Myotonic dystrophy: Mouse model shows that GABA receptors are implicated in sleepiness

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People with the inherited disorder myotonic dystrophy (DM) often experience excessive daytime sleepiness and fatigue, as well as altered responses to anesthetics that can put them at risk for complications when hospitalized.

Emory researchers, in collaboration with colleagues at Columbia and University of Florida, now have evidence from a mouse model of DM's central nervous system symptoms, indicating a link to the inhibitor neurotransmitter GABA—and a potential remedy. The results are published in *eNeuro.*

"Problems with sleep and anesthetic responses are two major concerns for people with myotonic dystrophy," says Gary Bassell, Ph.D., chair of cell biology at Emory University School of Medicine. "The behavioral, pharmacologic and molecular alterations we've uncovered help us understand where those aspects of the disorder come from."

The lead author of the paper is Kamyra Edokpolor, Ph.D., a former Emory graduate student, with contributions from anesthesiologist Paul Garcia's lab at Columbia and neurogeneticist Eric Wang's lab at University of Florida.

The *eNeuro* paper describes how the DM model mice have enhanced sensitivity to GABA. They display a stronger response to benzodiazepines, a class of anti-anxiety and anti-insomnia drugs that act through GABA. Additional findings suggest that drugs that counteract benzodiazepines, such as the repurposed antidote flumazenil, might work against DM's prolonged sleep and daytime sleepiness.

"The acute effects of flumazenil were recently tested in a small-scale clinical trial in people with DM, but our results suggest that that study needs to be revisited," Wang says. "Specifically, the wake-promoting effects of flumazenil in DM may be taking place on a more extended timescale than previously investigated."

DM is known as a muscle disease, but it affects the entire body. In addition to classic symptoms of myotonia (difficulty relaxing a contracted muscle) and muscle weakness or wasting, people with DM also often have cardiac or gastrointestinal problems, and many report both long sleep times and drowsiness or attention problems during the day.

DM is caused by abnormally expanded DNA repeats; in type 1, three letters of the genetic code (CTG) are repeated over and over dozens of times, and in type 2, four letters (CCTG) are repeated. The repeats are longer in people with myotonic dystrophy than in healthy controls; age of onset and severity vary with the length of the repeat. The expanded repeats, inserted at one of two locations in the genome, interfere with cells' ability to express

many genes. They distort the process of RNA splicing in muscle and other tissues, leading to DM's array of symptoms.

In the paper, researchers wanted to focus on the nervous system, so they used mice with a knockout of the gene Muscleblind-like 2 (MBNL2). MBNL2 is part of a group of RNA binding proteins, which scientists think are diverted by RNA produced by the expanded repeats. MBNL2 expression is stronger in the brain than in other tissues. Thus, MBNL2 knockout mice do not have muscle problems, but they appear to recapitulate DM's central nervous system symptoms.

The researchers exposed the MBNL2-knockout mice to the anesthetic sevoflurane, the benzodiazepine diazepam (Valium), or the benzodiazepine-like drug zolpidem (Ambien).

"All of these drugs target GABA-A receptors, and we had some clues that these receptors might be affected in DM, because of years of reports on post-operative anesthesia complications," says Edokpolor.

Compared with controls, the MBNL2-knockout mice exhibited delayed recovery after sevoflurane-induced anesthesia, delayed emergence and recovery from zolpidem-induced sleep, and a stronger response to diazepam.

As a potential explanation for the increased GABA sensitivity, researchers were able to show that MBNL2 knockout mice display altered RNA splicing of a gene encoding a GABA-A receptor subunit (gamma 2). The splicing patterns for genes encoding other GABA-A receptor subunits were not affected. For gamma 2, the altered splicing pattern means that a shorter form of the protein is produced. The shorter form is more sensitive to benzodiazepines and has greater tonic, or constant, activity. This means that sleep-promoting signals induced by GABA could be over-active in DM.

Compared with control mice, MBNL2-knockout mice spent more time immobile and presumably sleeping during the day, when mice usually sleep. However, when administered flumazenil, the MBNL2-knockout mice spent less time sleeping, with the effect becoming stronger after 3 or 4 hours. Immobility time was reduced by about 20 percent after 3 hours. The wake-promoting effect contrasted with control mice, which displayed immobility for more time when given flumazenil.

Flumazenil was discovered at Hoffmann La Roche in the 1970s, and was approved in 1992 by the FDA as a countermeasure for benzodiazepine overdose—the same year that the mutation for myotonic dystrophy type 1 was mapped. When used as an antidote, the drug is thought to displace benzodiazepines from their GABA receptor binding sites in the brain. The drug is not approved for other indications.

The authors became interested in flumazenil because starting in 2013, Emory sleep researchers David Rye and Lynn Marie Trotti repurposed the drug as an "off label" wake-promoting agent for sleep disorders, including idiopathic hypersomnia. Investigators subsequently learned that a few patients with DM also experienced benefits, leading to a clinical study of flumazenil. In that study, the 12 participants did not report wake-promoting effects, but efficacy was primarily assessed after intravenous dosing for up to 2 hours.

More information: Kamyra S. Edokpolor et al, Altered behavioral responses show GABA sensitivity in Muscleblind-like 2 deficient mice: Implications for CNS symptoms in myotonic dystrophy, *eNeuro* (2022). DOI: 10.1523/ENEURO.0218-22.2022

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