Scientists discover how chemical repellants trip up insects

August 25 2010

Fire up the citronella-scented tiki torches, and slather on the DEET: Everybody knows these simple precautions repel insects, notably mosquitoes, whose bites not only itch and irritate, but also transmit diseases such as West Nile virus, malaria and dengue.

Now, Johns Hopkins scientists have discovered what it is in the bugs' molecular makeup that enables citronellal (the aromatic liquid used in lotions, sprays and candles) and DEET, to deter insects from landing and feeding on you. A better understanding of these molecular-behavioral links already is aiding the team's search for more effective repellants.

In separate studies published Thursday, August 26, in *Neuron* and Current Biology, the Johns Hopkins researchers reveal how mosquitoes and other insects taste DEET — a man-made compound that's been the most widely used insect repellent for more than 50 years — and smell citronellal, a commonly used botanical repellant.

Three taste receptors on the insects' tongue and elsewhere are needed to detect DEET. Citronellal detection is enabled by pore-like proteins known as TRP (pronounced "trip") channels. When these molecular receptors are activated by exposure to DEET or citronellal, they send chemical messages to the insect brain, resulting in "an aversion response," the researchers report.

"DEET has low potency and is not as long-lasting as desired, so finding the molecules in insects that detect repellents opens the door to identifying more effective repellents for combating insect-borne disease," says Craig Montell, Ph.D., a professor of biological chemistry and member of Johns Hopkins' Center for Sensory Biology.

Scientists have long known that insects could smell DEET, Montell notes, but the new study showing taste molecules also are involved suggests that the repellant deters biting and feeding because it activates taste cells that are present on the insect's tongue, legs and wing margins.

"When a mosquito lands, it tastes your skin with its gustatory receptors, before it bites," Montell explains. "We think that one of the reasons DEET is relatively effective is that it causes avoidance responses not only through the sense of smell but also through the sense of taste. That's pretty important because even if a mosquito lands on you, there's a chance it won't bite."

The Johns Hopkins study of the repellants, conducted on fruit flies because they are genetically easier to manipulate than mosquitoes, began with a "food choice assay."

The team filled feeding plates with high and low concentrations of color-coded sugar water (red and blue dyes added to the sugar), allowing the flies to feed at will and taking note of what they ate by the color of their stomachs: red, blue or purple (a combination of red and blue). Wild-type (normal) flies preferred the more sugary water to the less sugary water in the absence of DEET. When various concentrations of DEET were mixed in with the more sugary water, the flies preferred the less sugary water, almost always avoiding the DEET-laced sugar water.

Flies that were genetically engineered to have abnormalities in three different taste receptors showed no aversion to the DEET-infused sugar water, indicating the receptors were necessary to detect DEET.

"We found that the insects were exquisitely sensitive to even tiny concentrations of DEET through the sense of taste," Montell reports. "Levels of DEET as low as five hundredths of a percent reduced feeding behavior."

To add to the evidence that three taste receptors (Gr66a, Gr33a and Gr32a) are required for DEET detection, the team attached recording electrodes to tiny taste hairs (sensilla) on the fly tongue and measured the taste-induced spikes of electrical activity resulting from nerve cells responding to DEET. Consistent with the feeding studies, DEET-induced activity was profoundly reduced in flies with abnormal or mutated versions of Gr66a, Gr33a, and Gr32a.

In the second study, Montell and colleagues focused on the repellent citronellal. To measure repulsion to the vapors it emits, they applied the botanical compound to the inside bottom of one of the two connected test tubes, and introduced about 100 flies into the tubes. After a while, the team counted the flies in the two tubes. As expected, the flies avoided citronellal.

The researchers identified two distinct types of cell surface channels that are required in olfactory neurons for avoiding citronellal vapor. The channels let calcium and other small, charged molecules into cells in response to citronellal. One type of channel, called Or83b, was known to be required for avoiding DEET. The second type is a TRP channel.

The team tested flies with mutated versions of 11 different insect TRP channels. The responses of 10 were indistinguishable from wild-type flies. However, the repellent reaction to citronellal was reduced greatly in flies lacking TRPA1. Loss of either Or83b or TRPA1 resulted in avoidance of citronellal vapor.

The team then "mosquito-ized" the fruit flies by putting into them the gene that makes the mosquito TRP channel (TRPA1) and found that the mosquito TRPA1 substituted for the fly TRPA1.

"We found that the mosquito-version of TRPA1 was directly activated by citronellal," says Montell who discovered TRP channels in 1989 in the eyes of fruit flies and later in humans.

Montell's lab and others have tallied 28 TRP channels in mammals and 13 in flies, broadening understanding about how animals detect a broad range of sensory stimuli, including smells and tastes.

"This discovery now raises the possibility of using TRP channels to find better insect repellants."

There is a clear need for improved repellants, Montell says. DEET is not very potent or long-lasting except at very high concentrations, and it cannot be used in conjunction with certain types of fabrics. Additionally, some types of mosquitoes that transmit disease are not repelled effectively by DEET. Citronellal, despite being pleasant-smelling (for humans, anyway), causes a rash when it comes into contact with skin.

More information:
-- Lee et al.: “Avoiding DEET through insect gustatory receptors.” Publishing in Neuron 67, 1-7, August 26, 2010. DOI 10.1016/j.neuron.2010.07.006
-- Kwon et al.: "Drosophila TRPA1 Channel Is Required to Avoid the Naturally Occurring Insect Repellent Citronellal." Publishing in Current Biology 20, 1-7, September 28, 2010. DOI 10.1016/j.cub.2010.08.016

Provided by Johns Hopkins Medical Institutions