



# Bad Fish

## Part I—"Poisoned!"

*Cell and Molecular Biology Edition*

by

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One evening during a recent trip to Indonesia, Dr. Marshall Westwood from the Montana Technical Institute sat down to a meal of pufferfish and rice. Within an hour of returning to his hotel room, Dr. Westwood felt numbness in his lips and tongue, which quickly spread to his face and neck. Before he could call the front desk, he began to feel pains in his stomach and throat, which produced feelings of nausea and eventually severe vomiting.

Fearing that he had eaten some "bad fish" for dinner, Dr. Westwood called a local hospital to describe his condition. The numbness in his lips and face made it almost impossible for him to communicate, but the hospital staff managed to at least understand the address he gave them and they sent an ambulance in response. As Dr. Westwood was rushed to the hospital, his breathing became increasingly difficult.

### Doctor's Notes

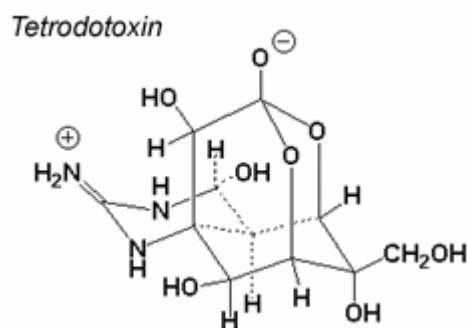
The patient presented in the ED with severe headache, diaphoresis, motor dysfunction, paresthesias, nausea, and an ascending paralysis that spread to the upper body, arms, face, and head. The patient was cyanotic and was hypoventilating. Within 30 minutes of presenting in the ED, Dr. Westwood developed bradycardia with a BP of 90/50. Atropine was administered in response to the bradycardia. IV hydration, gastric lavage, and activated charcoal followed a presumptive diagnosis of tetrodotoxin poisoning based on the clinical presentation in the ED. Five hours after intervention, the following vitals were noted:

- BP 125/79
- HR 78bpm
- Oxygen saturation: 97% on room air

### Follow-up

Within a few hours, Dr. Westwood's condition improved and he was on his way to a full recovery. After discussing his case with his physician, he learned that he had probably been the victim of a pufferfish poisoning. The active toxin in the tissues of this fish is a chemical called tetrodotoxin.

Tetrodotoxin is in a class of chemicals known as neurotoxins due to the fact that it has its effects on nerve cells (neurons). Specifically, tetrodotoxin blocks voltage-gated sodium ion channels on neurons.



## Questions

1. Explain why sodium ions need channels in order to move into and out of cells. Describe the process by which this transport occurs.
2. Describe the structure of a voltage-gated sodium ion channel.
3. Describe the function of the voltage-gated sodium ion channel. In your description, explain what is meant by channel gating and channel inactivation.
4. When nerve cells are at rest, there is an unequal amount of positive and negative charges on either side of a nerve cell membrane. This charge difference is called an *electrical potential*. Describe this "potential" when the neuron is at rest (*resting potential*).
5. What is happening to the electrical potential of a neuron when it generates an *action potential*? What is the function of the action potential in neurons?
6. Describe the role of sodium ions and sodium channels in the *action potential*.

## Mechanism of Action

TTX is an extremely potent neurotoxin that specifically blocks voltage-gated sodium ion channels on the surface of neurons. The molecule consists of a guanidinium group (a positively charged group with three nitrogen atoms), which gives the name to this class of neurotoxins: guanidinium toxins. The molecule also contains a pyrimidine ring and additional fused ring structures.

The channel binding is extremely tight ( $K_d = 10^{-10}$  nM). TTX mimics a hydrated sodium ion as it enters the mouth of the channel and binds to a peptide glutamate residue. The binding becomes tighter as the peptide complex changes confirmation in the second stage of the binding event. Following additional complex conformational changes, TTX forms an electrostatic attachment to the opening of the Na<sup>+</sup> gate channel.

TTX's tight hold on the channel complex is manifested in the occupancy time of TTX v. hydrated sodium ions at the complex. Hydrated sodium reversibly binds on a time-scale of nanoseconds, whereas TTX binds and remains attached to the complex on the order of tens of seconds. With the TTX molecule preventing sodium from entering the channel, sodium movement is effectively shut down and the action potential along the nerve remains blocked. The amount of TTX that can be placed on the head of a pin (less than one milligram) is enough to kill an adult.

## Questions

7. Describe how a sodium ion enters a voltage-gated sodium ion channel. How does this channel act selectively for this ion?
8. What would happen to a neuron if it were exposed to tetrodotoxin? Be specific regarding its effect on the ability of a neuron to communicate.
9. Describe the structure of glutamate. How do you think the guanidinium group of TTX and glutamate in the channel become involved in the binding of TTX to the channel?
10. What is the  $K_d$ ? What does it tell you about the binding of TTX to the Na-channel?

11. Explain what is meant by an electrostatic interaction between two molecules. From what you know about these interactions, would you guess that the TTX effects on the channel are reversible or irreversible? Explain your answer.
12. Explain how a conformation change in the channel complex might lead to tighter interactions between the TTX and the channel.



