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IT'S A REAL HEADSCRATCHER: HOW DO THE BODY AND BRAIN GENERATE AND PERCEIVE ITCH SENSATIONS?

Emerging research explores diversity in the triggers and nerve pathways that mediate itch; findings may help in the development of new treatments

Washington, DC — Got an itch? How your brain processes the sensation — and how well scratching relieves it — may depend on the source of the itch, according to a new study released today at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health. This and other recent itch research is providing insight into the neurobiology of itch, which, when chronic, substantially impairs the quality of life.

Although interesting to anyone who has experienced an itch due to a bug bite, dry skin, or other common trigger, the research has significant clinical importance. Itch, known medically as pruritus, is a major complication of liver, kidney, and other diseases and can be debilitating when chronic or severe. Itch is also a major side effect of certain drug treatments. Current anti-itching therapies are not always effective, particularly against nonhistaminergic forms of itch (ones not caused by the well-known itch inducer histamine).

The new finding shows:

- Separate nerve pathways in the skin — and possibly to the brain — become activated in response to histaminergic and nonhistaminergic "itchy" sources, and one of the pathways tends to be easier to relieve with scratching (Frauke Kosteletzky, abstract 775.9, see attached summary).

A panel presentation at the meeting focuses on the neurobiology of itch, and recent discoveries report:

- Animal and human research is identifying the range of sensations and behaviors common to itch, regardless of its source (see attached speaker's summary).
- Scientists have identified a gene associated with itch: gastrin-releasing peptide receptor (GRPR). In mice, GRPR inhibitors reduce scratching behaviors and prevent itching caused by painkillers (see attached speaker's summary).
- Different nerves transmit different sources of itch on the skin. This offers the potential to identify and produce therapies for specific kinds of chronic itches. This would spell particular relief for itches that are not caused by histamines and are thus unresponsive to antihistimines, a key itch reliever (see attached speaker's summary).

"Insight into how itch works and its similarities and differences from other sensations, like pain, increases our knowledge about the nervous system and puts us in a better position to design better drugs to help people suffering from a variety of disorders," said press conference moderator Ethan Lerner, MD, PhD, of Massachusetts General Hospital, who has elaborated the cellular mechanisms involved in itch.

Related Presentation:

Symposium: **The Neurobiology of Itch**
Monday, November 17, 8:30–11 a.m., Washington Convention Center, Ballroom B

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Abstract 775.9 Summary

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Study Finds that Not All Types of Itch Use the Same Neural Pathways

Findings promise to open new avenues of treatment for people suffering from chronic and debilitating nonhistaminergic itching

Researchers from the University of Erlangen-Nurnberg in Erlangen, Germany, have found that separate neural pathways in the skin — and possibly in the brain — become activated in response to two different types of "itchy" sources, and that one of these pathways tends to be easier to relieve with scratching. The study was released at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Although interesting to anyone who has experienced an itch due to a bug bite, dry skin, or other common trigger, these findings also have significant clinical importance. Itch, known medically as pruritus, is a major complication of liver, kidney, and other diseases and can be debilitating when chronic or severe. Current anti-itching therapies are not always effective, particularly against nonhistaminergic forms of itch (ones not caused by the well-known itch inducer histamine).

The tropical plant *mucuna pruiens*, or cowhage, has long been used for the study of nonhistaminergic itch. "Sticking cowhage spicules into healthy skin provokes a clear itch sensation, but not the 'flare reaction,' or reddening of the skin, characteristic of a histamine-mediated itch," says Clemens Forster, PhD, co-author of the study. "This suggests that other types of nerve fibers are causing the cowhage-induced itch."

In this study, Forster and his colleagues induced itch in healthy volunteers by applying histamine or cowhage to their forearms. The volunteers were then asked to rate the sensations of the itch before, during, and after scratching. "It took less time between the onset of the stimulus and the first itch sensations when histamine was given," says Forster. "However, the recovery after scratch was slower when the itch was induced by histamine."

