The Secret of Saltiness

Virginia researchers solve the mystery of why sodium chloride goes better on pretzels than sodium acetate does

WHY DOES TABLE SALT (SODIUM CHLORIDE) taste saltier than other sodium salts? This conundrum has long baffled researchers who study the sense of taste. At first it might seem that all sodium salts should taste the same, since physiologists have known for more than 30 years that the primary source of the salty taste is the positively charged sodium ions—not the negatively charged anions paired with them. But researchers have known for equally long that some sodium salts taste "saltier" than others. That meant anions must be playing some role in salt perception. No one knew what that role was. Until now.

In an article on page 724 of this issue of *Science*, physiologists John DeSimone and Gerard Heck and graduate student Qing Ye of Virginia Commonwealth University announce the answer to the "anion paradox." They found that anions exert their effect on taste by surrounding the taste receptor cells that make up the taste buds, changing the cells' chemical environment and thus modifying their sensitivity to sodium. "It really does explain a number of things that have been puzzles for a while," says membrane physiologist Sheella Mierson of the University of Delaware.

But that explanation has been achieved only at the end of a research trail that has proceeded by fits and starts for decades, while researchers doggedly plugged away at investigating the thousands of salt-sensitive taste cells grouped in taste buds on the surface of the tongue. Each bud contains 100 or so taste cells, which, when excited, trigger a nerve cell that carries the signal to the brain. In the 1950s, physiologist Lloyd Beidler at Florida State University showed that the basic salty taste could be accounted for by sodium ions. But it wasn't until the mid-1980s that a number of research groups showed precisely how that effect actually occurs: sodium ions enter the receptor cells through special sodium channels in the cells' outer membrane.

This discovery made sense in terms of what was already known about electrophysiology; sodium ions flowing into the taste cell change the voltage across the cell membrane, making the normally negative interior a bit more positive. This "depolarization" electrically excites the cell, leading to the release of a neurotransmitter that stimulates an adjacent nerve cell to relay the sensation of saltiness to the brain.

But that discovery didn't clarify the second effect Beidler observed: that the anion paired with sodium could change the perception of saltiness. "He showed that, at a given sodium concentration, sodium chloride was the most excitatory," says DeSimone. "If you tried sodium acetate or other sodium salts, you got a response, but it was almost always smaller. It was obvious the anion was having a major effect."

Though physiologists puzzled over the anion effect, none took a serious stab at explaining it until 1987, when physiologist Harry Harper, then with Stauffer Chemical Company, proposed that the anions might be diffusing around the taste cells through tight junctions: the semipermeable seals between cells in a layer called an epithelium, like the one on the surface of the tongue. By altering the ionic milieu around the cells, Harper posited, anions could modify the cells' response to salts.

While Harper's model provided an appealing theoretical explanation, there was little experimental evidence to back it up. And other possibilities remained—such as the idea that the anions exert their effect not by flowing around the receptors but by entering them directly. Last year, however, Sidney Simon and Ellen Elliot of Duke University published experiments ruling out that possibility. "We tried all the known inhibitors of anion channels [in cell membranes] that we could think of," says Simon. But none of the inhibitors changed the response to sodium chloride in the rat (their experimental animal). They concluded that chloride was not passing through channels into the taste cells—and therefore was probably flowing around them, as Harper had proposed.

But their reasoning was by elimination, and what was needed next was a direct test of the hypothesis. "After seeing [Simon and Elliot's results] Gary Heck and I and our student Qing Ye got to thinking... it should be possible to test this directly," recalls DeSimone. Heck had recently designed the technical equipment needed for the job: a tiny chamber, 5 mm in diameter, that can deliver solutions to a rat's tongue while electrodes measure the voltage across the surface layer of cells.

Using the chamber, DeSimone, Heck, and Ye did things no taste researcher had been able to do before. Instead of just putting a salt solution on the rat's tongue and measuring the response in the taste nerves, they could simultaneously measure electrical changes across the tongue's surface layer, or epithelium, directly. They could even control the voltage across the surface to see what effect that had on the nerve's response. And that kind of direct observation of the epithelium was just what was needed to confirm what Harper, Elliot, and Simon had proposed.

