Depressing sphingolipids

By Lev Osherovich, Senior Writer

European researchers have obtained the most compelling evidence yet that targeting sphingolipid metabolism could help treat depression.[1] The team has shown that two known antidepressants inhibit sphingolipid metabolism and now is planning to screen for other inhibitors that also elicit antidepressive effects.

Previous work has shown that some serotonin-specific reuptake inhibitor (SSRI) antidepressants have an inhibitory effect on a key lipid processing enzyme called sphingomyelin phosphodiesterase 1 acid lysosomal (SMPD1; ASM).[2] SMPD1 is a membrane protein facing the lysosomal lumen that converts sphingomyelin into ceramide (*see* **Figure 1, "Hitting ceramide for depression"**). Ceramide diffuses out of the lysosome to other cellular membranes to modulate signaling pathways involved in cell growth, inflammation and intracellular responses to bacterial infection.

A team, co-led by the **University of Erlangen-Nuremberg**'s Johannes Kornhuber and the **University of Duisburg-Essen**'s Erich Gulbins, has now shown that inhibiting SMPD1 has effects on the nervous system that could influence depression.

Gulbins is professor of molecular biology and medicine at Duisburg-Essen, and Kornhuber is professor of psychiatry and psychotherapy at Erlangen-Nuremberg.

"What was known before is that antidepressants like fluoxetine and amitriptyline functionally inhibit SMPD1," said Kornhuber. "These compounds enter the lysosome and cause the detachment of SMPD1 from the membrane, leading to its proteolytic degradation." However, according to Kornhuber, no direct link between this mechanism of action and the antidepressant effect of the compounds had been shown until now.

The team thus set out to test whether some of the effects of widely used antidepressants could be a result of SMPD1 inhibition.

Ceramide's touch

The researchers first observed that cultured human neurons treated with the generic SSRI antidepressants fluoxetine and amitriptyline had lower *in vitro* SMPD1 activity and ceramide levels than untreated neurons.

Eli Lilly and Co. markets fluoxetine as Prozac, whereas amitriptyline is no longer marketed as an antidepressant because of safety issues. Both compounds are suspected to have additional mechanisms of action on top of their SSRI activity.

The team then engineered mice with altered levels of *Smpd1* to test the enzyme's effect on the nervous system. *Smpd1* knockouts had lower ceramide levels and *Smpd1* overexpressing mice had higher ceramide levels than wild-type controls.

Treatment with fluoxetine or amitriptyline reduced ceramide levels in *Smpd1*-overexpressing mice but did not alter ceramide levels in *Smpd1* knockouts, suggesting *Smpd1* was needed to mediate the drugs' effects on ceramide.

The group then examined the effects of *Smpd1* activity on neurogenesis and neuronal survival, which are compromised in animal models for depression. In a mouse model for stress, animals overexpressing *Smpd1* had lower levels of hippocampal neurogenesis and neuronal survival than wild-type controls.

Antidepressants improved neurogenesis and neuronal survival in wild-type and *Smpd1*-overexpressing mice but not in *Smpd1* knockouts.

In addition, *Smpd1* activity correlated with depressive behavior in mice. In assays of stress-induced depression, antidepressants had little effect on mice lacking *Smpd1* but alleviated depression-associated behavior in wild-type controls and *Smpd1*-overexpressing animals.

Finally, the team directly injected ceramide into the hippocampus

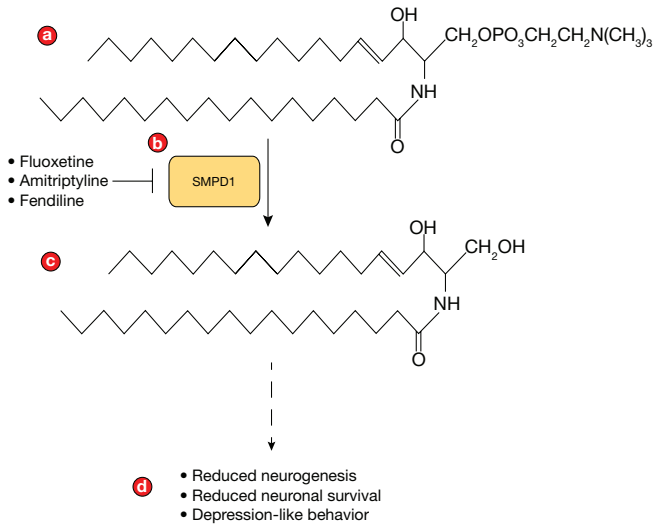

Figure 1. Hitting ceramide for depression. Gulbins *et al.* have evidence that blocking the production of ceramide, a phospholipid signaling molecule, has antidepressive effects.

