Chapter 19

TOXINS: ESTABLISHED AND EMERGENT THREATS

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INTRODUCTION

In its definition of "toxin," the 1993 Chemical Weapons Convention includes "any chemical which through its chemical action on life processes can cause death, temporary incapacitation or permanent harm to humans or animals," regardless of its origin or method of production.[1] Because there is no consensus on the inclusion criterion for toxins, international law regards a wide range of biological and chemical substances as toxins.

An array of toxins exists among the species of all kingdoms (Table 19-1). Many of these toxins have well-characterized and therapeutic effects and have been employed as medical treatments and scientific tools. However, many can have nefarious applications, especially when used outside of their therapeutic indices.

The wide spectrum of toxins includes the following three categories: 1) bacterial toxins (eg, botulinum neurotoxin and staphylococcal enterotoxin), which are high-molecular–weight proteins produced in large quantity by industrial microbiological methods; 2) snake poisons, insect venoms, plant proteins, and marine algae, which are either naturally occurring or chemically synthesized (eg, curare, batrachotoxin [BTX], and ricin); and 3) small molecules, such as potassium fluoroacetate, which are synthesized by chemical processes and produced by living organisms. This chapter focuses on the second toxin group.

Nature of the Threat

An attack involving a mass-casualty–producing weapon, whether biological or chemical, can no longer be anticipated only from hostile states. Some nonstate and terrorist entities have limited moral or social reservations about attacking civilian populations with the intent of causing large numbers of casualties. Agents considered "classical" chemical or biological weapons, such as mustard gas, organophosphorous nerve agents, botulinum toxin, and anthrax, threaten the health and safety of civilian and military populations. Throughout the 20th century, numerous countries have developed and stockpiled chemical and biological agents. Changes in the geopolitical climate over the last 30 years have made it possible for these weapons to fall into terrorists' hands.

Any toxin is a putative, mass-casualty–producing weapon, and to objectively estimate the threats they might pose, toxins must be evaluated against several criteria. First, the potential weapon agent must be suitably toxic. Groups who intend to injure or kill will not waste time or limited resources on agents that are harmless irritants to humans. Marginally toxic compounds must be stockpiled in very large quantities (tons) to produce an effective weapon. Similarly, toxins that produce mild effects following intoxication, or effects for which there are readily available treatments or antitoxins, are less likely threats. Many toxins can be discounted as potential candidates for weaponization based on this criterion alone.

Second, the requirement to stockpile toxin suggests that terrorists must possess the storage capability to maintain toxin potency and prevent toxin degradation. Unstable toxins with short half-lives or toxins that require special handling or storage conditions are typically undesirable. Terrorists' surroundings must be considered when assessing the stability of a potential toxin threat. For example, terrorists operating out of caves in mountains or tent encampments in the desert will not possess the necessary equipment to handle and store some toxins, but a small cell of college students or a state-sponsored group might have access to storage containers, a variety of solvents and acids to properly buffer a toxin for long-term storage, temperature- and humidity-controlled environments, and other special handling equipment.

Third, for a toxin to create mass casualties, a source of the toxin must be readily available. It is unlikely that terrorist groups would tend large snake farms, for example, to harvest snake toxin for weaponization. In addition to other logistical challenges, such an undertaking would be conspicuous and time consuming. However, if a commercial source of a particular toxin is available, the toxin becomes more attractive to a terrorist organization, particularly if the organization has the secure infrastructure available to acquire, purify, concentrate, and properly store toxin stocks. Many toxins have been chemically synthesized and are commercially available to researchers and scientists. Commercially available toxins are typically sold in small quantities for research purposes and are not cost prohibitive; however, some terrorist organizations are able to purchase and store toxins for future weaponization, and the chemical reactions for the synthesis of many toxins have been published in scientific literature and are therefore available to these organizations. Chemical synthesis begins with readily available, simple, and nontoxic compounds, which could be easily and inexpensively obtained from many scientific supply houses. In many cases, the requisite knowledge, skills, and apparatus to perform such synthesis are not trivial; however, for the well-equipped and skilled terrorist, there are no impediments to the synthesis and storage of very large quantities of toxin.

A suitable delivery method must also be designed in advance of bioweapon deployment for toxins to cause a significant threat. While some toxins are lipid soluble

TABLE 19-1

LIST OF KNOWN TOXINS AND THEIR SOURCES

Toxin	Source
α-Aminitin	Death cap mushroom, *Amanita phalloides*
α-Latrotoxin	Black widow spider venom, *Latrodectus mactans*
Abrin, crystalline	Jequirity beans, the seeds of *Abrus precatorius*
Aconitine	Roots of monkshood, *Aconitum napellus*
Aerolysin	*Aeromonas hydrophila*
Aflatoxin	Molds *Aspergillus flavus* and *A parasiticus*
Anatoxin	Cyanobacteria, *Anabaena flosaquae*
Atelopidtoxin	*Atelopus zeteki*
Batrachotoxin	Frogs, *Phyllobates terribilis* and *P aurotaenia*
Bee venom (apamin)	Honey bees, *Apis mellifera*
Botulinum toxin type A-G	*Clostridium botulinum* bacteria
Brevetoxin	Dinoflagellate algae, *Ptychodiscus brevis* or *Gymnodinium breve*
Brown recluse spider venom	*Loxosceles reclusa*
C2 toxin, C3 toxin	*Clostridium botulinum*
C-alkaloid E	Calabash-curare arrow poison
Cholera toxin	*Vibrio cholerae*
Ciguatoxin	Dinoflagellate *Gambierdiscus toxicus*
Clostridium difficile toxin A and B	*Clostridium difficile*
Cobra neurotoxin	Indian cobra venom, *Naja naja*
Conotoxins	Pacific cone snails
Dendrotoxin	Green mamba snake, *Dendroaspis anguisticeps*
Dermonecrotic toxin, pertussis toxin	*Bordetella pertussis*
Diphtheria toxin	*Corynebacterium diphtheriae*
d-Tubocurarine	Tube-curare arrow poison
Edema factor	*Bacillus anthracis*
Enterotoxins, exfoliative toxins, toxic-shock toxin	*Staphylococcus aureus*
Epsilon toxin	*Clostridium perfringens*
Escherichia coli toxins (cytotoxic necrotizing factors, heat-labile toxin, heat-stable toxin, cytolethal distending toxin, heat-stable enterotoxin-1)	*Escherichia coli*
Exotoxin A	*Pseudomonas aeruginosa*
Fasciculin	Venom of the green mamba snake
Grayanotoxin	Rhododendron and other Ericaceae
Hemolysin	*Escherichia coli*
Histrionicotoxin	Colombian frog, *Dendrobates histrionicus*
Israeli scorpion venom (charybdotoxin)	*Leiurus quinquestriatus hebraues*
Kokór arrow poison	Colombian frog, *Phyllobates aurotaenia*
Lethal factor	*Bacillus anthracis*
Listeriolysin O	*Listeria monocytogenes*
Maitotoxin	Marine dinoflagellate, *Gambierdiscus toxicus*
Microcystin	Cyanobacteria, *Microcystis aeruginosa*
Nicotine	*Nicotiana* tobacco plants
North American scorpion venom	*Centruroides sculpturatus*
Ouabain	*Strophanthus gratus* seeds
Palytoxin	Soft coral, *Palythoa toxica*
Perfringolysin O	*Clostridium perfringens*
Picrotoxin (cocculin)	*Cocculus indicus, Anamirta cocculus*
Pneumolysin	*Streptococcus pneumoniae*
Pumiliotoxin	Formicine ants of genera *Brachymyrmex* and *Paratrechina* and frog *Dendrobates pumilio*
Pyrogenic exotoxins	*Streptococcus pyogenes*

(**Table 19-1** *continues*)

Table 19-1 *continued*

Ricin, amorphous and crystalline	Castor beans, the seeds of *Ricinis communis*
Russell's viper venom	*Vipera russelli*
Salmonella toxin, cytotoxin, enterotoxin	*Salmonella Typhimurium* and *S Enteritidis*
Saxitoxin	Dinoflagellate marine algae, *Gonyaulax catenella and G tamarensis*
Shiga toxin	*Escherichia coli/Shigella dysenteriae*
Staphylococcus aureus α-toxin	*Staphyloccocus aureus*
Streptolysin O	*Streptococcus pyogenes*
Strychnine	*Stryhnos nuxvomica* bark or seeds
Taipoxin	Australian taipan snake, *Oxyuranus scutellatus*
Tetanus toxin	*Clostridium tetani bacteria*
Tetrodotoxin	Puffer fishes and certain salamanders
Textilotoxin	Australian common brown snake, *Pseudonaja textilis*
Tityustoxin	Brazilian scorpion, *Tityus serrulatus*
Trichothecene Mycotoxin (T-2)	Fusarial species of fungus
Veratridine	Liliaceae
Western diamondback rattlesnake venom	*Crotalus atrox*

and readily absorbed through dermal layers (posing contact hazards), most are water soluble. Water-soluble toxins can be aerosolized for delivery to target populations, which allows toxin access to the more vulnerable inner surfaces of the lung. Aerosol particles between 0.5 and 5 μm in diameter are typically retained within the lung, but smaller particles are not retained in the airway and most are exhaled. Particles between 5 to 15 μm are generally sequestered in nasal mucosa or in the trachea. A large percentage of aerosol particles larger than 15 μm drop to the ground or onto flat surfaces in the environment. Water-soluble toxins are generally not volatile, and those particles falling onto the ground no longer pose a respiratory threat.[2]

Many cases of accidental exposure to toxins in humans, especially from marine toxins, occur by ingestion. Intoxication by agents such as tetrodotoxin (TTX; isolated from the Japanese puffer fish) or brevetoxin (PbTx), implicated in neurotoxic shellfish poisoning (NSP), suggest that water or food supplies could be targeted for large-scale delivery of weaponized toxins to civilian populations. Several recent publications have presented mathematical models of toxin weapons delivered into food or water supplies.[3] These data suggest that this means of toxin delivery would impose a significant financial burden to diagnose and treat the affected population, a compromise to key infrastructure, and a reallocation of resources to deliver clean supplies to the effected population.

Established Threats

Toxins of concern to the US military and the Department of Homeland Security comprise a group of structurally diverse substances that share many features with chemical warfare agents. Toxins and chemical warfare agents interfere with important biological processes (eg, synaptic transmission, DNA replication, and protein synthesis) and produce incapacitation and death following acute exposure.[4] Toxins that are generally considered to be battlefield or bioterrorist threats include anthrax, botulinum neurotoxin, staphylococcal enterotoxin B, T-2 mycotoxin, and ricin. These five biotoxins are thought to be most likely used in the event of warfare or bioterrorism, although they represent a small subset of all lethal toxins known.[5] Potency, ease of production, stability, and prior history of weaponization are all factors hostile forces must consider before deploying bioweapons.[4–6] The Centers for Disease Control and Prevention (CDC) have designated anthrax and botulinum neurotoxin as category A threat agents, and staphylococcal enterotoxin B and ricin as category B agents (Table 19-2).[7] Category A agents are defined as those that "can be easily disseminated or transmitted from person to person; result in high mortality rates and have the potential for major public health impact; might cause public panic and social disruption and require special action for public health preparedness."[7] Category B agents are defined as those that "are moderately easy to disseminate; result in moderate morbidity rates and low mortality rates; and require specific enhancements of CDC's diagnostic capacity and enhanced disease surveillance."[7] For example, T-2 mycotoxin, a category B agent, is specifically addressed by the CDC as a select agent and toxin and additionally regarded as a threat because of its documented use in Laos, Vietnam, and Cambodia during 1975–1978.[8] Category C agents, the third highest priority, "include emerging pathogens that could be engineered for mass dissemination in

TABLE 19-2

CENTER FOR DISEASE CONTROL AND PREVENTION CLASSIFICATION OF BIOTERRORISM AGENTS/DISEASES

Category A	Category B	Category C
Anthrax	Brucellosis	Emerging future toxin threats
Botulism	Epilson toxin of *Clostridium perfringens*	
Plague	Food safety threats (*Escherichia coli, Salmonella species,* O157:H7, *Shigella*)	
Smallpox	Glanders	
Tularemia	Meloidosis	
Viral Hemmoragic Fevers	Psittacosis	
	Q Fever	
	Ricin toxin from *Ricinus communis*	
	Staphylococcal enterotoxin B	
	Typhus	
	Viral encephalitis	
	Water safety threats (eg, *Vibrio cholerae*, *Cryptosporidium parvum*)	

Data source: Bioterrorism agents/diseases: emergency preparedness & response Web site. Available at: http://www.bt.cdc.gov/agent/agentlist-category.asp. Accessed February 10, 2007.

the future because of availability; ease of production and dissemination; and potential for high morbidity and mortality rates and major health impact."[7] These emerging toxin threats are the focus of this chapter, toxins that possess the properties of the more well-known category A and B agents but that have not been considered likely threats to date (see Table 19-2).