The researchers also recorded the flow of blood to the volunteers' skin. "We found that cowhage, which induced a sharper and more stinging sensation, also provoked stronger responses of the sympathetic nervous system," says Forster. "This suggests that at least two different groups of peripheral nerve fibers and their pathways can be evoked by an itch. The two itch sensations may also be differently processed in the brain." These findings may one day lead to new treatments for people suffering from chronic and debilitating nonhistaminergic itching.

This research was supported by the German Research Foundation.

Scientific Presentation: Wednesday, November 19, 8–9 a.m., Washington Convention Center, Hall A-C

775.9, Sensations and vasoconstrictor reflexes during scratch induced relief of itch which was evoked by histamine and cowhage
F. KOSTELETZKY, C. FORSTER, B. NAMER, *H. HANDWERKER; Physiol & Exp Pathophysiol, Univ. Erlangen-Nurnberg, Erlangen, Germany
TECHNICAL ABSTRACT: Histamine is the most commonly used substance to induce itch under experimental conditions. In clinical situations pruritus is often not caused by histamine alone, also other mechanisms have an impact on that clinical condition. The intradermal application of cowhage spicules provides an alternative and histamine independent model to induce itch. This study compared the effects of cowhage spicules and histamine on the skin response and on the cognitive assessment of the sensations which were induced by an itch-scratch interaction. Cowhage spicules were fixed to a cotton applicator. Histamine application was done with inactivated spicules which had been charged by dipping them into a histamine solution (1%). Cowhage or histamine loaded spicules, respectively, were applied to the skin of the forearm by pressing the

head of the cotton applicator carrying the spicules against the skin (Johanek et al, J.Neurosci., 2007). Both types of spicules were applied in randomized order. The subjects were blind to that and were prevented from seeing the application site.
In the first test subjects had to fill in a questionnaire. Blood flow increases of the skin was measured by Laser Doppler Imaging. In a second test the individual itch sensation was assessed on electronic Visual Analogue Scale while the skin was intermittently scratched proximal to the application site. Skin blood flow of the finger as a measure of vasoconstriction was recorded by laser Doppler flowmetry.
Cowhage induced an itch sensation, which feels significantly more stinging, sharp and pricking. Histamine produced a distinctly stronger flare response regarding size and intensity as compared with cowhage. After application of histamine the itch sensation rose faster than after application of cowhage. Also the peak of itch was reached earlier in case of histamine. In contrast, after scratching itch sensation reappeared later following histamine compared with cowhage.
The application of spicules induced a significant drop in the skin blood flow (vasoconstriction) which recovered within one minute. With start of the itch sensation the vasoconstriction during cowhage was stronger as compared with histamine. Each scratch cycle induced an additional short lasting vasoconstriction which was stronger during histamine, but the recovery to baseline level was quicker during cowhage.
These findings further support the hypothesis that different pathways mediate the sensations of itch induced by either cowhage or histamine. They are also differently modulated when scratching is applied to the affected skin which can be treated as a clue that the two itch sensations are also differently processed in the brain.

Speaker's Summary

Speaker: Robert LaMotte, PhD **(203) 737-2720**
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New Haven, Conn.

Itch in Humans and Animal Models (301.2)
Symposium: The Neurobiology of Itch
Monday, November 17, 8:35–8:55 a.m., Washington Convention Center, Ballroom B

Our studies of itch provide new information about the kinds of behavioral responses in animals and sensory responses in humans that occur when an itchy or painful chemical is applied to the skin.

Studies of the neurobiology and pharmacology of itch in humans require the use of valid experimental models in animals. For a model to be valid, experimental stimuli that elicit different types of sensation in human, particularly itch and pain, should evoke correspondingly different types of behavior in animals. For example, histamine, a chemical that can contribute to allergic responses and hives, evokes itch when injected into human skin. Capsaicin, the active ingredient in hot peppers, evokes burning pain but not itch. A well-known model of itch, in certain strains of mice, measures the amount of scratching with the hind limb that occurs in response to an itchy chemical, such as histamine, applied to the nape of the neck. However, we discovered that when capsaicin was injected into the neck, the mice also scratched, and a greater dose of the chemical evoked more scratching, We realized that only one type of behavior was available to the mouse when a chemical was applied to the nape of the neck, namely, hindlimb scratching, In contrast, if a chemical was applied to the skin of the cheek, we found that two different behaviors were directed toward the site of an injection: In response to histamine, the mouse scratched the cheek with the hind limb; in response to capsaicin, the mouse wiped the cheek with the forelimb. This simple test therefore provided a behavioral differentiation of itch from pain in the mouse (Shimada and LaMotte, Pain, in press).

