Lithium Takes Indirect Route to Inhibit GSK-3β

Adapted from a story that originally appeared on the Alzheimer Research Forum.

20 January 2008. Lithium is an old and valued drug for mood disorders, whose mechanism of action remains murky. Inhibition of the glycogen synthase kinase 3 β (GSK-3β) has been implicated, but lithium inhibits this enzyme only weakly and requires much higher concentrations to block it in vitro than to elicit a therapeutic effect in vivo. Now, work from the lab of Marc Caron at Duke University Medical Center, Durham, North Carolina, published in the January 11 issue of Cell, shows that lithium can inhibit GSK-3 in vivo, but does so indirectly. It disrupts its signaling through the upstream kinase Akt. Specifically, lithium breaks up a magnesium-dependent association of Akt with the scaffolding protein β-arrestin 2 (β-ARR2). That results in activation of Akt, which then phosphorylates and inhibits GSK-3β.

There are some provisional links between members of this pathway and schizophrenia, including genetic association studies implicate AKT1 as a susceptibility gene (see SchizophreniaGene entry), and studies that suggest that antipsychotic drugs regulate Akt/GSK-3β signaling (e.g., Roh et al., 2007; Li et al., 2007; SRF related news story).

First author Jean-Martin Beaulieu used β-arrestin 2 (βARR2) knockout mice to probe the role of this scaffolding protein in lithium action. βARR2 organizes a signaling complex on G protein-coupled receptors that includes the Akt kinase. The complex does not require G proteins for activity, and thus represents an alternative signaling pathway utilized by GPCRs. Beaulieu and colleagues show that lithium injection into the striatum of mice led to activation of Akt and the subsequent inactivation of GSK-3β kinase. Lithium had no such effect in βARR2 knockout mice, nor did the mice show the expected behavioral effects of lithium treatment. The knockout mice were also refractory to chronic changes in the activity of Akt or GSK-3, and associated expression of the β-catenin gene, upon prolonged lithium treatment.

In-vitro immunoprecipitation studies showed that βARR2 was required to see interaction of Akt with protein phosphatase 2A in a signaling complex. Lithium prevented the association of Akt with βARR2 or Akt and two different subunits of PP2A. A similar effect was seen in vivo. Lithium broke up the complex by competing with magnesium, which was required for the interaction. Importantly, all these effects occurred at concentrations of lithium that are attained therapeutically. The effects of lithium appeared specific to the GPCR-mediated regulation of the Akt pathway, and the drug did not interfere with other functions of either βARR2 or G protein-dependent receptor signaling. The results indicate that lithium may not generally inhibit the pathway, but instead offers a means of precisely targeting selective GPCR functions that rely on arrestin-containing signaling complexes.

These pathways are not currently front and center in schizophrenia research, but several groups, in particular the laboratories of Joseph Gogos and Maria Karayiorgou at Columbia University, New York, have investigated the role of AKT in both schizophrenia etiology and antipsychotic drug effects (see SRF related news story). And, as Beaulieu and colleagues point...
out, lithium is used to enhance the effects of antipsychotic drugs in refractory cases. "Identification of the Akt:bArr2:PP2A signaling complex as a molecular target of lithium thus provides a mechanism by which this pharmacological agent may enhance the actions of other drugs acting through Akt/GSK3 signaling by preventing the inhibition of Akt by PP2A," they write.—Pat McCaffrey.

Reference:

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Comment by: Takeo Yoshikawa, Akihiko Takashima
Submitted 30 November 2006

In this study, Karayiorgou and Gogos’s group have conducted a meticulous anatomical analysis of pyramidal cell dendritic structures in the prefrontal layer V cortex, as well as genome-wide expression and pharmaco-behavioral analyses, focusing on prefrontal functions in Akt1-deficient mice. The study examines the reduced (or altered) AKT1-GSK3β signalling theory of schizophrenia, proposed by this (Emamian et al., 2004) and other groups.

AKT1 as a genetic susceptibility gene for schizophrenia shows promise in the Caucasian population but this is not reflected in Asian populations as evidenced by our results (Ide et al., 2006). In addition, even in Caucasians, true causal variants have not been identified. Because of this, schizophrenia researchers are interested in observing disease-relevant phenotypes in Akt1-deficient mice. In this study, they have detected morphological and functional alterations of frontal cortex-related traits in mutant mice using state-of-the-art techniques.

