NEWS

Selective enzyme block reveals new treatment for fragile X

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A new drug that selectively blocks one form of an enzyme called GSK-3 prevents seizures and improves cognitive deficits in a mouse model of **fragile X syndrome**.

Researchers presented the unpublished findings today at the **2017 Society for Neuroscience annual meeting** in Washington, D.C.

The new compound's specificity minimizes the risk of side effects associated with existing GSK-3 inhibitors. For example, lithium **increases bed-wetting** in children with the syndrome.

"We thought that if we developed a better GSK-3 inhibitor that is more selective and also more potent, we could give a lower dose, and so it would be safer than lithium and much better tolerated," says lead investigator **Florence Wagner**, director of medicinal chemistry at the Broad Institute in Cambridge, Massachusetts, who presented the findings.

GSK-3 regulates the development of neurons and helps to fine-tune the connections between them. It is a type of enzyme called a kinase, which adds tags called phosphate groups to other proteins.

GSK-3 is **overactive in the brains** of fragile X mice. Numerous drugs that block it **improve features of the condition** in these mice.

However, GSK-3 comes in two slightly different forms, alpha and beta, and most GSK-3 inhibitors block both of them. Inactivating both forms boosts the activity of a protein called **beta-catenin**, which leads to increased cell proliferation.

This suggests that one undesirable side effect of GSK-3 blockers may be a heightened risk of cancer. The few existing inhibitors that affect only one form of GSK-3 often affect many other kinases, which also ups the chances of side effects.

Small molecule screen:

Wagner and her colleagues set out to find more specific drugs for GSK-3. They began by screening a library of 320,000 small molecules for their effects on GSK-3's enzymatic activity.

They found one compound called BRD4953 that stymies GSK-3 but not any of 517 other kinases.

They then examined the 3-D structures of the alpha and beta forms of GSK-3. They found that the region of the enzyme that attaches to BRD4953 is slightly different between the two GSK-3 forms. They tweaked the structure of BRD4953 to come up with two new versions: one that preferentially binds to the alpha form of GSK-3, and another that preferentially binds to the beta form. Neither compound affects the activity of other kinases.

The researchers tested the new compounds in cancer cells grown in culture. They found that neither compound activates beta-catenin.

They then moved on to a mouse model of fragile X syndrome.

When exposed to a loud sound, about 80 percent of fragile X mice have seizures. But when the researchers injected the mice with the alpha-specific compound an hour before exposing them to the sound, only 31 percent of the mice had seizures. Treatment with the beta-specific compound had no effect.

“It was really interesting to see here that the biology of those two very closely related kinases is different,” Wagner says. “They’re not just functionally redundant.”

Better and better:

Another drug developed for fragile X, called **CTEP**, initially prevents seizures in fragile X mice but loses its ability to do so after five days of treatment. Wagner and her colleagues found that the alpha-specific compound does not lose its effectiveness.

Wagner’s team also explored the effects of the GSK-3 blockers on learning and memory.

When placed in a chamber with a dark compartment or a well-lit one, mice typically prefer to spend time in the dark. But if they receive a painful foot shock upon entering that compartment, they are reluctant to re-enter it again 6 to 48 hours later.

Fragile X mice, by contrast, are less hesitant to re-enter, suggesting they have memory problems. After being treated with the alpha-specific compound, however, they perform similarly to control mice on this test.

Neurons in brain slices from fragile X mice fire unusually easily in response to electrical stimulation. Treatment with the alpha-specific compound normalizes their excitability. The compound also normalizes another hallmark of fragile X syndrome: **excessive protein production**.

Encouraged by these findings, Wagner’s team has licensed the compound to a drug company that will formulate it for potential use in clinical trials. She is also collaborating with a team of researchers to test the compound’s effects in mice missing **SYNGAP1**, a top autism candidate gene, because these mice also have overactive GSK-3.

*For more reports from the 2017 Society for Neuroscience annual meeting, please **click here**.*