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## Part II—"Oh No! Not Again"

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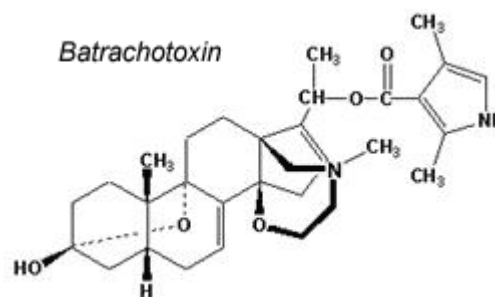
After recovering from his TTX poisoning, Dr. Marshall Westwood decided to take a vacation. An avid birder, he decided to go to Papua New Guinea with Bill Whitlatch, an ornithologist friend of his from Montana Technical Institute.

Three days into their trip, Bill netted bird with an orange body and black wings and head for closer study. Dr Westwood was very curious and asked Bill if he could have a closer look at the bird. After handling the bird and then later wiping his mouth with his hand, Dr. Westwood noticed that his fingers and lips were going numb. His mind immediately flashed back to the disastrous trip to Indonesia and he began to panic. Luckily, the symptoms faded before they progressed into anything more serious.



His friend Bill used a key to identify the animal as a pitohui. The pitohui are small, social songbirds that live in Papua New Guinea. They are generally about 23 centimeters long with strong legs and a powerful beak. Their encounter was the first time anyone had scientifically realized the birds' toxicity.

Before releasing the bird, Dr. Westwood collected feather and tissue samples to bring back to the lab. After returning to Montana, he set out to isolate the toxic compound that he believed was being produced by the pitohui. It appeared that the active ingredient was a homobatrachotoxin. Homobatrachotoxin is a steroidal alkaloid that is similar to batrachotoxin, the toxic principle of the Central American poison arrow frog *Phyllobates aurotaenia*. Batrachotoxin and homobatrachotoxin are both known to act on the voltage-sensitive sodium channels in excitable tissues.



You and your colleagues received a call from Dr. Westwood asking if you could help elucidate the mechanism of action of this toxic compound. One of the hypotheses is that this toxin acts similarly to TTX.

## Questions

13. In your first experiment, you have determined that the receptor affinity for this toxin is not as great as it is for TTX. What does this mean about the value of  $K_d$  for this toxin relative to what is known for TTX?
14. In your second experiment, you generated action potentials in squid axons in the presence of this new toxin. You found that after depolarizing, the membrane potential remained positive for an extended length of time and the repolarization was often extremely delayed. Draw a graph (membrane potential in mV vs. time) to illustrate this effect.
15. As you continued to experiment with higher concentrations of the toxin, you found cases when the cell could not repolarize at all, or if it began to repolarize, it would immediately depolarize again. Using this description and the description in the previous question, describe how this toxin acts on voltage-gated sodium ion channels.
16. In your last experiment, you conducted binding studies with homobatrachotoxin and tetrodotoxin. You find that tetrodotoxin can prevent homobatrachotoxin from producing its effect on squid axons, but protein-binding studies show that the two toxins do not bind to the same spot on the ion channel. Explain how tetrodotoxin is able to inhibit the homobatrachotoxin effect.

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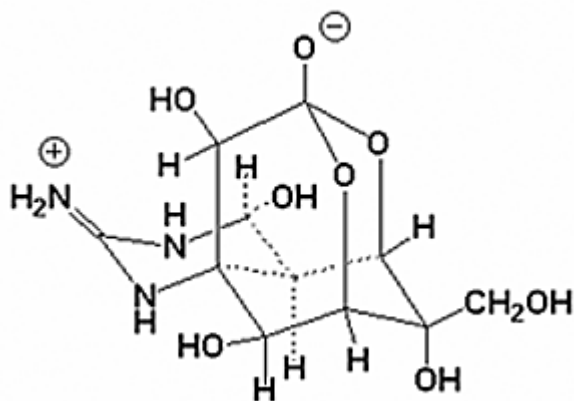
## Part III—"Pharmacology"

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In this section, you (or your group) will become a small pharmaceutical company that is trying to develop a drug that can be used in the hospital to treat tetrodotoxin poisoning. There is no antidote to tetrodotoxin poisoning. Therefore, it is extremely important that your company is successful in this endeavor. In your report, you need to include the following:

- **Your Company Name:**
  - *What do you want to call your company? Be creative!*
- **Name of Your Drug:**
- **Mechanism of Action:**
  - *Describe how your drug will work.*
  - *Be sure to provide details regarding its action. You are now familiar with the activity of this toxin at the level of molecules and cells, so your description should contain details of how your drug works at that level.*

Some of your information will come from your understanding of what tetrodotoxin does to neurons. There is no right or wrong answer to how your drug will work because there currently is no drug to treat this problem. You may want to do some research on drugs that have actions at neurons (specifically sodium ion channels) and diseases that involve sodium ion channels. This will help you develop your drug.

**Date Posted:** 04/10/03 nas

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