To further strengthen AKT1 as a candidate disease gene in schizophrenia, several issues need to be addressed in the near future. For instance, if a reduction of AKT1 signalling occurs in the brain, tau should be hyper-phosphorylated by activated GSK3β, which in turn will lead to the formation of neurofibrillary tangles (NFTs) as seen in Alzheimer’s disease. Therefore, it would be interesting to determine whether Akt1-deficient mice show a similar pattern of tau phosphorylation. Accumulating evidence suggests that hyper-phosphorylated tau may affect a variety of neuronal functions. Our recent biochemical analyses failed to reveal any significant reduction of AKT-mediated signalling in the prefrontal cortex of schizophrenic brains or the expected inverse correlation between phosphorylation levels of AKT and tau (Ide et al., 2006). This highlights the difficulty of examining protein phosphorylation status using postmortem brains, where results are often confounded by multiple, uncontrollable factors.

Another important but poorly understood point is the functional relationship among subspecies of the AKT family (at least AKT1, AKT2 and AKT3) and GSK3 (GSK3α and GSK3β) (for example see Sale et al., 2005). We look forward to continuing multidisciplinary studies aimed at unravelling the role of the AKT cascade, including the clarification of downstream

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Implicit in the findings of Schmid et al. is the idea that the relationship among ligand, receptor signaling, and cellular context is an extremely complex one that will take a great deal more work to tease out. Thus, Dr. Bryan Roth has proposed on a number of occasions (see, for example, Gray and Roth, 2007; Abbas and Roth, 2005) that novel approaches for drug discovery may prove more effective in producing schizophrenia drugs that have greater therapeutic efficacy with lower side effect liability. Since it will likely be many years before the field has a detailed understanding of the "nitty-gritty" of the receptor signaling and trafficking relevant to schizophrenia and its treatment, we have suggested a number of approaches that are less reliant on such information.

For example, approaches based on screening for drugs that either mimic the gene expression profiles of gold standard drugs such as clozapine or normalize schizophrenia-associated changes in gene expression are being explored. Another approach is behavior-based screening, in which targeted screens are performed with drugs to find those that have efficacy in animal disease models. A further related approach, exemplified by Psychogenics' Smartcube(TM) (the associated database is called Smartbase[TM]) involves injecting drugs and monitoring the resulting behavior using computer-based machine learning to generate a multidimensional behavioral signature for gold standard drugs. Drugs can then be screened to look for those that mimic gold standard drugs in terms of their signatures. Though Psychogenics does not appear to have done much (at least publicly) with this approach, it represents the sort of innovative thinking that may prove fruitful in future behavior-based drug discovery efforts since it is not dependent on knowing anything about the mechanism. In the end, at least in the near future, we believe such approaches may prove extremely useful in drug discovery efforts since they do not rely on extensive mechanistic knowledge of the processes underlying schizophrenia.

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Reevaluation of the dopamine D<sub>2</sub> receptor in the treatment of schizophrenia: Novel intracellular mechanisms as predictors of antipsychotic efficacy

Since the advent of antipsychotic medications, there have been many speculations about their precise mechanisms of therapeutic action. Although it is apparent that blockade of dopamine D<sub>2</sub> receptors (D<sub>2</sub>R) is crucial to the efficacy of all current antipsychotic medications, it is not clear which signaling events downstream of the D<sub>2</sub>R must be blocked for the therapeutic actions of antipsychotics and which events, when blocked, lead instead to side effects.

The best characterized D<sub>2</sub>R-mediated signaling pathways involve coupling of the receptor to pertussis toxin-sensitive G proteins of the G<sub>i</sub> and G<sub>o</sub> subfamilies (Sidhu and Niznik, 2000), through which D<sub>2</sub>R activation results in a decrease in cyclic AMP (cAMP). D<sub>2</sub>R activation can also have a number of other effects, including enhancement of specific potassium currents, inhibition of L-type calcium currents, mediation of extracellular signal-regulated kinase 1 (ERK1) and potentiation of arachidonic acid release (Beom et al., 2004; Missale et al., 1998; Perez et al., 2006; Hernández-López et al., 2000). There is growing evidence that D<sub>2</sub>Rs can interact with a number of membrane-bound or intracellular proteins, which may further modulate signaling specificity (reviewed in Terrillon and Bouvier, 2004; Ferré et al., 2007a). In particular, D<sub>2</sub>R heteromerization may result in a switch from G<sub>i</sub>/o coupling to G<sub>s</sub> (i.e., through D<sub>2</sub>R and cannabinoid 1 receptor interaction) (Kearns et al., 2005) or to coupling with G<sub>q</sub> (as suggested in D<sub>2</sub>R and D<sub>1</sub>R interactions) (Rashid et al., 2007). Moreover, heteromerization between D<sub>2</sub>R and other receptors such as the adenosine A<sub>2A</sub> receptor may allow for reciprocal modulation of D<sub>2</sub>R function (Ferré et al., 2007a; Ferré et al., 2007b). It also has been suggested that calcium signaling mechanisms may modulate D<sub>2</sub>R's signaling efficacy; interaction between D<sub>2</sub>R and calcium-binding protein S100B results in enhanced D<sub>2</sub>R intracellular signaling (Liu et al., 2008; Stanwood, 2008).