Emergent Threats

The group of biotoxins not considered immediate threats with the potential to cause human illness and death is potentially very large and includes the sodium channel toxins BTX,[9] PbTx,[10] saxitoxin (STX),[11] TTX,[12] and pumiliotoxin.[13] Others include palytoxin (PTX), which alters the sodium-potassium exchanger (sodium-potassium ATPase),[14] and the nicotinic receptor agonist, anatoxin-A.[15] Because these toxins are employed as pharmacological tools for studying ion channel properties, active efforts to optimize their synthesis are being developed.[16] If these efforts are successful in generating large quantities of toxin, members of this group will need to be reevaluated for their potential as threat agents.

TOXINS

Palytoxin

Synthesis

PTX is an extremely potent marine neurotoxin that acts on sodium-potassium ion pumps. First isolated from the zoanthid coral (genus *Palythoa*) by Moore and Scheuer,[17] PTX has long been categorized as a marine animal toxin. It has been identified in several species living in close contact with zoanthid anemones (eg, some dinoflagellates, *Ostreopsis* species);[18] Polychaete worms;[19] several species of xanthid crab (*Lophozozymus pictor* and *Demaina toxica*),[20] and several species of fish.[21–23] PTX is found in the red alga *Chondria aramata*.[24,25] PTX has also been associated with the blue humphead parrotfish,[26] filefish, and serranid fish.[27] The primary source is most likely a bacterium associated with soft corals that inhabit the digestive tract of filefish (Figure 19-1). PTX is a large (molecular weight 2,678.5), water-soluble, nonproteinaceous polyether, with molecular formula $C_{129}H_{223}N_3O_{54}$. PTX has an exquisitely complex structure (see Figure 19-1). It was first elucidated and synthesized in 1982[28] and is currently available from several commercial sources.

Mechanism of Action and Toxicity

PTX affects all excitable cells by inducing the activity of a small conductance (9–25 pS), nonselective, cationic channel, which triggers secondary activations of voltage-dependent calcium channels and of sodium-calcium exchange. In addition to electrically excitable

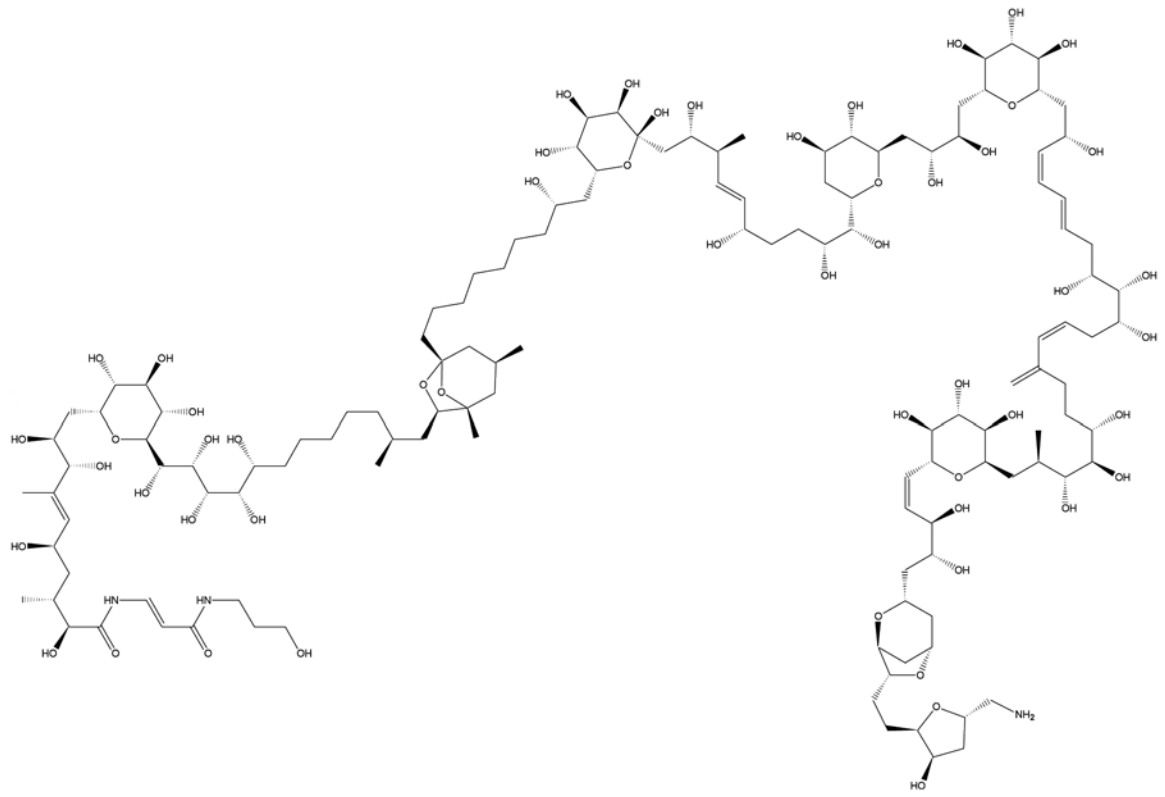

Fig. 19-1. Structure of palytoxin.
Illustration: Courtesy of Richard Sweeny.

cells (muscle, heart, neurons), PTX affects virtually all cell types that rely on the sodium-potassium ATPase exchanger to maintain electrolyte balance, membrane potential, and electrical/ionic gradients. The sodium-potassium ATPase exchanger pump has been suggested to be a major molecular target of PTX.[29] PTX leads to contractions of striated skeletal and smooth muscle cells, neurotransmitter release by nerve terminals,[30] potassium release and hemolysis of red blood cells, and blood vessel vasoconstriction. PTX leads to contraction in both smooth and skeletal muscle as a result of slow and irreversible depolarization of the plasma membrane in these cells from an induction of an inward, sodium-dependent current.[25,30] A cardiotonic effect in cardiac muscle and depolarization of muscle membranes occurs as a result of PTX intoxication.[30] PTX causes a depolarization and a decrease in the amplitude, upstroke velocity, and duration of action potential in papillary muscle of the heart secondary to an increase in sodium permeability of the cardiac cell membrane. Membrane depolarization of the plasma membrane drives sodium into the cells, promoting calcium influx through L-type calcium channels and by the sodium-calcium exchanger. Evidence suggests PTX binds to the sodium-potassium ATPase exchanger at ouabain receptor sites. This active transport ion pump is converted to an open ion channel, diminishing ion gradient across the membrane.[22,29–31]

PTX affects adrenergic neurons and red blood cells, increasing norepinephrine and potassium release, respectively.[30] PTX also effects blood vessels through its interactions on vascular smooth muscle, nerve terminals, and vascular endothelial cells, leading to vasoconstriction, an increase in systemic blood pressure, and massive pulmonary hypertension. In addition, depolarization of the plasma membrane opens L-type calcium channels, promoting calcium influx and contractions.[25] Perivascular nerve terminals undergo membrane depolarization, releasing norephinephrine that binds to alpha-1-adrenoceptors on smooth muscle cells. Activation of phospholipase C by norephinephrine binding induces mobilization of intracellular calcium stores and activates protein kinase.[25] PTX also acts on vascular endothelial cells by releasing nitric oxide and induces the release of prostaglandins from the aorta.[25]

PTX is a rapid-acting, lethal neurotoxin most commonly introduced by ingestion. The median lethal dose

(LD_{50}) in humans is estimated to be 0.15 μg/kg body weight. Intoxication by PTX affects the sodium-potassium ATPase exchanger pump by converting the active ion transport process into a relatively nonspecific cation channel.[25,29] At the cellular level, PTX action leads to membrane depolarization, the most likely cause of smooth muscle contraction in vitro and vasoconstriction in vivo.[14] Clinical signs and symptoms of PTX intoxication include vasoconstriction, hemorrhage, ataxia, muscle weakness, ventricular fibrillation, ischemia, and death.[25,30] Challenge by intravenous (IV) or subcutaneous injection has been shown to be the most effective route of exposure for inducing intoxication by PTX in test animals, although a number of fatalities involving human intoxication by ingestion have been reported.[27,32–34]

Toxin Exposure, Health Effects, and Treatment

PTX can cause a diverse array of clinical signs and symptoms, including skin irritation, generalized weakness, muscle spasms, sweating, skin irritation, abdominal cramps, nausea, vomiting, diarrhea, temperature dysesthesia, and paresthesias ("pins and needles"). More severe signs and symptoms include acute respiratory distress, vasoconstriction, hemorrhage, ataxia, generalized muscle weakness, tonic contraction of all muscle groups, elevated muscle enzymes, myoglobinuria, rhabdomyolysis, tremors, seizures, cyanosis, bradycardia, ventricular fibrillation, ischemia, renal and cardiac failure, and death. Because PTX is an extremely potent vasoconstrictor, it affects all muscle and neuronal cell types. A depolarization of membrane potential occurs in cells, with sodium entering the cells in exchange for potassium.[31]

Physical Examination. After PTX intoxication, an initial decrease in blood pressure followed by a rise in systemic blood pressure has been observed.[35] In addition, after ingesting PTX, some poison victims have reported tasting metal.[34] Bradycardia has been reported in acute poisonings. PTX can also lead to myocardial damage. Furthermore, PTX displays cardiotonic properties in cardiac muscle, leading to depolarization of excitable membrane, including cardiac muscle, as described above.[22,34] Electrocardiograms (EKGs) have shown negative T waves in leads III and aVf following human ingestion of PTX; however, echocardiography remained normal during the clinical course.[26] In one clinical case report of PTX intoxication, serum cardiac enzyme, creatine kinase MB isozyme, was reported to be 8% on the fourth hospital day.[26] On respiratory examination, patients may experience acute dyspnea, tachypnea, and shallow breathing.[22,34] While coronary vasoconstriction is usually a primary factor leading to death, respiratory failure can result in death when the essential muscles of respiration stop working.[36] Neurological examination may show seizures, tremors,[22,36] muscle spasms, and generalized weakness[23,34] secondary to depolarization of muscle or nerve membranes.[22] In addition, a cold-to-hot temperature reversal dysesthesia has been noted in ciguatera fish poisoning.[34,37] Circumoral and limb paresthesias have also been reported in patients,[22,34,37] in addition to restlessness and dizziness.[34]

Gastrointestinal symptoms are the earliest symptoms to manifest in PTX intoxication. Nausea, vomiting, abdominal cramps, and diarrhea are common complaints.[22,34] Patients may complain of dark brown to black urine, secondary to myoglobinuria,[36] anuria, and renal failure.[34] PTX can also cause eye and skin irritation,[38] cold sweats,[34] and excessive perspiration.[22] While contractile responses are seen in both smooth and skeletal muscle,[22] increased skeletal muscle tone, cramps, and severe myalgya[23,36] are hallmarks of PTX intoxication. A prominent rhabdomyolysis may also occur, leading to myoglobinuria.[26] Additionally, PTX has caused a dose-dependent contraction of the human umbilical artery,[39] but there is no data concerning teratogenicity. PTX is also a known tumor promoter, even at low levels.[40]

Laboratory Findings and Monitoring. Laboratory examination can reveal elevated liver enzymes in serum creatine phosphokinase (CPK), aspartate aminotransferase, and lactate dehydrogenase.[22,26,36] These should be monitored as indicators of muscle damage. One case report showed that serum aspartate aminotransferase was elevated to 3,370 IU/L on the third day after ingestion of PTX-containing fish, and serum lactate dehydrogenase was elevated to 7,100 IU/L on the fourth day.[26] Serum levels should be monitored for hyperkalemia and hyponatremia due to PTX effects on the sodium-potassium exchanger. In addition, hemolysis has been shown to develop within hours after potassium release from human erythrocytes.[31] Urinalysis is typically positive for blood but with few or no red blood cells, an early indicator of hemolysis. A dark urine color and myoglobinuria may also be present. Serum aldolase, serum myoglobin, and urinary myoglobin should all be monitored.