We also obtained new information about the sensations that humans report when algesic and itchy chemicals were applied to a minute region of the superficial skin on the forearm by means of a single spicule or needle-like hair of a tropical plant called cowhage (Mucuna pruriens). The cowhage spicule, with a tip diameter of a few thousandths of a millimeter and inserted only a few tenths of a millimeter into the skin, contains an itchy substance, recently identified as a cysteine protease (Reddy et al. 2008). Forty-five subjects were asked to categorize the quality and perceived intensity of sensation at regular intervals of time.

We discovered that a single native spicule could produce a significant itch typically lasting 5 -15 minutes that was typically accompanied by sensations of pricking/stinging or, less frequently, burning. The nociceptive sensations were usually of lesser magnitude or duration than the itch. A spicule that had been heated to inactivate the protease evoked no sensation after its insertion into the skin. Surrounding the site of application of an active cowhage spicule on the arm was often a large area of "itchy skin" lasting tens of minutes to an hour or more and within which a light touch could elicit an itch or a fine springy filament could elicit a greater than normal pricking pain followed by an enhanced itch sensation. Thus, a very small amount of chemical applied to a minute region of the hairy skin could not only elicit itch and pain but temporarily alter the way in which the nervous system processed mechanically evoked sensations in the skin.

In the poster presented at this meeting by Sikand et al., it is shown that a single heat-inactvated spicule, soaked in histamine or capsaicin and then dried, elicited the same qualities of itch and nociceptive sensations as cowhage and, at the higher doses of chemical, the same magnitude of itch. These sensations were accompanied in many instances by a surrounding area of itchy skin having the same properties as

that evoked by cowhage. Thus, when histamine, protease, and even capsaicin were applied by means of a spicule, each was capable of eliciting similar qualities of itch and pain and enhanced sensory states in which mechanically evoked sensations were altered. It is possible that each chemical applied by means of a spicule may be activating the same types of nerve endings supplying the superficial layers of the skin.

Speaker's Summary

Speaker: Zhou-Feng Chen, PhD **(314) 747-5093**
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St. Louis, Mo.

Cellular/Molecular Mechanisms Mediating Itch (301.5)
Symposium: The Neurobiology of Itch
Monday, November 17, 10:20–10:55 a.m., Washington Convention Center, Ballroom B

Itchiness is one of the most common symptoms encountered in clinics. And chronic itch impairs the life quality of millions of people. Unfortunately, the majority of chronic itch problems cannot be relieved by anti-histamine drugs. Why we cannot stop scratching remains a big puzzle for researchers. In addition, itch has long been considered a milder form of pain that is difficult to be separated from a pain response. It has been unclear whether pain and itch are transmitted by the same sets or by distinct sets of genes and neurons.

Our laboratory identified the first gene for the itch sensation in the central nervous system. Called gastrin-releasing peptide receptor (GRPR), the gene exerts its effects in the most superficial layer of the spinal cord, a region that also is critical for transmitting pain and temperature sensation. Mice lacking the gene don't scratch as well as their littermates, and inhibiting GRPR activity reduces scratching behaviors evoked by agents that cause itching. But when GRP, the peptide that enhances GRPR activity, was injected into the spinal cords of mice, unexpectedly, we found these mice scratched like crazy.

Although GRPR is expressed in a region known to be important for relaying pain information, mice lacking GRPR demonstrate normal pain responses. These studies indicate that although GRPR is critical for transmitting itch information from the skin to the brain. It may not be involved in the transmission of pain signals. These results were published last year.

Our findings that GRPR is important for itch but not for pain sensation have important therapeutic implications. For example, spinal injection of pain killers such as morphine is widely used in the treatment of patients who suffer from severe pain. One of the well-known side effects of this procedure is itchy skin. This side effect has been thought to be inseparable from the good pain killer effect. We hypothesized that GRPR may be responsible for the generation of the itchy sensation but not involved in the pain response.