The interaction between D<sub>2</sub>R and arrestin has received increasing attention. Following D<sub>2</sub>R activation, D<sub>2</sub>R signaling is attenuated by recruitment of arrestin 3 to the cell surface where it binds to the receptor (Klewe et al., 2008; Lan et al., 2008a; Lan et al., 2008b), leading to inactivation and internalization of the D<sub>2</sub>R. Arrestin 3 also binds Akt—a serine/threonine kinase involved in multiple cellular functions and implicated clinically in schizophrenia (Arguello and Gogos, 2008; Beaulieu et al., 2005; Brazil and Hemmings, 2001; Brazil et al., 2004; Emamian et al., 2004; Kalkman, 2006). Following D<sub>2</sub>R activation by dopamine, the signaling scaffold formed by arrestin 3, while facilitating receptor desensitization and internalization, also recruits Akt into a complex with the phosphatase PP2A, which dephosphorylates and consequently inactivates Akt (Beaulieu et al., 2007a). Thus, D<sub>2</sub>R activation inhibits Akt activity through an arrestin-dependent but G protein-independent pathway (Beaulieu et al., 2007a; Beaulieu et al., 2007b). Curiously, the mood stabilizer, lithium, has been shown to disrupt the arrestin 3-Akt-PP2A complex, thereby preventing dopamine-induced dephosphorylation of Akt and blocking amphetamine-induced locomotion.

Related News: An Arrestin Development: Antipsychotic Drugs Hit Dopamine Signaling in New Way

Comment by: Zachary Z. Freyberg, Eneko Urizar, Holly Moore, Jeffrey Lieberman, Jonathan Javitch
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Moreover, amphetamine-induced locomotion is greatly diminished in arrestin 3 knockout mice, suggesting that this pathway is critical to at least some psychostimulant effects (Beaulieu et al., 2005).

Using newly developed BRET (bioluminescent resonance energy transfer) biosensors in assays that measure direct protein-protein interactions within the living cell, recent studies have demonstrated that antipsychotic medications prevent arrestin 3 recruitment by blocking D$_2$R activation (Klewe et al., 2008; Masri et al., 2008). Masri et al. (2008) hypothesized that antipsychotic drugs achieve their therapeutic effect through a common mechanism involving blockade of arrestin-mediated signaling (Masri et al., 2008). Masri et al. (2008) also demonstrated that nearly all antipsychotics tested (including haloperidol, clozapine, olanzapine, desmethylclozapine, chlorpromazine, quetiapine, risperidone and ziprasidone) behave as inverse agonists to decrease constitutive G protein signaling as well as to prevent the agonist quinpirole from inhibiting cAMP synthesis (via D$_2$R-mediated G$_i$/o signaling). The lone exception, aripiprazole, behaved as a partial agonist instead of as an inverse agonist of the G protein mediated effects. The latter finding is consistent with previous studies highlighting aripiprazole’s ability to differentially modulate various G protein-mediated effector pathways, a property termed “functional selectivity” (Mailman, 2007; Urban et al., 2007). Using the BRET assay, Klewe et al. (2008) and more recently Masri et al. (2008) demonstrated that all antipsychotics, including aripiprazole, block arrestin 3 recruitment. This finding has led Masri et al. (2008) to suggest that blockade of arrestin 3 recruitment to the D$_2$R, and not modulation of G-protein-mediated pathways, is a common and specific property of all current antipsychotics and may be used to predict the antipsychotic efficacy of drugs in development (Masri et al., 2008). This hypothesis remains to be tested and at present appears to lean heavily on the evidence for aripiprazole’s atypical effects on constitutive (non-agonist-dependent) D$_2$R-mediated G-protein signaling. Indeed, the fact that lithium acts to prevent arrestin-mediated signaling in response to amphetamine but is not an effective antipsychotic in monotherapy suggests that antipsychotic action may be more complex than simple blockade of D$_2$R-mediated arrestin signaling. In addition, the ability of antipsychotics, including aripiprazole, to block agonist binding to the D$_2$R and thus activation of the receptor, makes it likely that agonist-induced activity in multiple signaling pathways will also be blocked by these drugs.

