TRP channel blamed for burning cold after a tropical fish meal

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Ciguatera is one of the most common forms of food poisoning, occurring after consumption of fish contaminated with ciguatoxins. New work by Vetter *et al* (2012) reveals the key molecular players that underlie the altered temperature sensation associated with ciguatera. In particular, they show that ciguatoxins act on sensory neurons that express TRPA1, an ion channel implicated in the detection of noxious cold.

Imaging yourself in the following idyllic settings: a white sandy tropical beach, blue sea and sky; and a barbecue on which your catch of the day, a 4-kg red snapper, is being grilled for dinner. But then, just a few hours after savouring the delicious fish meal, you undergo another unforgettable but far less heavenly experience: it starts with severe nausea, vomiting and diarrhoea, followed by disturbing neurological symptoms including headache, numbness and burning of the skin. You contracted ciguatera, a food-borne disease that affects an estimated 500 000 persons each year, particularly in the tropical and sub-tropical coastal regions (Dickey and Plakas, 2010).

So what causes ciguatera? The prime culprits are dinoflagellates of the genus *Gambierdiscus*, small microalgae that produce a group of fat-soluble toxins called ciguatoxins and grow on macroalgae in coral reefs (Yasumoto *et al*, 1977). *Gambierdiscus*-containing macroalgae serve as food for herbivorous fish, which results in the introduction of

ciguatoxins into the food web. These smaller, herbivorous fish are on the menu of large, carnivorous reef fish, such as mackerel, red snapper, or barracuda, which can accumulate ciguatoxins in the fatty parts of their body over years, apparently without any distress or obvious sign of disease. However, when such a mouth-watering but toxin-loaded catch appears on your menu, just a few bites (or sips of fish broth) can be sufficient to induce ciguatera (Figure 1A). Disturbingly, affected fish looks, smells, and tastes normal, and ciguatoxins are resistant to grilling, drying, or cooking of the fish, so there is no straightforward method to predict whether your tropical culinary dream will be followed by a

Figure 1 Ciguatera and cold allodynia. (A) Ciguatoxins, polycyclic polyether toxins, are produced by dinoflagellates of the genus *Gambierdiscus*, which reside in macroalgae in tropical coral reefs. These *Gambierdiscus*-containing macroalgae are eaten by herbivorous fish, which in turn serve as food for larger carnivorous fish, which accumulate the toxins in their bodies. Human consumption of these toxin-loaded fish causes ciguatera. (B) Ciguatoxins cause cold allodynia by increasing the cold sensitivity of TRPA1-expressing nociceptor neurons. *Left*, sensory nerve ending of a normal TRPA1-expressing nociceptor. At temperatures in the warm or innocuous cold region, the membrane potential (E_m) is around -65 mV, and both TRPA1 and the voltage-gated Na^+ (Na_v) channels are closed. Cooling $< 10^\circ\text{C}$ causes sufficient TRPA1 activation to cause action potential (AP) firing, experienced as burning cold. *Right*, ciguatoxin causes modification of the voltage-gated Na^+ channels, resulting in significant opening at rest and depolarization of E_m to around -55 mV. Increased neuronal excitability and depolarization-induced activation of TRPA1 result in action potential firing and burning pain at innocuously cold temperatures.

ciguatera nightmare. Yes, there are commercial kits available to test for the presence of the toxin in fish, but these are considered too cumbersome, unreliable, and/or expensive to be of general practical use. And yes, there are persistent rumours of tropical fishermen feeding part of their catch to the cat first...

Apart from the gastrointestinal torment, which mostly fades away after a day or so, one of the most striking and disturbing symptoms of ciguatera is a form of oversensitivity to cold, termed as cold allodynia (Isbister and Kiernan, 2005). For instance, ciguatera sufferers have reported that a refreshing dive in the ocean actually caused burning pain, or that drinking cool beer felt like too hot coffee. The mechanisms whereby ciguatoxins provoke this peculiar form of altered temperature sensitivity, which can last for weeks to years, were fully unknown.