Ordinarily, the membrane-associated phospholipid sphingomyelin [**a**] is cleaved by sphingomyelin phosphodiesterase 1 acid lysosomal (SMPD1; ASM) [**b**] to yield ceramide [**c**]. Gulbins *et al.* showed that in cell culture and mice, accumulation of ceramide correlated with depressive behavior and reduced neuronal growth and survival [**d**].

The team also showed that two known antidepressants—fluoxetine and amitriptyline—lowered ceramide levels and decreased activity of SMPD1 compared with no treatment. Fendiline, a nonselective calcium channel blocker, also reduced SMPD1 activity and normalized depression-like behavior.

of wild-type mice and saw an increase in depression-like behavior compared with what was seen in vehicle-treated controls.

"We hypothesized that high levels of ceramide were needed to produce depressive behavior," said Kornhuber. "When we overexpressed *Smpd1*, these mice showed spontaneous depressive behavior. When we injected ceramide into the hippocampus of mice, this also generated a depressed mood."

Results were reported in *Nature Medicine*.

Getting selective

Overall, the findings suggest high ceramide levels contribute to cellular and behavioral correlates of depression. The findings also argue that commonly used antidepressants have an additional mechanism of action—inhibition of SMPD1—that is distinct from the inhibition of serotonin reuptake.

"We believe that lowering ceramide is an important mechanism of antidepressant action. Does every antidepressant drug work this way? Probably not; but many antidepressants do."
—Johannes Kornhuber, University of Erlangen-Nuremberg

From a drug discovery standpoint, the results open up the possibility of directly targeting SMPD1 or other enzymes that affect ceramide levels to treat depression.

In their *Nature Medicine* paper, Kornhuber and Gulbins also reported preliminary efforts to identify small molecules that modulate ceramide levels and depression in mice.

The team screened a panel of 250 neurologically active compounds for effects on ceramide levels in cell culture and found other molecules that acted like fluoxetine and amitriptyline. Among these was fendiline, a calcium channel blocker that had not previously been tested as an antidepressant. In mouse models for depression, fendiline mimicked the effects of fluoxetine and amitriptyline.

Kornhuber cautioned that fluoxetine, amitriptyline and fendiline are likely to have additional biochemical effects beyond inhibiting SMPD1 that contribute to their antidepressive effects.

"We believe that lowering ceramide is an important mechanism of antidepressant action," said Kornhuber. "Does every antidepressant drug work this way? Probably not; but many antidepressants do." He noted that other SSRIs tested by the team had antidepressive effects but did not alter ceramide levels.

The next step is to identify selective SMPD1 inhibitors and test their effect on hippocampal ceramide levels and depressive behavior in mice.

Kornhuber further noted that complete inhibition of SMPD1 could have undesired consequences.

"If you have low levels of ceramide, it's bad for the central nervous system," said Kornhuber, noting that a genetic defect in ceramide synthesis causes certain forms of Niemann-Pick disease. "But if you lower SMPD1 levels by as much as 80%, it's well tolerated."

What remains unclear is whether excess intracellular ceramide leads to depression in humans. Kornhuber and Gulbins found that changes in ceramide levels caused by tinkering with *Smpd1* expression led to changes in the activity of protein kinase B (PKB; PKBA; AKT; AKT1), a kinase that participates in a broad range of intracellular signaling pathways.

Uncovering the downstream mechanisms of ceramide signaling in depression will require further cell culture and *in vivo* studies.

The findings described in the paper have not been patented.

Osherovich, L. *SciBX* 6(27); doi:10.1038/scibx.2013.675
Published online July 18, 2013

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