Pain Research Forum

Progress through collaboration

Metabolomics Uncovers a New Driver for Neuropathic Pain

A product of sphingomyelin breakdown fuels allodynia in rats

by Megan Talkington on 30 Jan 2012

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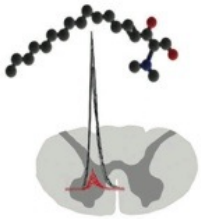


In the hunt for the molecular underpinnings of pain, there are plenty of suspects to investigate. There are DNA sequences, the transcripts produced from them, and the proteins they encode. Then there are metabolites that proteins break down and build up, and that function as molecular signals. A new study focuses its gaze at this level—the panoply of small molecules in biological tissues—and comes up with a fresh clue to the causes of chronic pain.

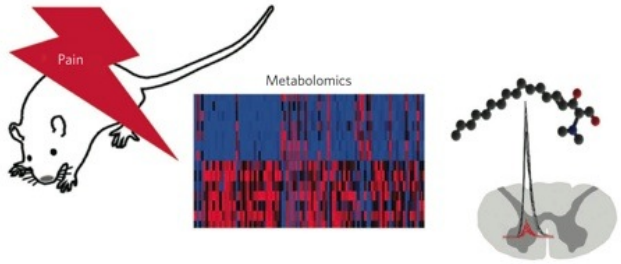
Using mass spectrometry to profile tissue metabolites, researchers led by Gary Siuzdak at The Scripps Research Institute and Marianne Manchester at the University of California, San Diego (UCSD), both in La Jolla, California, found multiple changes in the proinflammatory sphingomyelin/ceramide pathway in the spinal cord of rats with nerve injury-induced persistent pain. One overproduced metabolite was *N,N*-dimethylsphingosine (DMS), a sphingomyelin breakdown product not previously associated with pain. When administered to animals, DMS caused painful hypersensitivity that mimicked the effects of nerve injury. The study, published online January 22 in Nature Chemical Biology, identifies DMS as a new pain mediator, and should heighten interest in the sphingomyelin/ceramide pathway as a source of novel candidate targets for pain treatment.

Metabolomics aims to survey all the small molecules in a given tissue—the sugars, amino acids, hormones, and more. Thanks to the sensitivity of mass spectrometry, tens of thousands of chemical components can be identified in tissue extracts. Many represent unknown compounds, or compounds with unknown biological functions. The use of metabolomics to attack pain biology, and the discovery of a novel pain mediator by this

method, makes for a "compelling story," said Daniela Salvemini of Saint Louis University School of Medicine in Missouri. Salvemini, who was not involved in the work, told PRF that the study "confirms and extends the importance of the ceramide pathway in pain."

Ceramide is produced by the breakdown of sphingomyelin, a phospholipid present in cell membranes, including in the myelin sheath around nerve axons. Salvemini and others have shown previously that ceramide, and its metabolite sphingosine-1-phosphate (S1P), mediate inflammatory pain in rodent models (for recent results, see **Doyle et al., 2011**). Ceramide and S1P function as second messengers that sensitize nociceptive neurons in response to nerve growth factor (NGF) and the inflammatory cytokine tumor necrosis factor-α (TNF-α) (**Zhang et al., 2002**; **Zhang et al., 2006**; **Zhang et al., 2006**; **Joseph and Levine, 2004**). Blocking S1P or its receptor can relieve nociceptor hyperexcitability and pain (**Mair et al., 2011**; **Doyle et al., 2011**; **Chi and Nicol, 2010**). In the spine, ceramide is upregulated in astrocytes and microglia by chronic morphine treatment. There is evidence that ceramide and S1P contribute to opioid-induced hyperalgesia and tolerance, and inhibiting production of the metabolites blocks the ill effects of long-term opioid treatment (**Ndengele et al., 2009**; **Bryant et al., 2009**; **Muscoli et al., 2010**). On the other hand, some experiments have demonstrated an antinociceptive role for S1P in the spine (**Coste et al., 2008**).

