SOUTHWESTERN NEWS

Media Contact: Amanda Siegfried
(214) 648-3404
Amanda.siegfried@utsouthwestern.edu

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UT Southwestern researchers reveal mechanisms of smooth-muscle contraction

DALLAS – April 5, 2004 – Researchers at UT Southwestern Medical Center at Dallas are the first to use genetically engineered mice containing a fluorescent molecule to examine in real time the chemical reactions that result in smooth-muscle contraction.

Smooth muscle, found in the walls of blood vessels and in internal organs such as lungs, stomach and the bladder, contracts as the end result of a series of chemical reactions. In a new study, UT Southwestern researchers report that one set of chemical reactions resulting in the contraction of the smooth-muscle cells is augmented by a second chemical pathway that kicks in when the first pathway is limited.

"Understanding the underlying chemical signals involved in this process may have implications in treating conditions such as hypertension and other smooth muscle related conditions where there is too much contractile activity," said Dr. James Stull, chairman of physiology at UT Southwestern and senior author of the study.

The research appears in an upcoming issue of the *Proceedings of the National Academy of Sciences* and was to be posted online this week.

Dr. Stull and his colleagues discovered that when one of the chemicals in the primary contraction mechanism – a protein called calmodulin – is in short supply, a second series of chemical reactions kicks in to take up the slack. The result is that the strength of the contraction of smooth-muscle cells remains robust.

The first step in the primary chemical pathway for muscle contraction is for calcium in the muscle cell to combine with calmodulin. Then, the calcium/calmodulin complex "activates" a protein called myosin light chain kinase (MLCK). If not activated, MLCK cannot transfer phosphate to the motor protein myosin. Myosin needs the phosphate – in a process called phosphorylation – to initiate contraction in smooth-muscle cells.

When the researchers treated smooth muscle cells from mice with the drug carbachol, the amount of calcium available within the cells increased. Because there is much more calmodulin than MLCK in cells, they expected the increase in calcium to lead to more MLCK activation, and that

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therefore the contraction would be stronger.

The researchers saw the strong muscle contraction, but they only saw a small increase in MLCK activation, not enough to account for the muscle response. They discovered that because MLCK was competing for calmodulin with other calmodulin-binding proteins, there was only enough calmodulin available in this system to activate a small portion of the MLCK.

"Surprisingly, there is not enough calmodulin for all of its targets," Dr. Stull said. "So the signaling system has recruited a second pathway to enhance the limited activation of MLCK, which leads to a strong muscle contraction."

At the end of the primary chemical pathway, an enzyme called phosphatase can remove the phosphate from the myosin, hampering the muscle cell contraction. But the second chemical pathway inhibits the phosphatase from removing the phosphate.

"In this second pathway, the phosphates are no longer taken away from the myosin, which allows more phosphorylated myosin to remain, leading to a stronger muscle contraction," Dr. Stull said.

To track the progress of this intricate chemical dance, researchers genetically engineered a mouse containing a fluorescent molecule, or biosensor that directly monitors the calcium/calmodulin activation of MLCK in real time in smooth-muscle cells.

"These studies demonstrate the feasibility of producing transgenic biosensor mice for investigations of signaling processes in intact systems," Dr. Stull said.

Other UT Southwestern researchers involved in the study were Dr. Kristine Kamm, associate professor of physiology and a longtime collaborator for studies on muscle; Drs. Kim Lau and Gang Zhi, assistant professors of physiology; and postdoctoral researchers Drs. Eiji Isotani, Jian Huang, Yusuke Mizuno and Ramaz Geguchadze. Dr. Anthony Persechini from the University of Missouri-Kansas City also contributed. The research was supported by the National Institutes of Health and the Moss Heart Fund.

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