PTX may be isolated using successive column chromatography or thin layer chromatography.[34,36,40] In addition, a nuclear magnetic resonance spectrometry method can be used, in combination with gradient enhancement and 3D Fourier transform, to elucidate hydrogen and carbon nuclear magnetic resonance signals of PTX.[41] A rapid and sensitive neutralization assay has been developed to detect PTX.[42] This assay uses the hemolytic properties of the toxin to specifically

induce neutralizing monoclonal antibody.

PTX toxicity has been studied in several animal species, each showing similar sensitivities[43,44] and clinical effects to humans. In general, most experimental animals show clinical signs of drowsiness, weakness, vomiting, respiratory distress, diarrhea, convulsions, shock, hypothermia, and death within 30 to 60 minutes of IV injection. Early signs of PTX poisoning in dogs include defecation and vomiting.[30] Rats and nonhuman primates have demonstrated similar sensitivity to IV PTX challenge with 24-hour LD_{50} of 89 ng/kg and 78 ng/kg, respectively.[43]Following IV administration of PTX, nonhuman primates become drowsy, weak, and ataxic. Vomiting sometimes occurs (incidence not reported),[43] followed by collapse and death.

PTX causes a moderate skin reaction in rabbits[45] as well as an increase in histidine decarboxylase activity in mice after topical PTX application to the skin.[46] Based on histamine release data in rat mast cells, PTX may have immunological effects.[47] It causes a depolarization of the membranes of myelinated fibers, spinal cord, and squid axons; induced norepinephrine release from adrenergic neurons[48] and clonal rat pheochromocytoma cells[49]; and causes a temperature-dependent potassium loss from rat erythrocytes, followed by hemolysis in a matter of hours.[50]

PTX also leads to dysrhythmias and vasospasm in animals. It exerts cytotoxic effects in rat aortic smooth muscle, leading to surface granularities, vacuoles, rounding, and cell death; increased release of lactate dehydrogenase; increased ionic conductance to sodium and potassium; and profound membrane depolarization on electrophysiological recording.[14] Finally, PTX has a direct cardiotoxicity in vivo, resulting in atrioventricular block, extrasystoles, ventricular tachycardia, coronary vasoconstriction, and ventricular fibrillation. The shape and rhythm of the EKG is abnormal, showing S-T segment elevation most likely due to coronary vasoconstriction.[35] Death from PTX appears to be secondary to coronary artery vasoconstriction, reducing blood flow to cardiac tissues, resulting in necrosis. This leads to cardiac failure and progressive myocardial ischemia, ventricular fibrillation, and cardiac arrest observed by EKG in nonhuman primates following IV exposure to PTX.[43]

Food poisoning incidents by accidental PTX ingestion are not uncommon in Japan,[26,36] and clinical signs and symptoms have been reported after cases of human PTX ingestion.[26,27,34,36] The patients in a Taniyama et al case report suffered severe muscle pains, dyspnea, apnea, and discharge of black urine.[27] Symptom onset occurred 3 to 36 hours following ingestion. On laboratory findings, serum CPK levels were above the normal range and were reported to be 700–23,800 IU/L. All of the patients observed in the study recovered. Reported muscle pains abated, CPK levels returned to normal, and urine color resolved, although recovery took approximately 1 month (Exhibit 19-1).

In cases of accidental poisoning it is difficult to ascertain how much PTX the victim ingested. Toxin distribution and concentration, the precise quantity of food consumed, and the amount of toxin ingested cannot be adequately determined, as PTX toxicity by ingestion has not been thoroughly studied. An Okano case report involved a 55-year-old male who consumed the raw meat and liver of a blue humphead parrotfish contaminated with PTX. The patient developed progressive weakness and myalgia in his extremities 5 hours after ingesting the toxin. Rhabdomyolysis and myocardial damage developed with serum CPK levels elevated to 40,000 IU/L by the third day following ingestion. Serum aldolase, serum myoglobin, and urinary myoglobin were similarly elevated. Elevated myosin light chain levels and alterations in the EKG were noted.[26] After mannitol-alkaline diuresis once daily for a period of 4 days, the patient recovered. Weakness and myalgias subsided within 4 weeks.

PTX is less toxic by ingestion than by other routes of exposure.[51] Its stability and the potency differences from various routes of entry must be further studied to estimate the threat of PTX.

Treatment. Life support may be required to minimize respiratory and cardiovascular compromise after PTX intoxication. Treatment of PTX-intoxicated victims consists of rapid diagnosis, decontamination with copious amounts of water, and general supportive care. Any patient suspected of ingesting PTX should be monitored in a controlled setting until all signs and symptoms of toxicity have abated. In cases of oral exposure, syrup of ipecac is not recommended due to the rapid nature of PTX absorption. Activated charcoal should be given emergently in aqueous slurry for suspected ingestion only in patients who are awake and able to protect their airways. In patients at risk for seizures or mental status changes, activated charcoal should be administered by personnel capable of airway management to prevent aspiration in the event of spontaneous emesis. Activated charcoal is only useful if administered within approximately 30 minutes of ingestion. Cathartics are not recommended due to the vomiting, diarrhea, and electrolyte imbalance caused by PTX.

Oxygenation, hemoglobin, hematocrit, plasma free hemoglobin, urinalysis, and other indices of hemolysis should be monitored. Transfusion of blood or packed red blood cells may be necessary to treat hemolysis. Early treatment should be aimed at controlling acute metabolic disturbances (hyperkalemia, hyponatremia,

EXHIBIT 19-1

ADVERSE EFFECTS OF HUMAN PALYTOXIN INTOXICATION

- A 49-year-old Filipino male fell ill minutes after ingesting crab containing PTX. Early symptoms were dizziness, nausea, fatigue, cold sweats, and an oral metallic taste. The patient complained next of paresthesias in the extremities, restlessness, vomiting, and severe muscle cramps. The patient suffered episodes of severe bradycardia (heart rate 30 bpm), rapid and shallow breathing, cyanotic hands and mouth, anuria, and eventual renal failure at the hospital. He was treated with atropine, diphenhydrimine, meperidine, and epinephrine without success. The patient died 15 hours after ingestion.

- A 54-year-old Asian male and a 79-year-old Asian female ingested parrotfish (*Ypiscarus ovifrons*) containing PTX. Both patients presented with dyspnea, myalgia, convulsions, and myoglobinuria on the first day of admission. Labs revealed elevated serum creatine phosphokinase, lactate dehydrogenase, and serum glutamic-oxaloacetic transaminase. The male patient recovered after 1 week, and the female patient died 3 days later after complications of respiratory arrest.

- PTX-contaminated mackerel was ingested by a 35-year-old male. Within hours, he experienced excessive sweating, weakness, nausea, abdominal discomfort, diarrhea, circumoral and extremity paresthesias, temperature reversal dysesthesia, muscle spasms, and tremor. The patient was hospitalized 48 hours after ingestion when he developed tonic contractions. Endotracheal intubation was started after he developed respiratory distress. Creatine phosphokinase, lactate dehydrogenase, and serum glutamic-oxaloacetic transaminase levels were extremely elevated, and his urine was dark brown. The patient recovered 11 days after ingestion and received only symptomatic therapy throughout his hospital stay.

Data sources: (1) Alcala AC, Alcala LC, Garth JS, Yasumura D, Yasumoto T. Human fatality due to ingestion of the crab *Demania reynaudii* that contained a palytoxin-like toxin. *Toxicon*. 1988;26:105–107. (2) Noguchi T, Hwang DF, Arakawa O, et al. Palytoxin is the causative agent in the parrotfish poisoning. In: Gopalakrishnaknoe P, Tan CT, eds. *Progress in Venom and Toxin Research*. *Proceedings of the First Asia-Pacific Congress on Animal, Plant and Microbial Toxins* Singapore, China: National University of Singapore; 1987: 325–335. 3) Kodama AM, Hokama Y, Yasumoto T, Fukui M, Manea SJ, Sutherland N. Clinical and laboratory findings implicating palytoxin as cause of ciguatera poisoning due to *Decapterus macrosoma* (mackerel). *Toxicon*. 1989;27:1051–1053.

hyperthermia, hypovolemia). Subsequent treatment should focus on the control of seizures, agitation, and muscle contraction. Urine alkalinization with sodium bicarbonate and maintenance of adequate urine output may help prevent nephrotoxicity from red blood cell breakdown products. One case report involved gastric lavage with activated charcoal and forced mannitol-alkaline diuresis therapy.[26] In this case, the patient recovered without long-term sequelae (eg renal failure). However, urine alkalinization can cause alkalemia, hypocalcemia, and hypokalemia.

If central nervous system and respiratory depression occur, intubation, supplemental oxygenation, and assisted ventilation should be rapidly administered. Rapid administration of steroids may reduce the severity of effects. In case of seizure activity, benzodiazepines (diazepam or lorazepam) should be administered first. If seizures persist, phenobarbital should be considered. One should also monitor for hypotension, dysrhythmias, and respiratory depression and the possible need for endotracheal intubation. Healthcare providers should evaluate for hypoxia, electrolyte disturbances, and hypoglycemia, and consider starting IV dextrose. In the case of rhabdomyolysis, early aggressive fluid replacement is the definitive treatment and may prevent renal insufficiency. Diuretics (eg, mannitol or furosemide) may be needed to maintain urine output. Vigorous fluid replacement with 0.9% saline is necessary if there is no evidence of dehydration. The hypovolemia, increased insensible losses, and third spacing of fluid increase the fluid requirements associated with managing a patient with PTX intoxication. In addition, one should monitor for evidence of fluid overload, compartment syndrome, and CPK, and perform renal function tests.

Decontamination should be administered immediately in cases of PTX intoxication. For ocular exposure, the eyes should be irrigated with copious amounts of saline or water for at least 15 minutes. If symptoms of eye irritation, pain, swelling, lacrimation, or photophobia persist after irrigation, obtain an ophthalmology consult for further examination. In cases of dermal exposure, remove contaminated clothing and wash exposed areas thoroughly with soap and water.

Intoxication by PTX in laboratory animals can be managed by the administration of vasodilator agents. Intraventricular cardiac injections of papaverine or isosorbide dinitrate in animals will ameliorate the vasoconstrictive actions of PTX. IV injection of vasodilators is ineffective because of PTX's rapid lethality. Laboratory animals die within 3 to 5 minutes of receiving a lethal dose of PTX,[43] during which time the animals' circulation is compromised because PTX prevents adequate delivery of the vasodilator to effected tissues. There have been reports of some success protecting laboratory animals by pretreatment with hydrocortisone, but at most half of the test subjects showed resistance to the toxin following pretreatment.

Stability

PTX is soluble in water, pyridine, dimethylsulfoxide, and aqueous acidic solutions. The chemical stability and activity of dilute PTX, stored in glass or plastic, are unaffected by exposure to light and room temperature for short periods (up to several hours).[52] Reconstituted PTX can be stored at 4°C for 3 to 6 months, but no stability data exists for longer term storage. Lyophilized PTX from a commercial source is recommended to be stored at < 0°C and protected from light.

These storage and stability recommendations indicate that PTX is not a particularly stable substance, although the storage conditions are very mild. These mild storage requirements could make PTX desirable to potential terrorists who have limited specialized equipment to reconstitute and store PTX. The storage conditions are somewhat restrictive but not necessarily prohibitive, allowing a small but sufficient window of opportunity for terrorists to disperse a PTX weapon.

Protection

PTX is extremely potent once it is introduced to the body; however, it is not lipid soluble and therefore not likely to present a contact hazard by absorption through the skin. The probable routes of human exposure to PTX in a bioterrorism incident would be inhalation of PTX vapor or ingestion of contaminated food or water. Human fatalities due to accidental PTX intoxication have been reported[22,34,36]; however, more testing must be done to fully understand how to protect against PTX intoxication.

