

Rapid Antidepressive-Like Activity of Specific Glycogen Synthase Kinase-3 Inhibitor and Its Effect on β -Catenin in Mouse Hippocampus

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Background: Inhibition of glycogen synthase kinase-3 (GSK-3) is thought to be a key feature in the therapeutic mechanism of several mood stabilizers; however, the role of GSK-3 in depressive behavior has not been determined. In these studies, we evaluated the antidepressive effect of L803-mts, a novel GSK-3 peptide inhibitor, in an animal model of depression, the mouse forced swimming test (FST).

Methods: Animals were intracerebroventricularly injected with L803-mts or with respective control peptide (cp) 1 hour, 3 hours, or 12 hours before their subjection to FST.

Results: Animals administered L803-mts showed reduced duration of immobility at all three time points tested, as compared with cp-treated animals. Expression levels of β -catenin, the endogenous substrate of GSK-3, increased in the hippocampus of L803-mts-treated animals by 20%–50%, as compared with cp-treated animals.

Conclusions: Our studies show, for the first time, that in-vivo inhibition of GSK-3 produces antidepressive-like behavior and suggest the potential of GSK-3 inhibitors as antidepressants.

Key Words: GSK-3, forced swimming test, β -catenin, bipolar disorder, depression

Glycogen synthase kinase-3 (GSK-3) is a ubiquitous cellular serine/threonine protein kinase that was initially shown to regulate glycogen metabolism and to inhibit insulin signaling pathway (for review see Eldar-Finkelman 2002; Grimes and Jope 2001; Woodgett 2001). Recent studies implicated GSK-3 in neuronal cell signaling and showed that dysfunction of the enzyme might be causative in central nervous system disorders, including schizophrenia, stroke, and Alzheimer's disease (Beasley et al 2001; Bhat and Budd 2002; Hernandez et al 2002; Kozlovsky et al 2002; Lucas et al 2001; Mandelkowitz et al 1992). A notable development in the field was the implication of GSK-3 in psychiatric disorders. This was based on the findings that lithium or valproic acid, mood-stabilizers frequently used as first-line treatment for bipolar affective disorders, were effective inhibitors of GSK-3 (Chen et al 1999; Klein and Melton 1996). It was therefore proposed that the biological action of these drugs is mediated through inhibition of GSK-3 (Jope and Bijur 2002; Manji and Lenox 2001). Indeed, a number of studies lend support to this view by showing remarkable parallels between consequences of GSK-3 inactivation and treatment with lithium or valproic acid. For example, GSK-3 was shown to facilitate cell death (Pap and Cooper 1998), whereas treatment with lithium or valproic acid exerted neuroprotective effects and enhanced cell survival (Bijur et al 2000). Additionally, treatment with lithium or valproic acid significantly increased the accumulation of β -catenin, a substrate of GSK-3, in cultured cell (Chen et al 1999; Stambolic et al 1996) and in rat brain (Gould et al 2004). Lithium and valproic acid, however, are not specific toward GSK-3 and

inhibit additional cellular targets, such as inositol monophosphatase and histone deacetylases (Berridge et al 1989; Phiel and Klein 2001).

Although there are no data directly linking GSK-3 with monoamines, we speculated that some mood stabilizers and antidepressants might share common mechanisms influencing GSK-3 activity. This hypothesis can be further supported by previous work. First, lithium has been shown to potentiate the action of antidepressants, and this has been recently related to its enhancement effect on serotonin function (Nixon et al 1994; Rouillon and Gorwood 1998; Wegener et al 2003). Second, the GSK-3 substrate CREB (cyclic adenosine monophosphate [cAMP] regulatory element-binding protein transcription factor) has been shown to modulate antidepressant drug activity (Chen et al 2001a; Newton et al 2002). Finally, brain-derived neurotrophic factor (BDNF), a target modulated by antidepressants (Chen et al 2001b; Russo-Neustadt et al 2000) and producing antidepressive-like activity in preclinical behavioral models (Shirayama et al 2002; Siuciak et al 1997), was shown to inhibit GSK-3 in BDNF-treated cells (Mai et al 2002). It is thus suggested that inhibition of GSK-3 might contribute to antidepressants' activity.

We recently generated a novel class of peptide inhibitors of GSK-3 (Plotkin et al 2003). These inhibitors behaved as substrate competitive and were specific toward GSK-3; that is, they did not inhibit a selection of other protein kinases (Plotkin et al 2003). In the present study, we examined the impact of the GSK-3 inhibitor L803-mts on depressive behavior, using a widely accepted preclinical animal model of antidepressive drug activity, the forced swimming test (FST; Porsolt 2000; Porsolt et al 1977).