Despite the paucity of direct evidence for D$_2$R-arrestin coupling as the mechanism underlying the antipsychotic effects of drugs, the hypothesis remains quite intriguing. Given that Akt and its downstream target GSK-3 (glycogen synthase kinase-3) have been implicated in schizophrenia in a number of genetic and postmortem studies, and the Akt/GSK-3 pathway might represent an opening into alternative therapeutics of schizophrenia. Akt is a serine/threonine kinase that may have significant roles in synaptic physiology and neurodegeneration (Brazill et al., 2004). Recruited to the cell surface by binding to phosphatidylinositol 3,4,5 trisphosphate, Akt is activated via phosphorylation of 3-phosphoinoitoide-dependent protein kinase 1 (PDK1) and the rictor-mTOR complex (Brazill and Hemmings, 2001; Sarbassov et al., 2005). Once active, Akt phosphorylates GSK-3, thereby inactivating it. Since D$_2$R activation leads to inactivation of Akt, this also results in increased GSK-3 activity (Beaulieu et al., 2004; Lovestone et al., 2007). GSK-3 activity also plays an important role in modulating the dopaminergic response to amphetamine. Amphetamine’s stimulation of DAT-mediated dopamine efflux and subsequent D$_2$R stimulation likely results in Akt inactivation and increased GSK-3 activity. Rats treated with the specific GSK-3 inhibitor, AR-A014418, failed to display amphetamine-induced hyperactivity (Gould et al., 2004). Similarly, heterozygous GSK-3β knockout mice (expressing approximately half of...
wildtype levels of GSK-3β) displayed significantly reduced levels of locomotor activity following amphetamine treatment (Beaulieu et al., 2004). Additionally, treatment of dopamine transporter (DAT) knockout mice with multiple GSK-3 inhibitory drugs inhibited the ordinarily hyperactive behavior of the non-treated DAT knockout mice (Beaulieu et al., 2004).

In a mouse model, acute and chronic haloperidol treatment was shown to increase levels of active, phosphorylated Akt isoform Akt1 and increased phosphorylation and inactivation of GSK-3β (Emamian et al., 2004). Thus, it was suggested that haloperidol treatment may compensate for the decreased levels of endogenous Akt1 in the frontal cortex of people with schizophrenia (Emamian et al., 2004). Atypical antipsychotics also impact on the regulation of Akt and GSK-3β activities. For example, treatment with clozapine results in increased levels of phosphorylated GSK-3β (Kang et al., 2004; Sutton et al., 2007). Interestingly, however, differences between haloperidol and atypical antipsychotics have emerged in the kinetics of Akt/GSK-3 phosphorylation, the levels of proteins expressed following drug exposure, and the signaling pathways that are preferentially activated (Roh et al., 2007).

The abilities of antipsychotic drugs to activate distinct signaling pathways to mediate their ostensible differential pharmacologic effects would suggest clinical variation in their therapeutic effects. However, meaningful differences in the clinical effects of these compounds have not been clearly or consistently evident. The initial reports of superior efficacy of the so-called second generation or atypical antipsychotics on measures of psychosis (Kane et al., 1988), negative symptoms (Tollefson et al., 1997), cognitive deficits (Keefe et al., 1999), relapse prevention (Csernansky et al., 2002), adherence (Wahlbeck et al., 2001) and illness progression (Lieberman et al., 2005a), have not been borne out by more recent studies (Geddes et al., 2000; Lieberman et al., 2005b; Jones et al., 2006; Leucht et al., 2008). Indeed, the differences between antipsychotic drugs are most evident in the types, frequency and severity of side effects rather than in their therapeutic actions (Leucht et al., 1999; Allison et al., 1999; Henderson et al., 2005). In this regard the emerging pattern of variation in the molecular mechanisms of antipsychotic drugs in the face of their common clinical profiles resembles what was previously observed with the variability in neuroreceptor binding profiles (Kinon and Lieberman, 1996). The marked differences in the affinities and selectivity of the various antipsychotics for the receptors of different neurotransmitters were thought to underlie a rich pattern of clinical variation in the therapeutic actions of this group of drugs (Miyamoto et al., 2005). However, this hypothesis has not been supported by clinical studies (Lieberman, 2006; Lewis and Lieberman, 2008).

Nevertheless, there is reason to be hopeful that through functional selectivity, or other potential actions, the abilities of drugs to engage different signaling pathways will confer novel therapeutic effects that will improve the efficacy of treatments. In this context, the studies of Masri et al. (2008) and Klewe et al. (2008) highlight the plausibility that D2R/arrestin 3 modulation of Akt and GSK-3 activity is an important mechanism underlying psychosis and a potential target for future antipsychotic drugs. Further study of this pathway, including studies designed to reverse the effects of D2R antagonists or partial agonists (antipsychotic drugs) with systematic differential manipulation of the signaling pathways induced by D2R activation, is likely to be a fruitful path toward the development of novel treatments for schizophrenia-related disorders.

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