Surveillance

Currently, there are no specific PTX surveillance programs in place, but several public health surveillance programs may be adapted to monitor specifically for potential bioterrorist events. The CDC, in conjunction with state and local health departments, is developing the Enhanced Surveillance Program, which is designed to monitor data on hospital emergency department visits during special events to establish a baseline of patient symptoms. The goal of this program is to identify deviations from the normal patient visit data and report to state and local health departments for confirmation and appropriate epidemiological follow up. Data from patient visits was collected at the 1999 World Trade Organization Ministerial in Seattle, the 2004 Republican and Democratic National Conventions held in Philadelphia and Los Angeles, respectively, and the 2001 Super Bowl in Tampa, Florida, to test the Enhanced Surveillance Program. If the Enhanced Surveillance Program proves successful, it could serve as a model for a national surveillance program to quickly identify casualties from the types of weaponized toxins presented in this chapter.

Tetrodotoxin and Saxitoxin

Synthesis

TTX, and to some extent STX, have been used as tools in physiology and pharmacology research for many years, allowing investigators to study the physiological properties of ion channels, action potential generation and propagation, cellular membranes, and various aspects of neuroscience. TTX, a selective sodium channel blocker and potent neurotoxin, has been isolated from a wide variety of marine animals. Puffer fish and toadfish, members of Tetraodontiformes, are the best known sources of TTX, although the toxin has been detected in more than 40 species of fish.[53] TTX has also been found in the Australian blue-octopus (*Hapalochlaena maculosa*), xanthid crabs (*Eriphia* species), horseshoe crabs (*Carcinoscorpius rotundicauda*), two Philippine crabs (*Zosimus aeneus* and *Atergatis floridus*), mollusks (*Nassarius* species), marine algae (*Jania* species), epiphytic bacterium (*Aleromonas* species), *Vibrio* species, and from *Pseudomonas* species.[54] Additionally, TTX has been isolated in some terrestrial organisms, including Harlequin frogs (*Atelopus* species), Costa Rican frogs (*Atelopus chiriquiensis*), three species of California newt (*Taricha* species), and members of the family Salamandridae.[33,55,56] STX is the best-understood member of a much larger group of structurally related neurotoxins, the paralytic shellfish poisoning (PSP) toxins, which are found in dinoflagellates.[57–59] PSP is similar to NSP but more severe because paralysis is not a typical feature of NSP.[60] PSP is associated with red tide blooms but also may occur without red tide (Figure 19-2). [61]

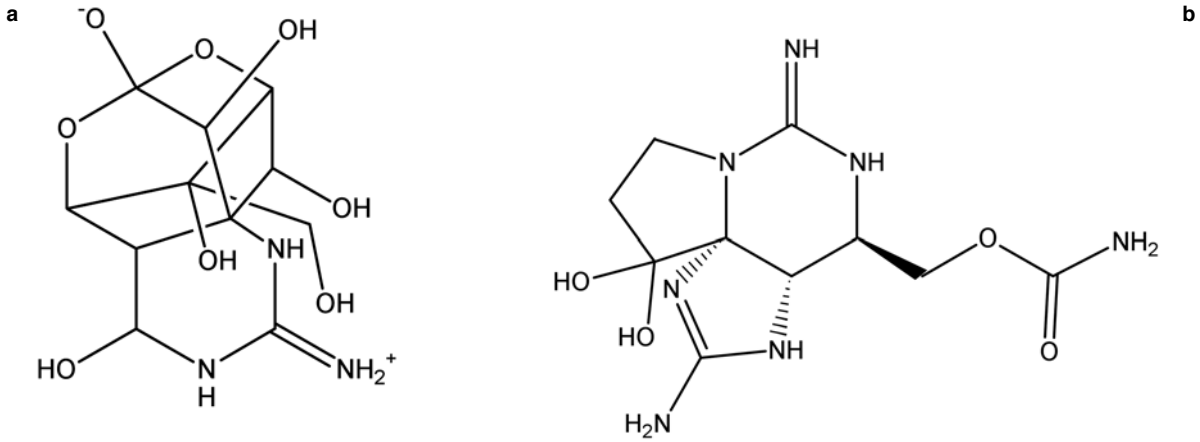

Fig. 19-2. Structures of tetrodotoxin (left) and saxitoxin (right). Illustration: Courtesy of Richard Sweeny.

From 1956 to 1958, nearly 500 Japanese citizens died from puffer fish ingestion, prompting the immediate elucidation of the toxin.[62] The structure of TTX (see Figure 19-2, left) was determined in 1964,[63–65] and Kishi synthesized the toxin.[66] TTX remains widely used in research today and is available to scientists from many commercial sources. STX synthesis (see Figure 19-2, right) was first published in 1977.[67] Like TTX, STX is a potent, selective, sodium channel blocker. STX, only one component of PSP toxins, is the product of the dinoflagellates *Gonyaulax catenella* and *Gonyaulax tamarensis*. STX has been isolated in certain mollusks that feed on *Gonyaulax catenella*[68] and is believed to bioaccumulate to cause toxicity in humans.

Mechanism of Action and Toxicity

Both TTX and STX are water-soluble, heat-stable molecules[61,69–72] and can be absorbed through the mucous membranes and small intestine.[73,74] Both inhibit neuromuscular transmission by binding to the voltage-gated sodium channel (Figure 19-3). As selective, voltage-dependent, sodium channel blockers, both toxins exert major neurotoxic effects by preventing action potential generation and propagation (see Exhibit 19-1). Six different binding sites on the voltage-gated sodium channel have been identified, each site corresponding to a locus on the protein where groups of neurotoxins can bind (Figure 19-4). Both TTX and STX occupy binding site 1,[75] which is on the S6 transmembrane domain. This domain forms the mouth of the pore in the three-dimensional structure of the channel on the extracellular face (see Figure 19-3). TTX and STX will bind irreversibly to the sodium channel, occluding the pore. In this way, TTX and STX act as sodium channel blockers, sterically preventing sodium ion access through the channel. In the context of the brief description of action potential generation above, prevention of sodium ion movement by either toxin has catastrophic effects on normal neuronal function. The end result is blockade of nerve conduction and muscle contraction (see Figure 19-4). The toxins are reversible and do not lead to damage of the nerve or skeletal muscle.[73,74,76] Another similar feature is that these toxins inhibit cardiac and smooth muscle at higher concentrations. One difference between the two toxins is that STX lacks the emetic and hypothermic action of TTX[77]; the mechanism behind this difference is not well understood. Other cardiovascular effects for these sodium channel toxins have been noted. STX has been demonstrated to induce hypotension by direct action on vascular smooth muscle or through blocking vasomotor nerves.[78] It also decreases conduction at the AV node.[79] Both toxins have effects in the brain. STX inhibits the respiratory centers of the central nervous system[79] while TTX action produces blockade of sodium channels in the axon of the magnocellular neurons of the neurohypophysis, inhibiting release of vasopressin. Children appear to be more sensitive to STX than adults.[80,81]

As a selective sodium channel blocker, TTX binds its molecular target tightly with extremely strong kinetics (Kd = 10-9 nM). Toxicology of TTX and STX is reported in the literature based primarily on mouse data. Both toxins are extremely potent, with an approximate LD_{50} 8 to 10 μg/kg in mice.[69] Toxicity studies in mice examined intoxication by IV administration, while the route of exposure in humans is generally through ingestion. Deaths have been reported following human ingestion of both toxins,[61,70] and it is estimated that 1 to 2 mg

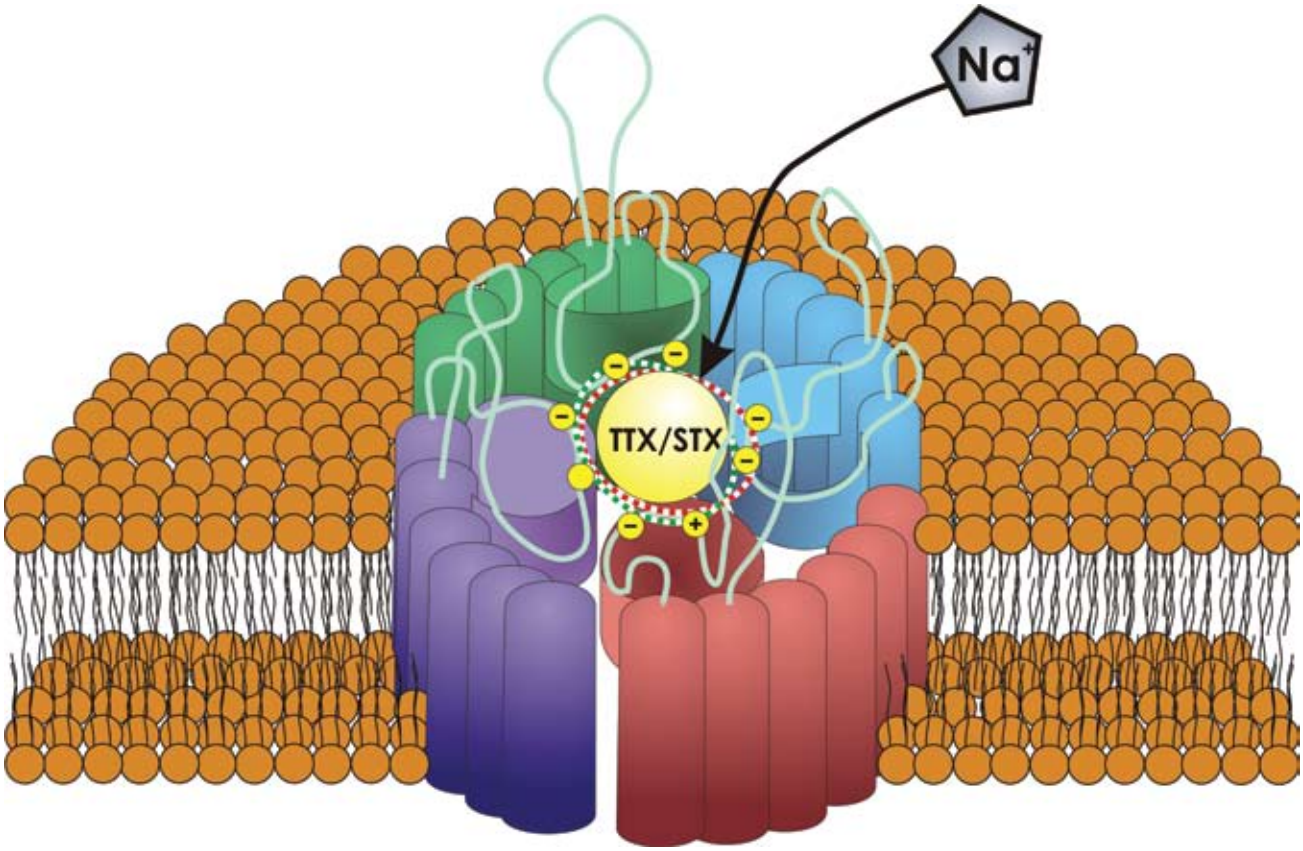

Fig. 19-3. Three-dimensional representation of a voltage-gated sodium channel sitting in a phospholipid bilayer membrane. The linear protein folds to form a pore in the cell membrane, providing a central, electrically charged aperture through which sodium ions can pass. The toxins bind to regions of the channel structure occluding the pore, preventing sodium ions from entering and traversing the channel pore.
Na^+: sodium ion
STX: saxitoxin
TTX: tetrodotoxin

of TTX is a lethal dose for an average adult human.[69] Respiratory toxicity of STX is less well understood in every model system than systemic toxicity; however, data from aerosol deposition studies in mice exposed to STX aerosol give LC_{50} (lethal concentration; the concentration of the chemical in air that kills 50% of the test animals in a given time) values < 1 μg/kg.[71] Thus, in these studies, STX is at least 10-fold more toxic to mice by aerosol exposure than by systemic administration. The mechanism of this enhanced toxicity is unknown.