To test this idea, we compared both scratching and pain killer effects between mice lacking GRPR and their normal littermates following spinal injection of pain killers such as morphine. We found that mice without the GRPR gene did not scratch at all. Importantly, these animals maintained the drug's normal pain killing effect. We then injected an inhibitor of GRPR into the spinal cords of mice receiving pain killing drugs, and the scratching behaviors evoked by the pain killers were completely eliminated. The pain killer effect, however, remained the same.

Our results suggest that pain and itch can be separated from one another, and provide us with molecular clues to explain why pain and itch are different sensations that may be relayed by distinct pathways in the spinal cord. Our studies also suggest GRPR is required for morphine-induced itch.

The discovery of the first itchy gene in the spinal cord raises the hope that it may be possible to relieve itchiness in patients by blocking the GRPR function using GRPR inhibitors. Such treatment would not affect the body's pain pathway, and therefore would not inhibit the pain-killing effects of drugs such as morphine. Because GRPR can transmit itch signals evoked by histamine-independent itchy agents, it also

is possible that chronic itch problems, which cannot be relieved by anti-histamine-based medicine, may be relieved by targeting GRPR in the spinal cord.

Speaker's Summary

Speaker: Matthias Ringkamp, MD PhD **(410) 955-2250**
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Peripheral Neurons Mediating Itch (301.3)
Symposium: The Neurobiology of Itch
Monday, November 17, 8:55–9:10 a.m., Washington Convention Center, Ballroom B

The findings of our studies suggest that itch, i.e. the sensation that causes the desire to scratch, can be mediated by different types of nerve fibers innervating the skin in human.

Chronic itch can be a major complaint of patients suffering from a variety of different illnesses including dermatological, kidney or liver diseases, blood cell diseases or infectious diseases like HIV and Herpes zoster. Itch can be devastating to patients and lead to extensive loss of quality of life. Unfortunately, the treatment of itch is often unsatisfactory, as antihistamines which are very effective in inhibiting itch accompanying seasonal allergies or mosquito bites are often without effect. A better understanding of the mechanisms and the nerve fibers involved in mediating the sensation of itch is therefore expected to lead to the development of new strategies for the treatment of chronic itch.

Since antihistamines are often ineffective in the treatment of itch, we used the small spicules that cover the pods of the cowhage (Mucuna pruriens), a tropical plant, in our research. Insertion of such spicules into the skin produces the sensation of itch in humans. Previous work suggested that cowhage itch is not mediated through histamine, but a systematic study had not been performed.

In a first series of experiments we tested if cowhage induced itch in humans is mediated through histamine. We found that cowhage induced itch could not be blocked by pre-treating the skin with a cream containing an antihistamine. However, pretreatment of the skin with capsaicin, the ingredient of hot chili peppers prevented cowhage induced itch. This finding suggests that capsaicin sensitive nerve fibers that are also involved in mediating the sensation of pain may mediate cowhage induced itch. We also observed that cowhage itch is not accompanied by skin reddening ('flare') in the surrounding skin. As application of histamine usually produces a flare in the skin surrounding the application site, this finding suggested that cowhage and histamine produce the sensation of itch through activation of different sets of nerve fibers innervating the skin.

In electrophysiological recordings from nerve fibers we tested if cowhage and histamine activated the same type of nerve fibers innervating the skin. Histamine has previously been shown to activate a special class of nerve fibers, so called 'mechanoinsensitive' nerve fibers. In contrast to histamine, we found that cowhage exclusively activated a different type of nerve fiber that can also be activated by mechanical and heat stimuli. Activity in this type of nerve fiber may also explain the sensation of itch that can be produced by punctate mechanical and heat stimuli. Taken together, our findings demonstrate that the sensation of itch is not mediated exclusively through one but multiple types of nerve fibers.

As nerve fibers mediating the sensation of itch are also involved in mediating the sensation of pain, future research will focus on the mechanisms used by the brain to differentiate these quite different sensations.