In the new study, first author Gary Patti and colleagues found evidence that the ceramide pathway is also involved in neuropathic pain. Patti, who is now at Washington University School of Medicine in St. Louis, Missouri, performed an untargeted survey of the metabolome in rats after tibial nerve transection (TNT), a model of neuropathic pain in which mechanical hypersensitivity (allodynia) develops and persists for weeks after the nerve injury itself has healed. Working in the laboratories of Siuzdak and Manchester, he analyzed several tissues, including the damaged nerve, the dorsal root ganglia (DRG), the dorsal horn of the spinal cord, and plasma, all collected three weeks after injury. Strikingly, the vast majority (94 percent) of differences between injured and sham-operated animals showed up in the spinal cord, far from the original nerve lesion.



Mass spectrometry-based metabolic profiling of rat tissue revealed that the sphingolipid DMS acts as a pain mediator in the spinal dorsal horn. Image credit: Reprinted by permission from Macmillan Publishers Ltd: Nature Chemical Biology, advance online publication, 22 January 2012 (doi: 10.1038/nchembio.767).

Patti and co-first author Oscar Yanes found that, among the compounds whose levels were altered in the dorsal horn, many lay in the sphingomyelin/ceramide pathway. Ceramide, for example, was upregulated threefold, indicating an increase in sphingomyelin degradation. DMS—a compound that, like S1P, is a product of ceramide metabolism—was upregulated 3.5-fold.

Previous studies had shown that DMS is produced in some cancer cell lines and human brain tissue (**Igarashi et al., 1990**; **Nudelman et al., 1992**), but its roles were not understood. To see whether DMS might cause neuropathic pain, the group injected it into the spinal cord of healthy rats, achieving a concentration in the dorsal horn that was similar to that observed after nerve injury. They found that DMS administration elicited mechanical allodynia.

Further experiments suggested a possible mechanism of action for DMS: release of inflammatory mediators from astrocytes. When administered to rats, DMS caused an increase in spinal glial fibrillary acidic protein (GFAP) expression, a marker of astrocyte activation. In cultured astrocytes, DMS triggered release of the cytokines interleukin 1β (IL1β) and monocyte chemoattractant protein-1 (MCP-1), both of which have been associated with chronic pain (for other results on MCP-1, see **related PRF news story**, and for more on glial function in pain, see **related PRF news story**).

S1P—the ceramide metabolite implicated in pain by previous studies—showed no change in abundance after nerve injury or DMS injection. DMS is known to inhibit S1P production, but Patti said that the concentrations of DMS he observed (femtomoles of DMS per milligram of tissue) were below the levels needed to affect S1P. "We think we're tapping into a different mechanism that does not involve S1P," he said.

The results suggest that blocking DMS synthesis or action might offer a route to pain therapy. However, it remains to be

determined where DMS is produced or where it acts, and what enzyme or enzymes are responsible for its synthesis. Although inhibiting enzymes upstream in the ceramide metabolic pathway would be expected to decrease DMS levels, that would be a poor therapeutic strategy, Patti said, because it likely would have considerable unintended effects.

As with any untargeted "omics" scheme, this metabolomics experiment produced reams of data: besides DMS, 732 other compounds showed at least a twofold change in injured animals. "We need a prioritization scheme," Patti said. His hope is to profile the metabolome in a variety of pain models, as well as in human tissues, and compare the results. Towards that end, Patti, Siuzdak, and their colleagues have developed software (**Tautenhahn et al., 2011**) to enable meta-analysis of metabolomics data.

Note: Megan Talkington previously coauthored a scientific paper with Gary Siuzdak, one of the authors of the work covered in this story.

*Image credit: Adapted by permission from Macmillan Publishers Ltd: **Nature Chemical Biology**, advance online publication, 22 January 2012 (doi: 10.1038/nchembio.767).*

References:

Editors' Pick

Metabolomics implicates altered sphingolipids in chronic pain of neuropathic origin.
Patti GJ, Yanes O, Shriver LP, Courade J-P, Tautenhahn R, Manchester M, Siuzdak G
Nat Chem Biol. 2012 Jan 22.
PMID: 22267119.

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