As mentioned earlier, β -catenin is a substrate of GSK-3 (Miller and Moon 1996; Peifer and Polakis 2000) and was recently implicated in brain development, cognitive activity, and dendritic growth (Coyle-Rink et al 2002; Yu and Malenka 2003). Phosphorylation of β -catenin by GSK-3 enhances the degradation of the protein, whereas inactivation of GSK-3 stabilizes β -catenin and promotes its accumulation in the cell cytoplasm (Aberle et al 1997; Ikeda et al 1998; Yost et al 1996). The unphosphorylated β -catenin can then migrate into the nucleus, where it associates with the transcription factors of the Lef/Tcf family to stimulate gene expression (Behrens et al 1996). It is thus suggested that accumulation of β -catenin can serve as a marker for in-vivo

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inhibition of GSK-3. Having determined on the behavioral level the impact of the GSK-3 inhibitor L803-mts in FST, we sought to examine its effect on β -catenin levels in the mouse hippocampus.

We report that inhibition of GSK-3 produced antidepressive-like activity and led to accumulation of β -catenin in the mouse hippocampus.

Methods and Materials

Peptides

L803-mts (N-Myristol-GKEAPPAPPQS(p)P; Plotkin et al 2003) and a scrambled control peptide, cpL803-mts, were synthesized by Genemed Synthesis (San Francisco, California). The selectivity of the peptide inhibitor was tested against a selection of protein kinases, including mitogen-activated protein kinase, protein kinase C, cAMP-dependent protein kinase, casein kinase 2, and cycling dependent protein kinase (cdc2). Apparently, the peptide inhibitor did not inhibit these protein kinases (Plotkin et al 2003). Moreover, the fact that cdc2, the protein kinase most closely related to GSK-3, was not inhibited by L803-mts, further indicated the high specificity of the inhibitor. Because peptides rarely cross the blood–brain barrier (Prokai 1998), we used intracerebroventricular (ICV) injections to introduce the peptide into the brain.

Assessment of GSK-3 Activity In Vitro

Purified recombinant rabbit GSK-3 β (Eldar-Finkelman et al 1996) was incubated with peptide substrate PGS-1, derived from GSK-3 substrate glycogen synthase, together with L803-mts or cpL803-mts at indicated concentrations. The reaction mixture included Tris 50 mmol/L, pH 7.3, 10 mmol/L MgAc, 32 P[γ -adenosine triphosphate] (100 μ M), and .01% β -mercaptoethanol, and was incubated for 10 min at 30°C. Reactions were spotted on phosphocellulose paper (p81), washed with 100 mmol/L phosphoric acid, and counted for radioactivity, as previously described (Eldar-Finkelman et al 1996).

Animals and FST

C57BL/6J mice were housed in individual cages with free access to water in a temperature-controlled facility with a 12-hour light/dark cycle. Animals at age 14–16 weeks were used, and each experimental group consisted of 10–20 randomly chosen mice. At day 1, mice were subjected to pre-FST (see below). At day 2, mice were anesthetized with halothane (inhalation) and were unilaterally ICV injected with L803-mts, cpL803-mts (1 μ L of 25 mmol/L stock solution to reach a final concentration of approximately 50 μ mol/L in the brain). Animals were subjected to FST once 1, 3, and 12 hours after reagents were administered. The FST procedure used was similar to that initially described by Porsolt et al (1977). Briefly, animals were placed at day 1 in a large cylinder (30 cm \times 45 cm) of 25°C water for a 15-min period. At day 2 (24 hours later), treated mice were placed in the cylinder of water for a 6-min period. The duration of immobility was monitored during the last 4 min of the 6-min test. Immobility period was defined as the time spent by the animal floating in the water without struggling and making only those movements necessary to keep its head above the water. All testing took place between 11:00 AM and 3:00 PM. After completion of the FST, mice were killed, and hippocampi were removed, frozen in liquid nitrogen, and stored at -80° C. Animal care followed guidelines of the institutional animal care and use committee.

