuclear factor)-kB (Ozaki and Chuang 1997; Jope and Song 1997). In addition, lithium alters the level of various other mRNAs in the brain and neural cell cultures (e.g. for neuropeptides, tyrosine hydroxylase, glucocorticoid type II receptor) that are important in the regulation of several aspects of neural function (for review see Manji et al 1995; Lenox et al 1998). The further analysis of the question, which, if any, of these effects are critical for the therapeutic action of lithium will be decisive for the further development of the field.

**Effects of Mood Stabilizer on cellular resilience**

Although neuroprotective effects of mood stabilizers are known for some time (Bruno et al 1995; Nonaka et al 1998) the importance of actions of mood stabilizers on cellular resilience in the central nervous system was only recently fully appreciated (for recent reviews see Manji et al 2000, 2001). The neuroprotective and antiapoptotic effects of mood stabilizers are at least partly explained by the finding that lithium and valproate
upregulate the neuroprotective and anti-apoptotic protein bcl-2 (Chen et al 1999c). Some of these effects may be in part mediated via inhibition of GSK3β (King et al 2001). That neuroprotection could be also an important mechanism of action of mood stabilizers in vivo is suggested by the findings that lithium induces neurogenesis in adult rodent brain (Chen et al 2000) and increases N-acetyl-aspartate levels (a putative marker of neuronal viability and function) (Moore et al 2000) and total grey matter in human brain (Moore et al 2000b). These results are particularly important in view of the recent evidence from brain imaging and post mortem studies that mood disorders are associated with morphometric changes suggestive of cell loss and/or atrophy (Drevets et al 1997; Ongur et al 1998; Rajkowska et al 1999, 2000, 2001; reviewed by Manji et al 2000, 2001; Rajkowska 2000; Drevets 2001).

Conclusions

The original hopes that the investigation of the mechanism of action of lithium might provide a key to the understanding of the pathogenesis of affective disorders have been left unfulfilled. However, the recent progress in the unraveling of the effects of lithium and other mood stabilizers on signal transduction systems has identified a number of mechanisms shared by lithium and valproate, and, to a lesser extent, of carbamazepine (fig. 2). It has also offered a conceptual framework that might help to integrate the hitherto disconcerting and disparate actions of lithium on e.g. neurotransmitters and membrane transport systems (for review see Wood and Goodwin1987; Bunney and Garland-Bunney 1987) into a comprehensive model. Thus, many of the actions of lithium on neurotransmitter release may be ultimately explained by its modulation of PKC activity, which critically depends of various factors such as acute vs. chronic administration, brain region and particular isoenzyme type. On the other hand, lithiums effects on Na⁺-K⁺-ATPase, which may be mediated via its action on DARPP-32 (see above) illustrate, how the modulating actions of lithium ions on protein phosphorylation might explain effects on mechanisms of membrane transport. Since the processes of sensitization and de-sensitization of receptors are determined by the function of signal transduction systems (for review see Hadcock and Malbon 1993), it is conceivable that also the long disputed (for review see Bunney and Garland-Bunney 1987) effects of lithium on receptor sensitivity are mediated by its action on second messenger systems. The effects of lithium on immediate early genes point to a potential modulation by lithium of neural plasticity phenomena, which determine the processes of learning and memory. Memory complaints are often associated with lithium therapy.

The effects of mood stabilizers on signal transduction and particularly the potential modulation of phenomena of neural plasticity are also of great interest with respect to the concept that kindling-like sensitization of neural pathways might be responsible for the long-term course of affective disorders. The influence of psychosocial stress as a trigger for affective disorder is often pronounced in the first episode, but diminishes in the subsequent episodes, while the frequency and severity of episodes increases. This phenomenon exhibits a formal similarity to the processes of kindling and behavioral sensitization, in which repeated stimuli such as stress, drugs like cocaine or electrical stimulation evoke not an adaptive decrease but rather an gradual increase in response, until ultimately the reaction might occur spontaneously without any triggering event (for review see Post 1992). The opposite phenomena sensitization and adaptation (Hyman and Nestler 1996) are most likely determined by a distinct pattern of timing and intensity of the stimuli. The molecular mechanisms of these processes are not understood, but
alterations of components of signal transduction are almost certainly of pivotal importance. Little is known about the effects of mood stabilizers on such phenomena of neural plasticity and stress-induced behavioral sensitization, although first evidence indicates inhibitory actions of lithium on long-term potentiation (Aronica et al 1991), a molecular paradigm of learning (for review see Anwyhl 1989; Malenka 1994), and „learned helplessness“ (Taxeira et al 1995), a stress-induced depression-like behavior of rats. The application of modern molecular biological methods for unraveling the biochemical mechanisms of action of mood stabilizers in such animal models of affective disorders should help to identify among the multitude of biochemical effects those that are clinically relevant.

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Signal transduction systems

Neurotransmitter/-hormones e.g. 5HT, noradrenaline

Ca-Ions

Endopl. Ret.

R αs βγ
R αi/o βγ
R αq βγ

βγ αs
βγ αi/o
βγ αq

βγ

AC

PLC

ATP
Cyclic AMP
Proteinkinase A
Gene Transcription e.g. via CREB, AP-1

IP3
PIP2
DAG
Proteinkinase C
Ca-dependent Kinases

Neuroprotection
Synaptic Remodelling
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<th>Carbamazepine</th>
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<td>Rezeptor Sensitivity</td>
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<td>G-Proteins</td>
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Legends to the figures:

Fig. 1: Signal transduction Systems

Fig. 2: Comparison of the biological effects of mood stabilizers.