Toxin Exposure, Health Effects, and Treatment

Intoxication by TTX is the most common lethal marine poisoning[82] and most often occurs by the consumption of contaminated food. Ingestion of TTX-contaminated foods occurs throughout Southeast Asia and the Pacific, most commonly in Japan, where puffer fish is a delicacy. Additionally, neurologic illnesses associated with ingestion of Florida puffer fish have been reported since 2002. Signs and symptoms of TTX intoxication usually begin within 30 to 60 minutes after ingestion of the toxin. Anxiety, nausea, vomiting, and paresthesias of the lips, fingers, and tongue are all common. In cases of severe poisoning, clinical signs and symptoms include marked paresthesias, loss of consciousness, generalized flaccid paralysis, respiratory arrest, and death. Dizziness, dyspnea, and fixed, dilated pupils have also been reported. Patients with more moderate poisoning generally retain consciousness. There are reports of unresponsive patients who were nonetheless fully cognizant of events around them.[83]

PSP typically results from the consumption of mussels, clams, oysters, mollusks, starfish, sand crabs,

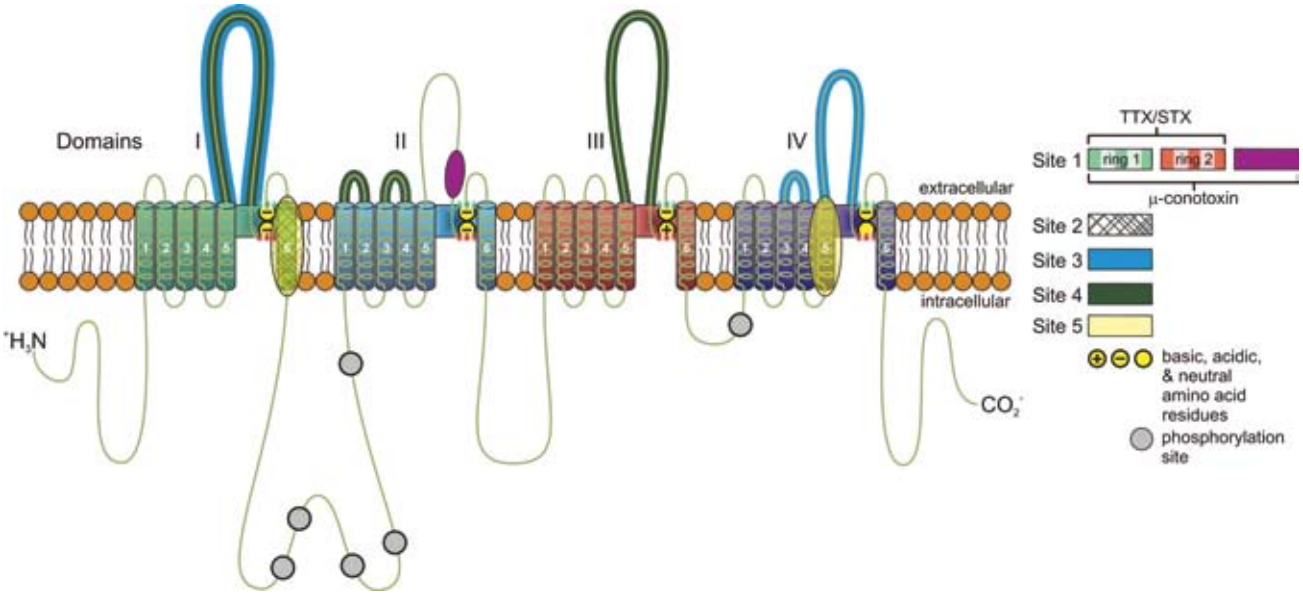

Fig. 19-4. Structure of the α-subunit of the voltage-gated sodium channel. The six transmembrane portions for each colored domain (I-IV) insert into the cell membrane and form the charged pore (shown above) through which ions can travel. The known toxin binding sites are color-coded and numbered, as are the phosphorylation sites and charged residues that form the selectivity filter of the channel. The lipid bilayer is illustrated in orange. Transmembrane segments 5 and 6 from each domain contribute to the channel pore and contributions from segment 4 form the voltage sensor. Amino acids between segments 5 and 6 from each domain form the filter (or gate) for ionic selectivity. The α-subunit illustrated here folds into four transmembrane domains (I–IV), colored green, blue, orange, and purple. The transmembrane domains are themselves comprised of six α-helical segments designated S1 through S6. Within each domain, the S4 segment has a primary structure containing positive charged amino acid residues at every third position. The S4 segment functions as the voltage sensor, detecting the depolarization of the cell membrane and initiating channel opening. When the α-subunit is properly folded in three dimensions, segments S5 and S6 form the channel pore. Amino acid residues between transmembrane segments S5 and S6 are predominately acidic (negatively charged) or neutral, which creates an electrically favorable tunnel to allow the passage of positively charged ions (eg, sodium ions) of a particular radius.

Six different binding sites on the voltage-gated sodium channel have been identified, each site corresponding to a locus on the protein where groups of neurotoxins can bind. TTX and STX bind to site 1 on the extracellular face of the sodium channel, occluding the pore and thereby preventing the movement of sodium ions through the pore. Batrachotoxin and the brevetoxins have similar physiological effects, mainly causing activation of the channel at more negative membrane potentials. Batrachotoxin binds to site 2 and brevetoxins to site 5.
STX: saxitoxin
TTX: tetrodotoxin

xanthid crabs, and various fish that have consumed the toxic marine algae dinoflagellates. Eating shellfish contaminated with STX, readily absorbed through the oral and gastrointestinal mucosa, can cause paralytic, neurotoxic, and amnestic symptoms.[80,84] STX causes symptoms very similar to several other dinoflagellate toxins (eg, PbTxs). Because STX and TTX share very similar mechanisms of action, as discussed above, it is not surprising that the symptoms of STX intoxication are almost indistinguishable from TTX intoxication. PSP can produce paralytic, neurotoxic, and amnestic symptoms in the range of mild to severe. Neurologic symptoms can include sensory, cerebellar, and motor. Mild symptoms of STX intoxication begin with paresthesia of the lips, tongue, and fingertips. These symptoms start within minutes of toxin ingestion. Nausea, headache, and the initial spread of paresthesias to the neck and extremities are common features. Moderate symptoms include limb weakness, dyspnea, hypersalivation, diaphoresis, and more neurologic involvement (eg, incoherent speech, ataxia, floating sensation, extremity paresthesias). Giddiness, rash, fever, tachycardia, hypertension, dizziness, and temporary blindness have been reported. Severe symptoms include muscle paralysis, severe dyspnea, choking sensation, and respiratory failure. As STX poisoning progresses, muscular paralysis and respiratory distress develop, and death from respiratory arrest occurs

within 2 to 12 hours, depending upon the severity of STX intoxication. As with TTX poisoning, many patients appear calm and remain conscious throughout the episode.[82]

Physical Examination. Fever has been associated with PSP,[85] hypothermia and sweating occur with TTX intoxication,[83,86–88] and both neurotoxins cause lip paresthesias.[89] TTX-induced circumoral paresthesia of the tongue and mouth occur within 10 to 45 minutes of ingestion.[90,91] Oral paresthesia, typically the first presenting symptom of TTX intoxication,[92] is followed by dysphagia,[90] aphagia, and aphonia.[93] STX causes ocular symptoms like temporary blindness,[61,94] nystagmus,[94,95] ophthalmoplegia, and iridoplegia.[96] TTX produces ophthalmoparesis,[97] blurred vision,[87,98] early stage miosis,[92,99,100] late stage mydriasis,[92,101] and absence of papillary light reflex.[91] TTX was reported to cause laryngospasm and dysgeusia.[36] PSP is associated with loss of the gag reflex, jaw and facial muscle paralysis, tongue paralysis,[96,102] dysphagia, and dysphonia.[94]

PSP can also cause tachycardia, T-wave changes on EKG,[94] hypertension,[103,104] or hypotension.[84] The cardiac enzyme creatine kinase MB has been shown to be elevated after PSP intoxication,[105] and mild tachycardia has been reported.[106] Puffer fish toxin may cause bradycardia, hypotension or hypertension,[92] dysrhythmias, and conduction abnormalities.[92,97,107,108] Chest pain is a common feature of both toxins.[93,99,106] TTX can also lead to cardiopulmonary arrest.[92,109]

Death from TTX or STX intoxication is caused by respiratory depression and paralysis of effector muscles of respiration.[79,96,107,108,110,111] Both TTX and STX intoxication cause dyspneic symptoms.[91,92,111,112] Apnea has been noted to occur within the first 2 hours after TTX ingestion[92,101] and even earlier with PSP,[113] suggesting the need for endotracheal intubation and mechanical ventilation. TTX blocks neuromuscular transmission, leading to skeletal muscle paralysis. Ascending paralysis may develop within 24 hours for either toxin.[91,99,106,114] Both toxins lead to the diminution of the gag reflex.[88,89,94] TTX has also been associated with acute pulmonary edema secondary to hypertensive congestive heart failure[115] and aspiration pneumonia.[99]

In addition to respiratory effectors, all voluntary muscles rapidly weaken with either toxin due to their effect on neuromuscular transmission; typically the upper extremities become weak, followed by the lower extremities.[89,92] Ascending paralysis follows[99] and patients may drop deep tendon reflexes, including absent Babinski signs.[90,91,100,112,113,116,117] Neurologic symptoms, such as paresthesias of the lips, tongue, face, neck and extremities, are the hallmarks of early intoxication, occurring within the first 30 minutes of ingestion.[95,96,104,107,109,114,118] Paresthesias of the lips, tongue, and throat usually precede the spread to the fingertips, neck, arms, and legs.[79,81] Lack of coordination, progressing to ataxia and dysmetria, has been reported for both toxins.[79,90,95,103] Seizures have been documented for puffer fish intoxication; these typically occur later in the progression of toxicity.[92,99] STX has also been associated with generalized giddiness, dizziness, incoherent speech, aphasia,[104] headaches,[81,104,113] asthenia,[79,113] and cranial nerve disturbances (eg, dysarthria, dipopia, dysphagia, fixed dilated pupils, absent ciliary reflex,[95,113] temperature reversal dysesthesia,[60] and neuropathies). STX-induced neuropathies consist of prolongation in distal motor and sensory latencies, decreased motor and sensory amplitudes, and reduced conduction velocities.[96] EEG abnormalities showing posterior dominant alpha waves intermixed with trains of short duration and diffuse theta waves have been demonstrated in TTX intoxication.[91] TTX causes central nervous neuropathies as well, manifested as blurred vision, ophthalmoplegia, dysphagia, and dysphonia.[93,97] Coma has been reported only after severe TTX poisoning but is less common.[112,117,119] Other symptoms reported with TTX intoxication include dizziness,[99] headaches,[110] and diabetes insipidus.[86]

Similar gastrointestinal complaints are experienced by patients early in TTX and STX poisoning by ingestion. Nausea, vomiting, diarrhea, epigastric pain, and hypersalivation are common to both TTX[83,88–90-,92,93,99,100,107,112,114,117] and STX[96,103,104,120,121] intoxication. Xerostomia has been reported in up to 20% of STX patients in one study.[103]

Hematologic abnormalities have been documented with puffer fish intoxication. Petechial hemorrhages and hematemesis are attributed to increased intrathoracic and intraabdominal pressure from violent episodes of emesis and wretching.[92,97] An isolated case of leukocytosis has been documented following TTX ingestion.[114] Hematologic abnormalities have not been reported for STX.