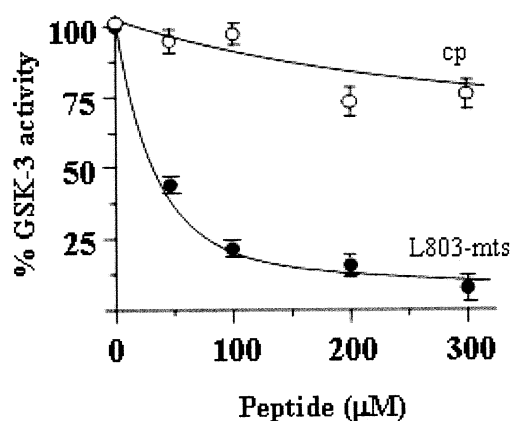


Figure 1. Effect of L803-mts and cpL803-mts on kinase activity in vitro. The ability of purified recombinant glycogen synthase kinase (GSK)-3 β to phosphorylate PGS-1 peptide substrate was measured in the presence of indicated concentrations of peptides L803-mts (filled circles) or scrambled control peptide cpL803-mts (cp, hollow circles). Results represent the percentage of GSK-3 activity in the absence of peptides. Results are mean of three independent experiments \pm SEM, where each point was assayed in triplicate.

Quantitation of β -Catenin in Hippocampus Extracts

Hippocampus tissue was homogenized with ice-cold buffer H (50 mmol/L β -glycerophosphate, pH = 7.3, 10% glycerol, 1 mmol/L ethyleneglycol bis-(β -aminoethyl ether)- N,N' -tetraacetic acid, 1 mmol/L ethylenediaminetetraacetic acid, 10 mmol/L NaF, 5 mmol/L NaPPI, 25 μ g/mL leupeptin, 25 μ g/mL aprotinin, 500 nmol/L microcystine LR, and 1% Triton \times 100). The extracts were centrifuged for 20 min at 15,000g, and supernatants were collected. Equal amounts of proteins (30 μ g), as determined by Bradford analysis (Bradford 1976), were boiled with Laemmli sample buffer and subjected to gel electrophoresis (10% polyacrylamide gel), transferred to nitrocellulose membranes, and immunoblotted with specific monoclonal antibodies for β -catenin (Transduction Laboratories, Lexington, Kentucky).

Statistics

Data were analyzed by one-factor analysis of variance (ANOVA) with Origin Professional 6.0 (Microcal Software, Northampton, Massachusetts). Data were deemed significant

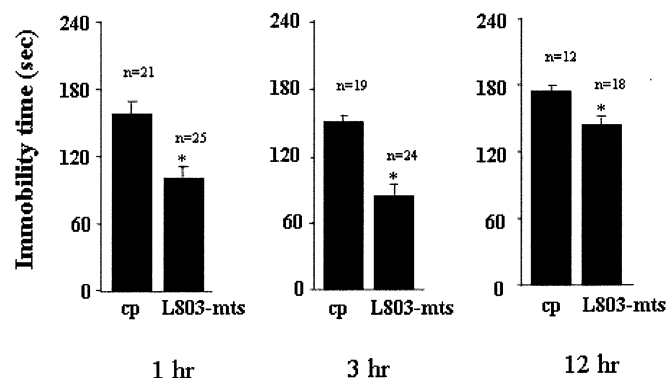


Figure 2. Effect of L803-mts on animal behavior in the forced swimming test. Bar graphs represent mean of immobility \pm SE from indicated number of animals, subjected to forced swimming test 1 hour, 3 hours, and 12 hours after administration of L803-mts or cpL803-mts (cp) as indicated. * p < .05, L803-mts treatment versus cp treatment.

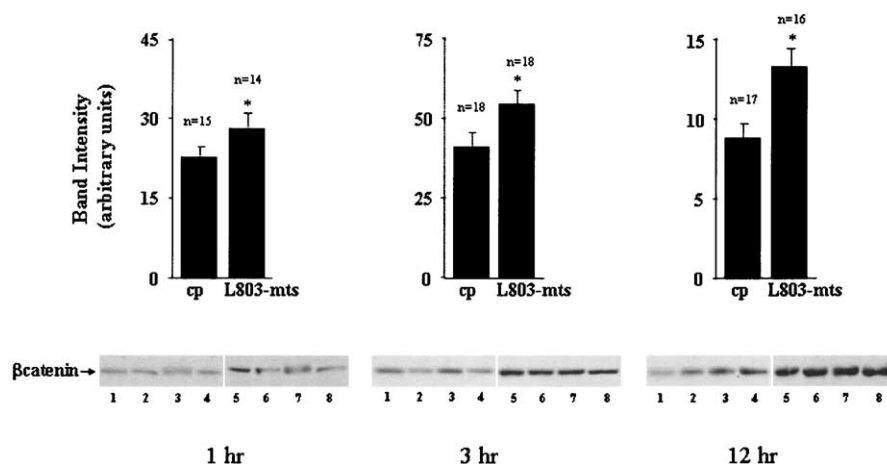


Figure 3. Beta-catenin levels are up-regulated in mouse hippocampus by L803-mts. Hippocampal tissue extracts were prepared as described in Methods, and equal amounts of protein aliquots as from indicated time points were subjected to gel electrophoresis and immunoblotted with antibody against β -catenin. Densitometry analysis of β -catenin from indicated number of hippocampi is shown in bar graphs and represent mean value \pm SE (upper panel). * $p < .05$, L803-mts treatment versus cp treatment. Representative gels of β -catenin from cp-treated animals (lanes 1–4) or L803-mts-treated animals (lanes 5–8) are shown for the indicated time points.

when $p < .05$. Data are expressed as group mean with standard errors.

