The Effect of *Kcs1* Deletion on the Therapeutic Action of Valproate

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Bipolar disorder (BD) is a chronic recurrent neuropsychiatric illness characterized by fluctuations between manic and depressed mood states. A number of medications are used to treat BD. However, none of the drugs prescribed to treat BD are completely effective, and the development of more effective drugs is hindered by the lack of knowledge of the therapeutic mechanism of action of these drugs and the underlying molecular mechanism that causes BD. Therefore, it is important to understand the therapeutic mechanisms of action of current drugs. Mood stabilizers such as lithium, valproate (VPA) and lamotrigine are used to treat BD. Despite the wide usage of VPA, many side effects have been identified. A large number of patients have failed to respond satisfactorily to VPA. The molecular mechanism of the therapeutic action of VPA in BD is yet to be identified. A known target of VPA is inositol metabolism (Vaden et al., 2001). Therefore, this work focuses on the effects of VPA on inositol metabolism. VPA decreases *de novo* synthesis of inositol by indirectly inhibiting myo inositol phosphate synthase (MIPS) activity *in vivo* in yeast (Vaden et al., 2001). The baker's yeast *S. cerevisiae* has been used as a eukaryotic model organism to study the effect of VPA on inositol metabolism (Vaden et al., 2001). Yeast cells grown in the presence of VPA exhibited decreased intracellular inositol and an increase in the expression of the structural gene. Inositol hexakisphosphate (IP6) IP6 affects the regulation of nucleosome displacement of *INO1*. *KCS1* is an inositol hexakisphosphate kinase that convert IP6 to IP7.

In yeast, VPA causes inositol depletion by indirectly inhibiting MIPS encoded by *INO1* (Vaden et al., 2001). Factors that regulate *INO1* expression in yeast include the transcriptional activators Ino2p/ Ino4p (Koipally et al., 1996), intracellular inositol levels (Heyken et al., 2005) and chromatin remodeling complexes (Shen et al., 2000). The INO80 chromatin remodeling complex positively regulates chromatin remodeling at the yeast *INO1* promoter (Ford et al., 2007). In yeast, IP6 affects the regulation of nucleosome arrengement of *INO1* (Shen et al., 2003), suggesting that IP6 may alter inositol depletion. *KCS1* converts IP6 to IP7 (Luo et al., 2003), and loss of *KCS1* leads to increased intracellular IP6 levels (Mulugu et al., 2007). Therefore, the disruption of these pathways may alter inositol synthesis.

In this study growth of *KCS1*was compared to WT. First, deletion mutants of genes that affect inositol metabolism were screened for inositol dependency. Cells were plated on both YPD and SM plates in the presence and absence of inositol and incubated at 30°C. *kcs1*Δ was identified as a potential candidate.

The growth of *kcs1*Δ was compared to that of the MIPS mutant *ino1*Δ. Isogenic WT, *kcs1*Δ and *ino1*Δ cells were grown in liquid SM at 30°C in the presence and absence of inositol. Both *kcs1*Δ and *ino1*Δ exhibited a marked decrease in growth compared to the WT in liquid I-SM medium. Growth was restored by supplementation of inositol. Because growth of *kcs1*Δ depends on inositol, it can be hypothesized that the mutant is more sensitive to VPA. To test the VPA sensitivity of *kcs1*Δ, growth of *kcs1*Δ can be compared to that of WT in the presence of VPA. Cells can be grown in liquid

media to the logarithmic phase of growth, at which time VPA (range 0 - 0.6 mM) can be added.

This study revealed that the deletion mutant of *KCS1* depends on inositol for growth. *KCS1* is an inositol kinase in the PI cycle. Kcs1p phosphorylates IP5 to IP6, IP6 to IP7, and IP7 to IP8 (Luo et al., 2003; Saiardi et al., 2000). Loss of IP6Ks results in pleiotropic cellular defects, including aberrant DNA recombination, abnormal vacuolar morphology, altered gene expression, increased chemotaxis, osmotic stress, altered protein phosphorylation, and decreased telomere length (Bennett et al., 2006). The results shown here indicate that *KCS1* is necessary for inositol biosynthesis. It has been found that many BD patients fail to respond to VPA satisfactorily. Mutations in genes that affect inositol metabolism, such as *KCS1* may affect patients' responsiveness to VPA. Studies on the genetic factors that could alter the responsiveness to VPA may create new avenues to improve VPA in the near future.

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