The first thing the team found was that different salt solutions had different effects on the voltage across the epithelium. When sodium acetate and sodium gluconate were put in the chamber, the inside of the epithelium became more positive relative to the tongue's surface. The reason seemed to be that the positively charged sodium ions were carried into the

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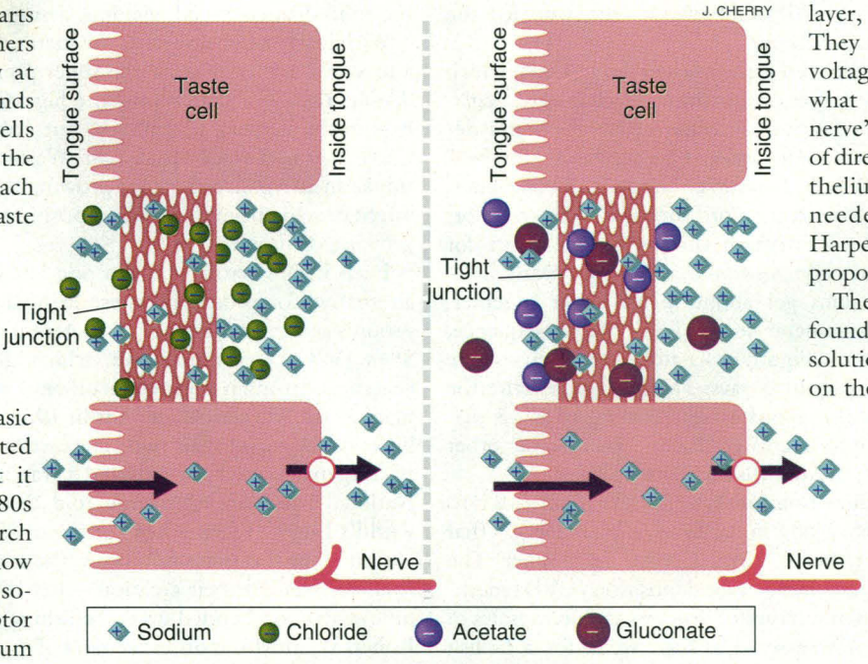

Salty sensations. *Chloride ions pass easily into the interior of the tongue's epithelium* (left). *Larger acetate and gluconate ions pass through with difficulty.*

tissue through the taste cells or through the tight junctions, but the negatively charged acetate and gluconate ions were unable to follow at the same rate.

Sodium chloride, on the other hand, gave only a small change in the voltage, because chloride (a smaller ion than acetate or gluconate) followed sodium through the tight junctions into the epithelium, where its negative charge acted to balance some of the sodium's positive charge.

Furthermore, the team found that the voltage differences were correlated with the nerve's response to salts: the bigger voltage changes associated with gluconate and acetate ions reduced the nerve's response. That makes sense, says DeSimone, because a lot of positive charge building up around the taste cells inside the epithelium would increase the voltage across the taste cell membrane, creating a condition called hyperpolarization. Hyperpolarization would, in turn, make it more difficult for sodium to trigger the electrical excitation that stimulates the nerve. And that would neatly explain why it requires a higher concentration of sodium acetate or gluconate than of sodium chloride to get the same degree of "salty" taste.

"So far everything was consistent," says DeSimone, "but that wasn't the final proof. The final proof is to go in there and actually control that [voltage]. Don't let the salts determine it, you determine it." If the anions act by influencing the voltage across the epithelium, DeSimone and his colleagues reasoned, they should be able to hold that voltage constant—and in that way equalize the responses to all sodium salts. When they did the experiment, that's what they found. They used a technique called a voltage clamp, in which they injected current to keep the voltage steady. A constant voltage proved to be the great equalizer: the nerve's response to all the salts was the same, confirming the hypothesis that the flow of anions through tight junctions was causing the taste differences.