Results

Effect of L803-mts and cpL803-mts on GSK-3 Activity In Vitro

GSK-3 β activity was assayed in the presence of L803-mts or its respective scrambled control peptide, cpL803-mts. As shown in Figure 1, L803-mts significantly inhibited purified GSK-3 β with an effective concentration of 40 μ mol/L required for 50% inhibition (IC₅₀). In contrast, cpL803-mts did not inhibit GSK-3 β activity at the range of concentrations tested (up to 300 μ mol/L).

Effect of GSK-3 Inhibition on FST

L803-mts was ICV injected 1 hour, 3 hours, or 12 hours before FST. Figure 2 shows that pretreatment with L803-mts significantly shortened immobility periods by 37% \pm 3.7% (1 hour), 44% \pm 5.7% (2 hour), and 16% \pm .6% (12 hours), as compared with control-treated animals, which became immobile after a brief duration of swimming ($p < .05$ for all). Forced swim test performed with vehicle- (1 λ dimethyl sulfoxide) or saline-treated animals resulted in values similar to those obtained in the cpL803-mts-treated animals (161 \pm 8.3 sec, $n = 10$, and 170 \pm 11.5 sec, $n = 5$, respectively).

β -Catenin in Hippocampus of L803-mts-Treated Mice

We next examined whether β -catenin levels are affected by L803-mts in the mouse hippocampus. Hippocampal tissue extracts were analyzed by Western blot technique with monoclonal anti- β -catenin antibodies. L803-mts treatment increased the amount of β -catenin in hippocampus extracts in a time-dependent fashion (Figure 3). Increases of 20% or 30% in β -catenin levels were observed after 1 hour or 3 hours treatment with L803-mts, respectively ($p < .05$ for both). The protein levels increased by 50% 12 hours after administration of L803-mts ($p < .05$, Figure 3).

Discussion

In these studies, we show that a selective GSK-3 inhibitor administered ICV produced a rapid antidepressant-like effect in mouse FSTs. To the best of our knowledge, this is the first study to show that in-vivo inhibition of GSK-3 provokes antidepressant-like activity. We further demonstrate that inhibition of GSK-3 led to accumulation of β -catenin in the mouse hippocampus. Up-regulation of β -catenin as a consequence of inactivation of

GSK-3 has been implicated in numerous studies using various cultured cells treated with *Wnt*, lithium, or valproic acid (Chen et al 1999; Ikeda et al 1998; Sakanaka et al 1998; Stambolic et al 1996). Beta-catenin, which is also an integral component of *Wnt* signal transduction, has been recently implicated as an important modulator in brain development and neural network signaling. It has been shown that overexpression of β -catenin enlarges neural tissue mass and affects development patterning of certain brain regions (Chenn and Walsh 2002; Coyle-Rink et al 2002; Yu and Malenka 2003). In addition, β -catenin was recently shown to be a critical mediator of dendritic morphogenesis and required for the effect of neural activity (Yu and Malenka 2003). Our studies indicated that up-regulation of β -catenin was associated with reduction in immobility duration in response to treatment with L803-mts; however, it is noteworthy that increases in β -catenin persisted 12 hours after L803-mts treatment, even though the effect of the inhibitor on immobility decreased. It is possible that once it is accumulated in the nucleus, β -catenin is protected from proteosomal degradation and phosphorylation by GSK-3, which occurs mainly in the cytoplasm. Although the functional role of β -catenin in depressive behavior is unknown, it is tempting to speculate that up-regulation of β -catenin, as detected in our experiments, can serve as a marker for the antidepressant behavior provoked by the GSK-3 inhibitor.

In summary, our studies indicate that specific GSK-3 inhibitors can alter depressive behavior in the FST animal model. Future studies should test the impact of the GSK-3 inhibitor in other preclinical models for depression (Nestler et al 2002). Nevertheless, our findings could trigger the exploration of a new class of antidepressant drugs based on GSK-3 inhibition and initiate testing of the potential therapeutic properties of selective GSK-3 inhibitors as antidepressants or mood stabilizers.

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