Laboratory findings and monitoring. Because TTX- and STX-intoxicated patients are diagnosed based on a high index of suspicion, clinical signs, and symptom presentation, laboratory findings and tests may be useful to determine etiology when patient history is inadequate and to monitor recovery. As a minimum, hemodynamic, acid-base, and fluid status, as well as serum electrolytes, blood urea nitrogen, creatinine, calcium, magnesium, phosphorous, urine output, CPK, EKG, and pulse oximetry should be monitored. Blood gases are helpful to monitor adequate oxygenation and ventilation. Lactic acidosis has also been reported in animals exposed to STX[105] and may be a useful parameter to monitor. Electromyography may show marked abnormalities and the cardiac

enzyme creatine kinase MB can be elevated. Serum electrolytes can be monitored for abnormalities due to dehydration, vomiting, and diarrhea. In addition, serum sodium, serum osmolality, and urine osmolality are useful for diagnosing suspected secondary diabetes insipidus in TTX intoxication.[86] CPK levels, which maybe elevated in STX intoxication, should be monitored. Urinary levels of TTX have been detected from suspected intoxication.[122]

STX has a direct action on the conducting system of the frog heart, producing decreases in heart rate and contractile force with severe bradycardia, bundle branch block, or complete cardiac failure. In cats, STX produces a reversible depression in contractility of papillary muscle.[77] In rats, TTX given intraarterially produces a rapid hypotension, beginning within 1 to 2 minutes and lethal by 6 minutes.[108] In several animal models, large doses of TTX cause conduction slowing, AV dissociation, and failure of myocardial contractility.[83] Seizures have been reported in several animals intoxicated with TTX.[35,83]

Dermatologic abnormalities, including pruritis, excessive diaphoresis,[104] and rash,[85] are reported for STX, while pallor,[93] bullous eruptions, petechiae, desquamation,[92,123] and diaphoresis[92,99] occur in puffer fish poisoning. Other abnormalities shared by both toxins include low back pain, muscle weakness, and elevated CPK levels.[102] Progression of any symptom is dependent on dose, route of exposure (ingestion or dermal), and rate of elimination, and not all individuals will react the same way to intoxication. Outbreaks of contamination may involve multiple toxins, so symptoms may appear to be characteristic of one toxin but clinical evidence may suggest the involvement of other toxins, further contributing to morbidity and mortality.[124]

Treatment. While there are no antidotes for TTX and STX intoxication, treatment is predominantly supportive and symptomatic. Good cardiovascular and respiratory support is critical,[83] and prognosis is excellent if supportive care is instituted early.[83,97] Activated charcoal can be administered after ingestion of either toxin, especially within 1 hour of ingestion of either toxin.[104,125] Cathartics and syrup of ipecac are not recommended for treatment of toxin ingestion. Most patients will recover with supportive care alone, but they should be monitored for signs of respiratory depression and neurotoxicity, requiring endotracheal intubation and mechanical ventilation. Electrolytes should be replaced, and fluids should be regulated according to arterial blood pressure and urinary output.[93,126] Fluid therapy can improve renal elimination of STX[105] because it is excreted into the urine.[106]

Hypoxia, acidemia, and conduction abnormalities should be corrected with careful EKG and blood gas monitoring. Bolus sodium bicarbonate may reverse ventricular conduction, slowing and dysrhythmias. Lidocaine IV can be given for ventricular tachycardia and ventricular fibrillation.[127] Bradycardia can be managed with supplemental oxygen and atropine; however, atropine alone may increase the lethality of TTX.[88,97] Adrenergic antagonists may prolong neuromuscular blockade of TTX and are not recommended.[128] Atropine can be given for asystolic cardiac arrest. Treatment with cholinesterase inhibitors has been attempted for TTX-induced muscle weakness, but data concerning their efficacy is scant. One study shows improvement of muscle weakness after TTX ingestion using IV edrophonium (10 mg) or intramuscular neostigmine (0.5 mg).[97,116]

Hemodialysis might aid recovery, but there is little data concerning the effectiveness of this treatment for TTX and STX intoxication. Hemodialysis was attempted because both toxins are low molecular weight, water-soluble molecules that are significantly bound to protein.[119] For example, an uremic woman who received regularly scheduled hemodialysis developed severe symptoms of TTX intoxication after eating fish soup. An hour after hemodialysis (and 21 hours after symptom onset), the patient recovered.[119] Hemodialysis was tried with mixed results for STX intoxication; one patient recovered and the other did not.[79] Desmopressin IV has been shown to be effective for TTX-induced central diabetes insipidus.[86] All other symptoms (hypotension, seizures, etc) can be managed as discussed previously.

Stability

TTX is water soluble at neutral pH and soluble in a dilute citrate or acetate buffer at acidic pH. In citrate or acetate buffers, it can be stored at −20°C for extended periods without loss of efficacy. It is unstable both in strong acid and alkaline solutions, and is rapidly destroyed by boiling at pH 2. TTX is likewise unstable in dilute hydrochloric or sulfuric acid, slowly protonating into the less toxic anhydrotetrodotoxin at equilibrium. It is relatively heat stable in neutral and organic acid solutions. Lyophilized TTX, available from commercial sources, should be refrigerated to maintain stability for long periods.

STX is remarkably stable[129,130] and readily soluble. Lyophilized STX is stable under the same storage conditions as TTX. Solutions of STX in acidic, aqueous solvent, or aqueous methanol, stored at a range of −80° to 4°C, are stable for several years. STX solutions stored at higher temperatures (37°C) are much less stable.

Protection

Cases of human poisoning by TTX and STX most commonly occur by ingestion of toxin-contaminated food, and poisoning by either toxin can result in paralysis, respiratory arrest, and death. Similar to PTX ingestion, it is often difficult to estimate the amount of toxin actually consumed. Relatively little toxin is usually consumed per accidental food poisoning case, yet deaths are not uncommon because of the toxicity of these compounds. Both TTX and STX are water soluble and stable under mild storage conditions, making them exceptional options for bioterrorist attacks targeting water, milk, or food supplies, especially fresh meats or vegetables.

The credibility of an aerosol TTX or STX threat is difficult to estimate, given the lack of inhalation toxicity research. It appears, however, that STX exhibits greater toxicity by inhalation[71] than by other routes of administration by a factor of 10. Whether this is a property of STX in particular or of all such toxins in general is not known at this time, and indeed the feasibility of weaponizing these toxins has not been explored. Given the known toxicity data, the threat cannot be discounted.

No antidote to TTX or STX poisoning is currently available for clinical use. Neostigmine has been suggested in some reports as a potential treatment for TTX poisoning;[83] however, no controlled trials have been conducted to investigate its efficacy.

Upon admission to intensive care facilities, treatment for TTX or STX intoxication involves careful observation and management of symptoms to avert respiratory arrest or cardiac failure.[131] In severe poisoning cases, atropine can be used to treat bradycardia,[107] and respiratory support may be indicated for periods of up to 72 hours. For cases of relatively mild intoxication, life-threatening complications are unlikely to develop after 24 hours following intoxication.

Surveillance

TTX and STX are presented together here because of the similarity in their sources, mechanisms of action, and clinical signs and symptoms of intoxication. Both are designated by the CDC as select agent toxins, or agents that have the potential to pose a severe threat to human health. STX was rumored to have been employed as the toxin in suicide capsules and injections provided to Central Intelligence Agency officers during the Cold War, notably U2 pilot Francis Gary Powers.[132] In 1969 President Nixon ordered the destruction of STX stockpiles.[133]

The extent of surveillance programs for TTX or STX is currently limited to state public health department monitoring for TTX- or STX-related food poisoning outbreaks, and no national program exists.

Brevetoxin

Synthesis

PbTxs are a family of marine neurotoxins found in the dinoflagellate *Karenia brevis*. *K brevis* produces nine known endotoxins, designated PbTx-1 through PbTx-9. During periods of algal blooms, like red tides, populations of the toxin-producing organism multiply, resulting in such high concentrations that they have been associated with human and animal intoxication. During these tidal blooms, the toxins are particularly poisonous to fish. Approximately 100 tons of fish per day were killed in a 1971 bloom off the Florida coast.[134] Other blooms have been noted in the Gulf Coast areas of Mexico, California,[135] and North Carolina (Figure 19-5).[136]

The PbTx family is composed of lipid soluble polyethers[137] and based on two different structural backbones (see Figure 19-5), PbTx-1 (brevetoxin A) and PbTx-2 (brevetoxin B). The other members of the family are derivatives of these parent chains and their chemical differences lie in the composition of the R-side chains. Each toxin subtype is an 11-member, heterocyclic, oxygen-containing, fused ring system ending with an unsaturated lactone on one end and an unsaturated aldehyde at the other. PbTx-1 is the only known toxin that is composed of five-, six-, seven-, eight-, and nine-member rings.[138] Synthesis of PbTx-1 and PbTx-2 was first accomplished by Nicoloau and colleagues.[139,140] PbTx-2 was the first to be synthesized,[139] validating the proposed structure of the molecule first advanced by Lin et al.[141] PbTx-1 was synthesized by the same group in 1998.[140] It is likely that the seven derivatives, PbTx-3 to PbTx-9, represent metabolites or biosynthetic modifications of one of the two parent chains, although at this time no specific pathways have been suggested.

Laboratory synthesis of PbTx-1 and PbTx-2 has been documented. These syntheses require many serial reactions to complete the complex macromolecule because, while the reactions are of moderate complexity, the overall yield is not very high. This last point is significant in the context of bioweapon production because terrorists might select compounds that could be easily synthesized with high yield, minimizing the skills and expertise required to produce toxin. PbTx-1 synthesis begins using D-glucose and D-mannose to synthesize two advanced intermediates, which are combined over Horner-Wittig conditions.[140] A total of 23 chemical reactions on D-glucose produces

yields that range from 64.5% to 94% per reaction. An additional six reactions on D-mannose, with approximately 90% yield per reaction, yields two advanced intermediate products, which are then bonded in four more synthesis reactions. Proper functionality and stereochemistry are established, and this synthetic PbTx-1 is identical to naturally occurring PbTx-1. The total synthesis of PbTx-2 has been reported by the molecular assembly of three subunits, requiring 108 total steps with similar step yields as PbTx-1 and an overall yield of 0.28%.[142]

Mechanism of Action and Toxicity

Similar to the mechanism of action of TTX and STX, PbTx also targets voltage-gated sodium channels. Active PbTx molecules bind on the α-subunit of the sodium channel at site 5, near the binding site of TTX and STX.[143,144] Binding of PbTx to the sodium channel alters the normal channel kinetics in two ways. First, it encourages the channel to open at more negative membrane potentials, which elicits sodium currents and causes the action potential to fire in the absence of membrane depolarization, a process that normally occurs in response to neurotransmitter binding to receptors. Second, PbTx inhibits the ability of the channel to inactivate itself.[138] Taken together, these effects can cause hyperactivity of the intoxicated neuron through increased duration of action potential firing because sodium channels open earlier (or spontaneously) and stay open longer. PbTx-induced sodium channel activation leads to acetylcholine release in the smooth muscles surrounding the airways, which leads to contraction and bronchospasm.[145,146,147]

PbTx-2 causes respiratory arrest and death in fish and mice.[148] PbTx-2 and PbTx-3 both produce symptoms of muscarinic-induced cholinergic crisis.[149] PbTx-3 is thought to be responsible for NSP and is more potent than PbTx-2 in mice, regardless of the route of exposure.[149] In contrast, PbTx-2 is more potent than PbTx-3 at neuromuscular blockade.[150] The principle mechanism of action appears to involve sodium-channel–mediated depolarization[151] rather than acetylcholine depletion.[150] PbTx produces a stimulatory effect on the nervous system and keeps sodium channels in their open states, while STX closes them.[151,152] PbTx also produces airway contraction and depolarization of airway smooth muscle.[145]

Toxin Exposure, Health Effects, and Treatment

Physical Examination. Human exposure to PbTx usually coincides with the red tide phenomenon and generally occurs through one of two routes: ingestion or inhalation. Intoxication by ingestion occurs through consumption of seafood containing high concentrations of PbTx and can result in NSP. Symptoms of NSP are generally mild, clinically resembling ciguatera, and include paresthesias of the face, throat, and extremities as well as a burning of the mucous membranes.[153–156] Abdominal pain, ataxia, seizures, and respiratory arrest may also develop. These toxins are heat stable and remain poisonous even after meals have been thoroughly cooked. PbTx is less potent than some of the neurotoxins presented here (eg, mouse LD_{50} of PbTx-1 is 95.0 μg/kg and the LD_{50} of PbTx-2 is 500 μg/kg intraperitoneal)[155]; therefore, PbTx ingestion is not lethal to humans.[156,157]

Exposure by inhalation occurs during red tide episodes, when wind can aerosolize PbTxs from the water-air interface.[158] These aerosols may contain additional contaminants, including subcellular fractions,