"It's an excellent study," says taste physiologist David Hill of the University of Virginia. "The results seem clear to everyone in the field." Questions remain about how the tight junctions restrict the flow of ions, points out Susan Schiffman, who studies the anion effect at Duke University. But that focus on the tight junctions is an important outcome of the work, says Delaware's Mierson—a view that goes beyond the solution of the anion paradox to address the bigger question of how the tongue's epithelium functions as a tissue. "The work treats the whole thing as a system," she says, and highlights the fact that important functions reside not only in cells but in the spaces between them. ■ MARCIA BARINAGA

How Plants Cope With Stress

When an insect munches on a plant the last thing it's looking for is a bad case of indigestion. But that's often what it gets, the result of a natural defense mechanism in which plants may produce proteinase inhibitors that disrupt the digestion of feeding insects, thereby encouraging them to seek their sustenance elsewhere. Despite the importance of this stress response to plant defenses—and the possibility that a better understanding of how the system operates might aid in the design of better pest control strategies—plant biologists were unable to identify the compound that triggers this stress response.

Now, in work described at last month's Third International Congress for Plant Molecular Biology in Tucson, Arizona, and also in a paper in *Science* (23 August, p. 895), biochemist Clarence Ryan and his colleagues at Washington State University in Pullman have isolated a new plant compound that seems to do the job. Even more exciting, the researchers have filled a major gap in plant biology. Previously, all the known plant hormones had been relatively simple compounds. Indeed, plants were thought to lack the capacity to use the more sophisticated polypeptide and protein hormones that so commonly serve to coordinate physiological responses in animals. But the molecule identified by the Ryan group is a polypeptide that behaves very much like animal polypeptide hormones. Says molecular biologist Mich Hein of the Scripps Research Institute in La Jolla, California, this discovery will prompt researchers to seek similar compounds in plants. "There have got to be more of these things," he notes.

CLARENCE RYAN

Dining out. *This tobacco hornworm may get more than dinner from tomato leaves.*

Twenty years ago, however, when Ryan began his search for the stress-triggering molecule, the idea that plants don't use sophisticated signaling molecules such as polypeptides was so ingrained that he never even thought about polypeptides. Instead, he focused on known plant hormones, including the auxins and cytokinins, which regulate growth and development, and also on carbohydrates, a logical choice for the stress signal molecule because they can be released from plant cell walls broken down by feeding insects. The trouble was none of these simple compounds worked. "It became clear that we had an unusual signal," Ryan says.

About 4 years ago then, he decided to take a different tack, ridding the plant extracts of all carbohydrates, as well as of the auxins and cytokinins, and looking for an entirely new class of compounds. The approach paid off. Eventually, Ryan's team, including Gregory Pearce, Daniel Strydom, and Scott Johnson, managed to isolate 1 microgram of a material that stimulates proteinase inhibitor production from about 60 pounds of tomato leaves. The material's chemical nature? It proved to be a peptide containing 18 amino acids. After watching how this newly identified peptide behaved in plants, the researchers dubbed it "systemin" because it is transported systemically throughout the plant, just as animal hormones are. Systemin also resembles animal peptide hormones in that it is very potent—as little as one part in 10 trillion can trigger a plant stress reaction—and it is synthesized as part of a larger precursor protein, from which it must be cleaved.

The next question was: How does systemin elicit plant stress reactions? And there Ryan says that his postdoc Edward Farmer may have hit on a clue when he showed that methyl jasmonate, a plant lipid, induces the production of proteinase inhibitors, just as systemin itself does. This was somewhat of a surprise, since methyl jasmonate had not previously been shown to be part of the stress response pathway. But in light of Farmer's results, Ryan speculates that systemin may work by stimulating the synthesis of methyl jasmonate, which is made from linolenic acid that can be released from membrane lipids by a lipase enzyme. More work will be needed to verify this scheme, Ryan cautions, but even if all the steps in the model are not confirmed, one thing is sure. Plants no longer have to take a back seat to animals when it comes to the sophistication of their hormonal pathways. ■ ANNE SIMON MOFFAT