In their paper, Vetter *et al* (2012) provide the first insights into the molecular mechanisms underlying cold allodynia induced by P-CTX-1, the most potent ciguatoxin present in the Pacific Ocean. First, they demonstrate that ciguatoxin-induced cold allodynia can be reproduced in a mouse model: following injection of minute quantities of P-CTX-1 into the sole of the paw, these mice exhibited clear signs of pain at moderately cool ambient temperatures, which were alleviated by warming. Next, they provide evidence that P-CTX-1 acts by sensitizing a specific subset of sensory neurons, characterized by the expression of the cation channel TRPA1. Finally, they show that pharmacological inhibition or genetic disruption of TRPA1 in mice strongly reduce the severity of cold allodynia upon P-CTX-1 injection.

TRPA1 is a member of the TRP superfamily of cation channels, many of which play key roles in the sensory system as molecular sensors of temperature and of a variety of chemical ligands (Talavera *et al*, 2008). TRPA1 was known to be expressed in a subset of pain-sensing neurons (nociceptors), where it acts as a sensor of a wide variety of pungent/irritating substances (e.g., mustard oil) and of noxious cold (Story *et al*, 2003; Jordt *et al*, 2004; Karashima *et al*, 2009). TRPA1, like most other temperature-sensitive TRP channels, is voltage dependent, and thermal activation reflects a temperature-induced shift of its voltage-dependent activation curve towards more negative potentials (Voets *et al*, 2004; Karashima *et al*, 2009).

Although TRPA1-expressing neurons show exquisite sensitivity to P-CTX-1, Vetter *et al* (2012) show that TRPA1 by itself is insensitive to P-CTX-1. Instead, P-CTX-1 responsiveness requires the combined presence of TRPA1 and of voltage-gated Na⁺ channels. In nociceptor neurons of non-intoxicated individuals, TRPA1 and the voltage-gated Na⁺ channels are largely closed at the resting membrane potential of around -65 mV, both at warm and at innocuously cool temperatures. Only upon cooling into the noxious cold range (<10°C), sufficient TRPA1 activation occurs to cause

depolarization beyond the threshold for action potential firing (Figure 1B). This creates the sensation of burning cold (Karashima *et al*, 2009), which represents an important alarm signal, warning the body for potential cold-induced tissue damage (frostbite). However, as Vetter *et al* (2012) demonstrate, poisoning with P-CTX-1 strongly deregulates the cold sensitivity of the TRPA1-expressing nociceptors, such that they greatly overdo their alarm function. Reconfirming earlier work (Isbister and Kiernan, 2005), they show that P-CTX-1 acts as a potent modifier of voltage-gated Na⁺ channels in these neurons. In particular, at a concentration as low as 1 nM, P-CTX-1 causes a hyperpolarizing shift of the voltage dependence of activation of Na⁺ channels. This has a dual effect on the cold sensitivity of the TRPA1-expressing nociceptors (Figure 1B): (1) modulation of the voltage-gated Na⁺ channels increases the excitability of the neurons, an effect that is further enhanced by the inhibitory effect of P-CTX-1 on voltage-gated K⁺ channels and (2) P-CTX-1-treated voltage-gated Na⁺ channels allow Na⁺ influx at the resting membrane potential of these neurons, resulting in membrane depolarization, which in turn facilitates the voltage-sensitive activation of TRPA1 (Karashima *et al*, 2009). These mechanisms explain why in individuals suffering from ciguatera innocuous cooling is already sufficient to provoke action potential firing of TRPA1-expressing nociceptors, and is perceived as a burning pain.

In addition to provide a molecular and cellular basis for ciguatera-associated cold allodynia, the study by Vetter *et al* (2012) also further strengthens the theory that TRPA1 is a relevant cold sensor *in vivo*, which has been disputed in several studies (Jordt *et al*, 2004; Knowlton *et al*, 2010). In particular, by using non-invasive functional MRI brain imaging, Vetter *et al* (2012) show for the first time significant differences in brain activity between wild-type and TRPA1-deficient mice when cooling their paw, and this difference is even more pronounced after the injection of P-CTX-1.

Some important open questions remain. For example, given that P-CTX-1 is also acting on voltage-gated Na⁺ channels in TRPA1-negative sensory neurons, it is surprising that the toxin did not cause increased sensitivity to other stimuli, such as heat or mechanical stimuli. Moreover, it would be interesting to know whether TRPA1, which is also highly expressed on sensory neurons that innervate the gastrointestinal tract, plays a role in the gastrointestinal symptoms of ciguatera. If so, then TRPA1 would be an even more appealing target for the development of specific drugs to create relief from ciguatera symptoms.

Conflict of interest

The author declares that he has no conflict of interest.

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