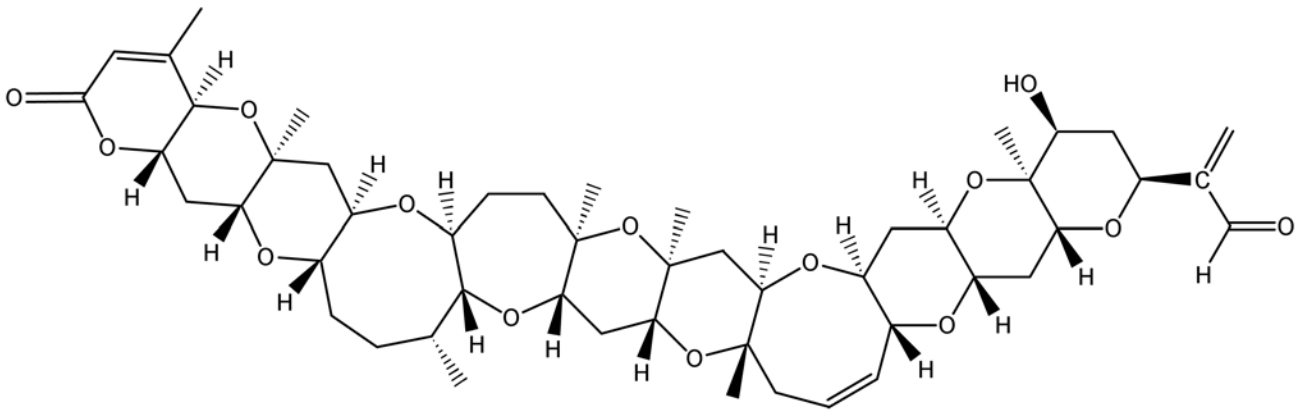

Fig. 19-5. Structure of brevetoxin A (PbTx-1).
Illustration: Courtesy of Richard Sweeny.

as well as bacteria, fungi, spores, and other materials. Symptoms of inhalation exposure include mydriasis,[159] ocular irritation,[157] lacrimation,[149] rhinorrhea,[160] coughing,[157] sneezing,[161] salivation,[149] bronchospasm, dyspnea, and burning sensations of the pharyngeal and nasal mucosa[162,163] in a concentration-dependent manner. PbTx-induced bradycardia can persist up to 12 hours in humans,[164] and the bronchospasms induced by PbTx may elicit an asthmatic attack in those with a preexisting history of exposure or hypersensitivity,[165] and respiratory irritation in the general population. The respiratory irritant zone of offshore red tide that has aerosolized has been estimated within a few kilometers of the beach.[161] In a study of 59 patients with asthma, exposure to aerosolized PbTx after walking along the beach for 1 hour during red tide was associated with significant increases in cough, wheezing, chest tightness, and eye and pharyngeal irritation, as well as abnormal pulmonary functional tests (eg, decreased forced expiratory volume 1[FEV1] and forced midexpiratory flow rate [FEF25–75]) compared to control subjects.[163]

Perioral, facial, and extremity paresthesias are common following PbTx ingestion.[136,157,166] A distorted or clouded sensorium,[159] dystaxias and generalized weakness,[136,157] temperature reversal dysesthesia (eg, warm objects feel cold), tremors,[136] seizures,[157,166] and coma have all been reported following PbTx ingestion.

The earliest symptoms of PbTx intoxication are gastrointestinal or dermatological, depending on the route of exposure. Ingesting these endotoxins can cause nausea, vomiting, abdominal discomfort, and diarrhea.[136,157,159,166] Swimming in red tides can produce pruritus.[157]

Although symptoms of PbTx exposure itself are relatively mild, the effects of inhalation exposure highlight the potential use of aerosolized neurotoxins during a bioterror attack. Toxicity in animal models by oral and parenteral routes has been shown to occur in the nanomolar to picomolar range.[137] Studies of atmospheric PbTx concentration in locations near red tide episodes have shown that concentrations less than 27 ng/m^3 are sufficient to cause symptoms in recreational beachgoers.[167]

PbTx released at a high concentration into a confined space with mechanically circulated air, such as shopping malls or subways, could have deadly effects, especially in individuals with respiratory ailments. Human deaths attributed to PbTx have never been reported,[157] so a minimum lethal dose in humans has not been determined.

Laboratory Findings and Monitoring. While no existing laboratory tests are useful for diagnosing PbTx intoxication, multiple methods are available to detect the toxin, including thin layer chromatography,[148] liquid chromatography/mass spectrometry, and immunoassay. A radioimmunoassay, synaptosomal assay using rat brain synaptosomes,[168] and an enzyme-linked immunoassay[169] have additionally been developed to detect PbTx.

A wealth of animal toxicity data exists for PbTx. This data shows that blood pressure responds biphasically depending on the dose. Low doses of IV PbTx lead to hypotension, while higher doses (160 $\mu g/kg$ IV) cause hypertension.[170] Bradycardia has been demonstrated in both cats and dogs.[159] Labored breathing and death have been reported in mice exposed to PbTx-2 or PbTx-3 by ingestion or injection.[149] Cat studies demonstrated bradypnea following PbTx intoxication,[170] and guinea pigs showed a biphasic tachypnea followed by bradypnea.[171] It is thought that PbTx-3 induces greater respiratory symptoms during red tide than PbTx-2.[149] A cholinergic syndrome (salivation, lacrimation, urination, and defecation) similar to nerve agent intoxication has been shown in mice injected with PbTx-2 or PbTx-3.[172] Both toxin subtypes produce tremors and muscle fasciculations in mice.[149] While a hemolytic agent has been associated with red tide dinoflagellates,[173] hemolysis is not a feature of PbTx in contrast to PTX intoxication.

Treatment. The route of exposure to PbTx should guide patient management. Inducing emesis is not recommended for PbTx ingestion. Activated charcoal can adsorb large molecules and is effective within 1 hour of ingestion, but it is ineffective once neurologic symptoms have occurred. Use of a cathartic with activated charcoal is not recommended because cathartics can cause gastrointestinal symptoms, electrolyte imbalances, and hypotension. Atropine has been suggested to reverse the bronchoconstriction induced by PbTx-3 as well as rhinorrhea, lacrimation, salivation, urination, and defecation.[149] No human data exists on the use of atropine in PbTx intoxication. Atropine does reverse PbTx-induced bradycardia in dogs but has no effect on blood pressure changes.[159] In the case of seizures, benzodiazepine treatment with diazepam, lorazepam, or midazolam should be administered, and the patient should be monitored for respiratory depression, hypotension, dysrhythmias, serum drug levels, and possible endotracheal intubation. If seizures continue, phenobarbital can be administered. In case of hypotension, isotonic fluids should be started while the patient is supine. Dopamine or norepinephrine can be used if hypotension persists.

In case of inhalation exposure, the patient should first be removed from the exposure, decontaminated, and monitored for respiratory distress. If cough or

dyspnea develops, monitor for hypoxia, respiratory tract irritation, bronchitis, or pneumonitis. Symptomatic treatment should consist of 100% humidified supplemental oxygen. The patient should be monitored for systemic signs of toxicity as well as the need for endotracheal intubation and assisted ventilation. Bronchospasm can be reversed using beta-2 adrenergic agonists. Ipratropium and systemic corticosteroids for bronchospasm should be started with continued monitoring of peak expiratory flow rate, hypoxia, and respiratory failure, or nebulized albuterol or ipratropium added to the nebulized albuterol. Systemic corticosteroids, such as prednisone, can reduce the inflammation associated with bronchospasm and asthma. For ocular or dermal exposure, eyes and skin should be flushed with copious amounts of water.

Stability

PbTx derivatives exhibit remarkably stable properties. In aqueous or organic solvent solutions, PbTx remains potent for months; culture media that contained growing *Karenia brevis* maintained its ability to intoxicate for similar periods. PbTxs are reportedly sensitive to air,[174] so commercial source PbTx is shipped in nitrogen-blanketed or evacuated containers. Lyophilized PbTx is stable for months without special storage conditions, and certain derivatives, such as PbTx-2 and PbTx-3, have been reported to be heat stable at extreme temperatures (300°C). The relative stability of PbTx and the ease with which lyophilized PbTx can be reconstituted make PbTx an attractive toxin to be weaponized.

Protection

No cases of paralysis or death from NSP have been reported.[157] Symptoms of PbTx intoxication as detailed above generally begin within 15 minutes of exposure, but may occur as late as 18 hours post-exposure, with symptoms potentially persisting for several days. Treatment for NSP or PbTx poisoning consists of supportive care; there is no antidote or antitoxin for PbTx exposure.

For individuals sensitive to PbTx inhalation exposure, a respiratory barrier or particle filter mask and departure from the area of exposure to an air conditioned or filtered environment should provide relief from inhalation exposure symptoms. The bronchoconstricitve airway response to inhaled PbTx in a sheep asthma model can be relieved by the use of histamine H1 antagonist diphenhydrimine, atropine, and the natural polyether brevenal.[165,175] This may direct further research and provide treatment options for both asthmatics and other susceptible persons exposed to aerosolized PbTxs. PbTxs can be easily oxidized by treatment with potassium permanganate ($KMnO_4$). This reaction is irreversible, proceeds quickly, leaves a nontoxic compound,[176] and is a potential means of detoxification.

Surveillance

Significant information is available on morbidity and mortality in aquatic animal populations exposed to red tide toxins, including domoic acid, PbTxs, STXs, and ciguatoxins. Much of what is known about gross and histopathologic analyses, diagnostics, and therapeutic countermeasures for these toxins has been gleaned from environmental population exposure studies.[177] Historically, marine mammals (pinnipeds, cetaceans, and sirenians), aquatic birds, sea turtles, fish, and invertebrates are environmental sentinel species. All are susceptible to toxin exposure via ingestion and immersion; however, marine mammals and sea turtles are particularly susceptible to respiratory exposure at the air-water interface, where aerosolization and concentration occurs. In addition, marine mammals have poor tracheobronchial mucociliary clearance compared to terrestrial mammals.

Although human and environmental impacts on coastal seawater quality and temperature can result in significant algal blooms, it is unlikely that a terrorist attack would attempt to directly impact red tides. However, an intentional chemical spill or factory attack could lead to subsequent algal blooms. Communication with marine mammal and sea turtle stranding networks, as well as other environmental agencies (eg, the Environmental Protection Agency, National Oceanic and Atmospheric Administration, etc), is critical in the early identification of adverse health effects on sentinel species.

A tampered freshwater source, such as a reservoir, would also have effects on fish, aquatic birds, and mammals in that system. A real-time, automated, biomonitoring, portable ventilatory unit developed by the US Army Center for Environmental Health Research measures gill rate, depth, purge (cough rate), and total body movement determined by amplified, filtered, electrical signals generated by opercular (gill) movements in bluegill (*Lepomis macrochirus*) and recorded by carbon block electrodes.[178] Biomonitor studies have already been conducted to determine the effects of PbTx-2 and toxic *Pfiesteria piscicida* cultures on bluegill.[179] Applications for this biomonitoring system have included watershed protection, wastewater treatment plant effluent, and source water for drinking water protection.

Batrachotoxin

Synthesis

BTX (Figure 19-6) is a steroidal alkaloid and the primary poison of the so-called Colombian Poison Dart Frogs of the genus *Phyllobates*. These frogs are brightly colored golden yellow, golden orange, or pale metallic green and they release BTX, as well as four other steroid toxins, through colorless or milky secretions from the granular glands in response to predatory threats. It is believed that *Phyllobates* do not produce BTX, but accumulate the poison by eating ants or other insects in their native habitats that have obtained BTX from a plant source. The natural sources of BTX have not been reported; however, frogs raised in captivity do not contain BTX and thus may be handled without the risk of intoxication,[180,181] suggesting that the toxin is the product of another organism. Recent field work has identified BTX in tissues of other, unexpected species, including the skin and feathers of some birds from New Guinea, *Ifrita kowaldi*, and three species of the genus *Pitohui*. The link between the toxin-bearing birds and frogs was hypothesized to be Melyrid beetles of the genus *Choresine*.[9,182–185] These beetles contain high concentrations of BTX and have been discovered in the stomach contents of captured toxin-bearing bird and frog species (see Figure 19-6).

