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Inhibition of glycogen synthase kinase-3 is necessary for the rapid antidepressant effect of ketamine in mice

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This study found that (a) administration of a relatively low dose of ketamine to mice inhibits brain glycogen synthase kinase-3 (GSK3), (b) this inhibition of GSK3 is necessary for rapid antidepressant-like effect of ketamine in the mouse model of learned helplessness because mice expressing constitutively active GSK3 are completely resistant to antidepressant-like effect of ketamine, and (c) administration of a dose of the GSK3 inhibitor lithium much higher than usually used produces a rapid antidepressant-like effect equivalent to ketamine. This demonstrates that inhibition of GSK3 is required for rapid antidepressant effect of ketamine in this model and raises the possibility that acute administration of specific GSK3 inhibitors may be sufficient to produce rapid antidepressant effects similar to ketamine.

Ketamine administration can produce rapid anti-depressant effects in patients with major depression and relieve treatment-resistant depression.^{1,2} These actions likely arise from ketamine's antagonism of glutamatergic *N*-methyl-D-aspartate (NMDA) receptors, as well as from additional mechanisms that have been suggested.^{3–5} We previously found that memantine, another NMDA antagonist, caused rapid inhibition of glycogen synthase kinase-3 (GSK3) in mouse brain,⁶ which raised the possibility that ketamine may have a similar action. This is relevant because much evidence links inadequate inhibition of GSK3 to major depressive disorder and bipolar disorder.⁷ Here, we report that ketamine rapidly inhibits GSK3 in mouse brain, and rapid antidepressant effect of ketamine in the learned helplessness model of depression-like behavior requires inhibition of GSK3 and is mimicked by acute high-dose administration of the GSK3 inhibitor lithium.

Using the subanesthetic dose of ketamine (10 mg kg⁻¹; i.p.) previously established to have rapid antidepressant effects in rodents,^{3,4} we found that ketamine robustly increased serine-phosphorylation of both GSK3 α and GSK3 β isoforms (Figure 1a), indicators of GSK3 inhibition.⁸ GSK3 inhibition was evident in the hippocampus and the prefrontal cortex 30 and 60 min after administration, times previously reported to be important for the rapid antidepressant action of ketamine.³ We confirmed previous reports^{3,4} that ketamine administered after inescapable shock training reduced subsequent failures to escape in the learned helplessness model of depression-like behavior (Figure 1b), indicative of a rapid antidepressant-like effect. To test whether inhibitory serine-phosphorylation of GSK3 induced by ketamine is required for this rapid antidepressant effect, we used GSK3 $\alpha^{21A/21A}/\beta^{9A/9A}$ knock-in mice with serine-to-alanine mutations to eliminate the inhibitory serines of GSK3 α/β .⁹ These mutations maintain GSK3 maximally active, but importantly within the physiological range as both GSK3 isoforms are expressed at normal levels, and these mice develop and reproduce normally with no overt phenotype. The antidepressant effect of

ketamine in the learned helplessness paradigm was completely absent in GSK3 knock-in mice, demonstrating that ketamine-induced serine-phosphorylation of GSK3 is necessary for its antidepressant action in this mouse model. To test whether other means of inhibiting GSK3 had a rapid antidepressant effect, we administered a high dose of lithium chloride (100 mg kg⁻¹; i.p.), a direct inhibitor of GSK3,¹⁰ to wild-type mice, which also reduced depressive-like behavior in the learned helplessness test.

These results demonstrate that ketamine rapidly inhibits GSK3 in mouse brain and that inhibition of GSK3 is necessary, and may be sufficient, for a rapid antidepressant effect in the learned helplessness model of depression-like behavior in mice. These findings add to the substantial evidence already linking active GSK3 to increased susceptibility to mood disorders and inhibition of GSK3 to therapeutic outcomes.⁷ These findings also raise the possibility that administration of specific inhibitors of GSK3 may provide a mechanism for rapidly controlling depression. This protocol is not feasible with lithium because of its toxic effects at serum levels above 1.5 mEq l⁻¹, but may be achieved with the application of new small molecule inhibitors of GSK3 that are potent and able to rapidly inhibit GSK3 in the brain.

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Figure 1.

Ketamine rapidly increases inhibitory serine-phosphorylation of glycogen synthase kinase-3 (GSK3), which is required for its antidepressant effect in the learned helplessness model of depression-like behavior in mice. **(a)** The inhibitory serine-phosphorylation of GSK3 α (on serine-21) and GSK3 β (on serine-9) was increased in the hippocampus and cerebral cortex 30 and 60 min after administration of 10 mg kg⁻¹ ketamine (i.p.). Total levels of GSK3 α and GSK3 β were unaltered by ketamine treatment, indicating ketamine modulates phosphorylation, not expression, of GSK3. **(b)** The learned helplessness model of depression-like behavior was tested as described by Li *et al.*³ Wild-type (WT) or GSK3 knock-in (KI) mice were subjected to random mild foot shocks, 24 h later ketamine (K; 10 mg kg⁻¹; i.p.) or lithium chloride (Li; 100 mg kg⁻¹; i.p.) was administered, and after 24 h mice were tested with escapable foot shocks. The number of failures to escape out of 30 trials from the escapable foot shocks and the 5-trial average latency to escape in 24 s trials are shown. Comparisons (**P* < 0.05; analysis of variance) demonstrate that ketamine significantly increased serine-phosphorylation of GSK3 and ketamine and lithium significantly reduced depression-like behaviors in wild-type mice, whereas ketamine was ineffective in GSK3 knock-in mice in which serine-phosphorylation of GSK3 is blocked.