BTX is commonly used by Noanamá Chocó and Emberá Chocó Indians of western Colombia for poisoning blowgun darts used in hunting. The most toxic member of *Phyllobates*, (*P terribilis*, *P aurotaenia*, and *P bicolor*), is *P terribilis*, which can bear a toxic load up to 1900 μg of toxin.[185] *Phyllobates* generally contain approximately 50 μg of toxin. Toxin is extracted by Chocó Indians by roasting captured frogs over a fire.[186,187] BTX is harvested from blisters that form on the frog from the heat of the fire and is weaponized by touching dart or arrow tips to the toxin. The toxin can be stockpiled by collection and fermentation in a storage container, and toxin stocks prepared in this way are reported to be potent for up to 1 year.[185]

Mechanism of Action and Toxicity

BTX is a neurotoxin that affects the voltage-gated sodium channels in a manner similar to the PbTx discussed above. Pathologic effects from BTX intoxication are due to the depolarization of nerve and muscle cells, which results from an increased sodium ion permeability of the excitable membrane.[188] BTX is lipid soluble, and activity is temperature dependent and pH sensitive. The maximum activity of BTX occurs at 37°C[185] and at an alkaline pH.[189]

BTXs bind sodium channels both in muscle cells and in neurons, modifying both their ion selectivity and voltage sensitivity.[188] The effect of toxin on the sodium channel is to make it constitutively open, causing the irreversible depolarization of cells.[190] However, effects are not observed in experiments where sodium ions are absent in intracellular and extracellular compartments. In addition, BTX alters the ion selectivity of the ion channel by increasing the permeability of the channel toward larger cations.[189] In-vitro muscle preparations treated with BTX have shown massive acetylcholine release in response to depolarization, as predicted. Ultrastructural changes have been observed in nerve and muscle preparations and are due to the massive influx of sodium ions that produce osmotic alterations.[191]

Toxin Exposure, Health Effects, and Treatment

BTX-tipped darts have been used to hunt game by several Indian groups with very effective results, although few Indian groups, notably the Chocó, have adopted its use in warfare. The Chocó fiercely resisted the Spanish in the late 16th Century, and it is not unlikely that BTX weapons were employed in warfare during that period.[185]

Physical Examination. Few published reports have described the systemic effects of BTX intoxication; however, the Chocó Indians claim that a human shot with a BTX-poisoned dart could run only a few hundred meters before dying.[185] In 1825 Captain Charles Stuart Cochrane, a Scottish explorer, described his encounters with the Chocó during an expedition around the lowland tropical rain forests of Colombia.

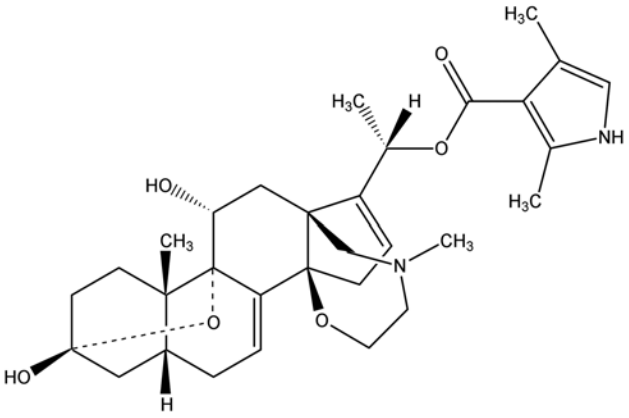

Fig. 19-6. Structure of batrachotoxin, the poison dart frog poison.
Illustration: Courtesy of Richard Sweeny.

In his work, Captain Cochrane writes that a dart envenomed with BTX will cause "certain death to man or animal wounded by it; no cure as yet having been discovered."[186]

Laboratory Findings and Monitoring. BTX is one of the most potent nonprotein poisons. It is cardiotoxic and neurotoxic to humans and animals. Cardiotoxic actions lead to irreversible depolarization of nerves and muscles, causing arrhythmias, fibrillation, and cardiac failure.[192] BTX produces a rapid succession of symptoms when given to animals, including ataxia, weakness, convulsions, paralysis, and cyanosis. Respiratory arrest from paralysis of respiratory effector muscles and cardiac arrest are the causes of death in cases of BTX poisoning.[187] At sublethal doses, symptoms in animals include strong muscle contractions, convulsions, salivations, dyspnea, and death,[193] death ensuing in mice of lethal challenge within minutes. BTX effects on cardiac muscle are similar to the cardiotoxic effects of digitalis, including interference with heart conduction causing arrhythmias, ventricular fibrillation, and other changes that can lead to cardiac arrest.[188]

Treatment. While there is no known antidote for BTX intoxication, treatment has been suggested by using an approach similar to that for treating toxins and chemicals with comparable mechanisms of action (eg, DigiBind [GlaxoSmithKline, SpA, Parma, Italy]).[194]

Stability

Collecting large quantities of the frog-based alkaloid toxins is difficult because a microgram-load of toxin is contained in a single specimen and because frogs bred and raised in captivity lose their toxic properties. Therefore, stockpiling BTX from natural sources may not be practical. BTX is notable because humans have weaponized it under primitive conditions and have successfully employed it in both hunting and warfare. However, the practicality of using such toxins as weapons of mass destruction is questionable.

Protection

BTX is a particularly deadly toxin; the LD_{50} in mice (subcutaneous) is 2 μg/kg.[195] Membrane depolarization can be blocked, or in some cases reversed, by treatment with sodium channel blockers (eg, TTX or STX), which allosterically block sodium currents through voltage-gated channels.[189] This presents an additional complication, because nerve conduction and action potential generation will be compromised.

Surveillance

As with several of the other toxins reviewed here, public health surveillance programs for BTX intoxication have not been established.

SUMMARY

The potential use of disease-producing microorganisms, toxins, and chemical agents has been of concern in both ancient and modern military conflicts, especially during the last century.[4–6] As of 2000, public reports assert that at least two dozen countries either have such chemical or biological weapons or actively seek them.[196] Covert attacks against unsuspecting civilian populations with any of the toxins reviewed here have the potential to produce large numbers of casualties. Management of these casualties will be difficult because treatment for exposure to the presented toxins generally only consists of supportive care. The key to mitigating the effects of a bioterror weapon is the realization that one has been used. Early characterization of the attack will allow appropriate steps to be taken to mediate the effects of the weapon both physically and psychologically on the civilian population. These critical measures include decontamination and evacuation of the affected area, early administration of antitoxin or treatments where available, and the allocation of clean food, water, or air supplies. Early determination of a bioterror attack by a weaponized toxin becomes more difficult when surveillance programs for these kinds of toxins are lacking.

Given a sufficient quantity of even mildly toxic material, bioterrorist attacks, in theory, could be conducted with virtually any toxin, resulting in numerous casualties and chaos in civilian populations. The potential of a toxin to be employed as an effective bioweapon, and therefore the need for a surveillance program for that toxin, should be evaluated using several criteria, including the toxicity of the compound, the ease of synthesis or commercial availability, and the ease of weaponization and delivery (ie, getting the bulk toxin into an appropriate form to introduce into the target population). The toxins reviewed here are sufficiently toxic, if employed effectively, to cause large numbers of casualties in populations unprepared for their release and without advance warning. In addition, most of these compounds are stable enough to be stockpiled with minimal specialized equipment and are also water soluble, allowing for easy dispersal of the toxins in food or water sources or via aerosol dispersion (Figure 19-7).

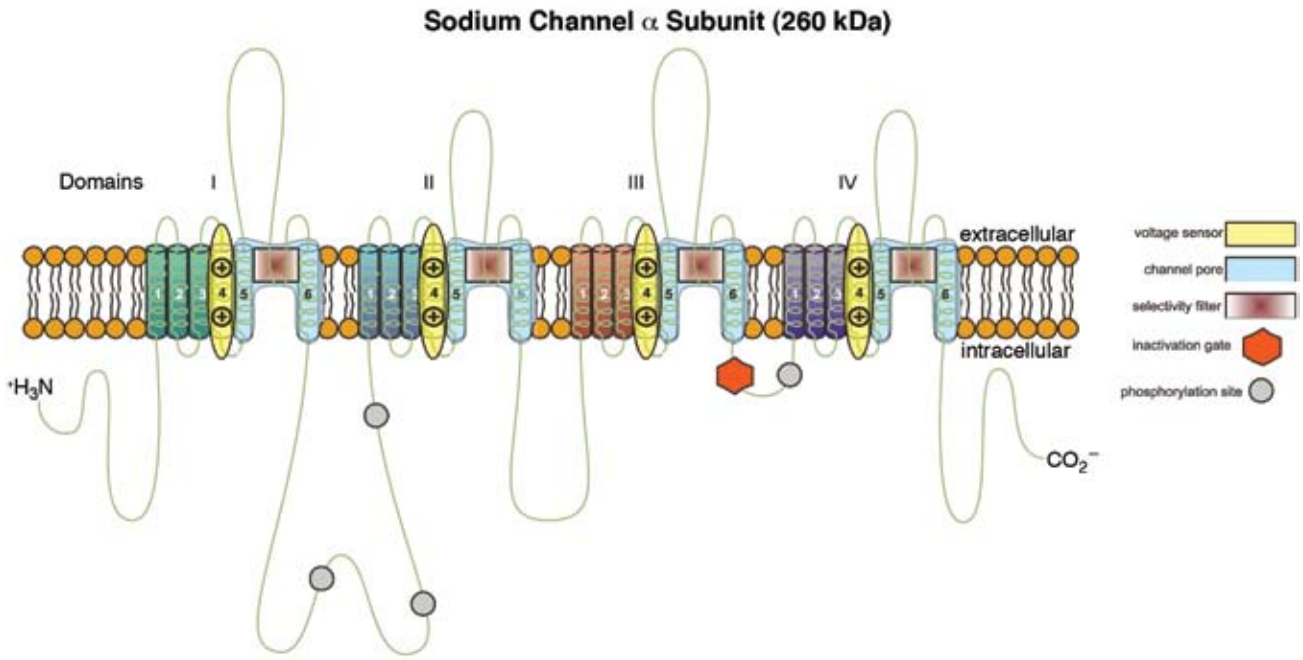

Fig. 19-7. Sodium channel proteins play an essential role in action potential generation and propagation in neurons and other excitable cell types. The resting membrane potential of a neuron remains around - 80 mV. When the neuron membrane becomes excited by the binding of neurotransmitters to their appropriate receptor molecules for example, the cell begins to depolarize and these voltage-gated sodium channels are activated. In response to membrane depolarization, these channels open, increasing cell membrane conductance and a large influx of sodium ions travels down the sodium concentration gradient. This large sodium current drives the membrane potential of the cell towards the reversal potential for sodium, approximately + 55 mV and is recognizable on electrophysiological recordings of neuron activity as the "spike" or action potential. Membrane potential is returned to resting by a combination of the termination of sodium influx due to loss of driving force on sodium, the eventual inactivation of the voltage-gated sodium channels, and the opening of potassium channels. The inactivated state is different from the closed channel state, with the inactivated-to-closed transition driven by the slight hyperpolarization of the cell membrane, which occurs in response to potassium current. Only closed channels are available to open.

Voltage-gated sodium channels are protein complexes composed of a 260 kDa α-subunit and one or more smaller, auxiliary β-subunits (β1, β2, or β3). The variable combinations of the α-subunit with multiple β-subunits allow the creation of a number of functionally distinct channels. The α-subunit illustrated here folds into four transmembrane domains (I–IV), colored green, blue, orange, and purple. The transmembrane domains are composed of six α-helical segments designated S1 through S6 (see Figure 19-4).

The use of biological agents against civilian populations is a legitimate issue of concern; attacks using biological agents have already occurred in the United States and abroad. We must anticipate terrorist groups employing toxins or other agents that are not considered classical weapon agents. Understanding the real strengths and weaknesses of toxins as weapons allows an educated and realistic assessment of the threat posed by toxins and can guide the administration of surveillance programs and contingency plans.

ACKNOWLEDGMENT

The authors thank Lieutenant Colonel Charles Millard, Walter Reed Army Institute of Research, and Dr Mark Poli, US Army Medical Research Institute of Infectious Diseases, for their insight and editorial contributions.

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