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Biochemistry of Tetrodotoxin

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ABSTRACT:

The puffer fish's ovaries and liver are the original sources of the strong neurotoxin tetrodotoxin (TTX). It is now abundantly obvious that TTX is generated by certain bacteria and enters the food chain rather than being created by puffer fish. Particularly in Japan, where puffer fish is regarded as the most delectable fish, the pharmacology of TTX has been investigated for a long time. However, several scientific studies into the cellular and molecular mechanisms behind TTX's specific and powerful blocking activity on the sodium channel have been started since the ground-breaking discovery of this effect. The fact that TTX has now been extensively employed in research labs to examine the sodium channel, other ion channels, as well as many facets of membrane excitability and synaptic transmission, is also significant.

This chapter provides the highlights of the most current TTX research investigations. Older literature is not covered because it has been thoroughly examined by numerous researchers. It is recommended that readers study these review articles. Thus, the current chapter discusses the most recent advancements in the fields of TTX sources, sodium channel action mechanisms, TTX-resistant sodium channels, TTX action and binding sites, and TTX therapeutic uses. It is also documented how the paralytic shellfish toxin saxitotoxin works by inhibiting sodium channels.

Keywords*:* Pseudmonas, Alteromonas, Shewanella, Neuroendocrine, Neurons

INTRODUCTION:

The liver and ovary of puffer fish produce tetrodotoxin (TTX). In Japan, where puffer fish is regarded as the greatest delicacy among marine delicacies, TTX is the source of intoxication from eating it. To serve puffer fish in a Japanese restaurant, you need a licence. Accidents happen when puffer fish enthusiasts who prefer having a numb feeling around the lips due to slight intoxication consume a small bit of ovary or liver to get the feeling but miscalculate the dose. Intoxication with puffer fish at such a restaurant is unheard of. Since there is no known pharmaceutical antidote, the only means to prolong life is to use artificial respiration since

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death results from diaphragm paralysis. Cardiac muscle is not significantly harmed by TTX because it is far less responsive than the nerve and skeletal muscle.

Today, it is known that many additional aquatic creatures, like the California newt, carry TTX. The fact that none of these creatures, including puffer fish, synthesise TTX is also becoming increasingly obvious. A few types of bacteria can produce TTX, which eventually makes its way up the food chain to these animals. There are a lot of different types of bacteria that produce TTX. Pseudoalteromonas, Vibrio, Shewanella, Pasteurella, Aeromonas, and Plesiomonas are a few of them (1–6). It is questioned if Vibrio alginolyticus is the source of TTX, though (7). Since bufonid frogs of the genus Atelopus generally possess both the steroidal bufadienolides and TTX in the skin, it is interesting to note that environmental conditions such symbiotic microbes play a significant role in the manufacture of TTX while the bufadienolides are continually synthesised (8).

PHARMACOLOGY OF TETRODOTOXIN:

Saxidomus giganteus, an Alaskan butter clam, but the toxin comes from the dinoflagellate Gonyaulax catenella (9, 10). One of the red tide poisons is STX. Similar to TTX, STX is widely distributed among marine animals. For instance, it is present in xanthid crabs like Zosimus aeneus along with other paralytic shellfish toxins including TTX, Neosaxitoxin, and Gonyautoxins (11–18).

MECHANISM OF ACTION ON SODIUM CHANNELS:

Although TTX has been known for a long time to inhibit nerve and muscle transmission (9, 19), the sodium channel did not receive considerable consideration as a potential TTX target until 1960. TTX was found to block the action potential using intracellular microelectrode techniques, but had no impact on the resting membrane potential, resting membrane resistance, or delayed rectification, which are indicators of the activity of potassium channels. This led to the theory that TTX blocks the sodium channel (20). The voltage clamp approach using lobster gigantic axons amply proved this theory (21). TTX fully and irreversibly blocks sodium currents while leaving potassium currents unaffected. Since then, TTX has developed into an extremely well-liked chemical instrument in the lab and has remained an effective tool. Numerous studies have been done on the activity of TTX, looking at its mechanism of sodium channel block as well as potential effects on receptors and ion channels other than sodium channels. Numerous review papers have been written about the use of TTX and its mode of action (19, 22–30). Readers are therefore urged to refer to these reviews for information on earlier works.

MOLECULAR STRUCTURE OF SODIUM CHANNELS:

The sodium channel is made up of subunits, 1, and 2. The major and most significant component is the subunit because, when the subunits of brain or skeletal muscle sodium channels are expressed in Xenopus oocytes, functional sodium channels are produced, albeit

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ones that are activated and deactivated more slowly than sodium channels found in native neurons or muscle.

Normal channel gating is only seen when the 1 and 2 subunits are both expressed (31–33). The component is made up of six -helical transmembrane segments (S1-S6) in each of its four homologous domains (I-IV) (24, 25, 34). A channel pore is thought to be formed by a loop that extends into the transmembrane space of the protein between transmembrane segments S5 and S6 of each domain. Voltage sensors are found in transmembrane segment 4 of each domain, the inactivation gate is situated in the inner loop between S6 of domain III and S1 of domain IV, and several phosphorylation sites by PKA and PKC are found in the inner loops between domains I and II and between domains III and IV, respectively.

TTX-Resistant Sodium Channels

Whereas the majority of sodium channels in neurons and skeletal muscle fibers is highly sensitive to the blocking action of TTX and STX with IC50 values. The sodium channels less sensitive or resistant to TTX/STX have been discovered in a number of tissues in the nanomolar range. Skeletal muscle and cardiac muscle with denervation serve as examples of classic situations (19, 26). As an illustration, the predicted IC50 values for blocking TTX were 1 M for rabbit Purkinje fibres (35), 9 M for rat cardiac cells (36), and 14 M for pig papillary muscle (37). These IC50 values are roughly two to three orders of magnitude greater than those for sodium channels that are TTXsensitive (TTX-S) in brain tissues and skeletal muscle.

However, it is also known that a variety of neurons include sodium channels that are TTX-resistant (TTX-R). These include sensory neurons from the bullfrog and garter snake, group C sensory neurons, rat nodose neurons, human and mouse dorsal root ganglion (DRG) neurons, and group C sensory neurons (44–48).

Since TTX-R sodium channels are connected to C fibres, which are responsible for sending pain signals to the brain, they did not begin to attract significant attention until the early 1990s. A substance may function as an effective antinociceptive if it blocks TTX-R sodium channels without affecting TTXS sodium channels. Along these lines, it is intriguing to note that in vitro recordings of C fibre action potentials in the presence of TTX were totally suppressed by capsaicin, demonstrating a strong link between the sensitivity of C fibres to capsaicin and their resistance to TTX (49). Thus, as soon as the first studies comparing the electrophysiology of TTX-S and TTX-R sodium channels in detail were published (50, 51), this area grew in popularity and underwent extensive research.

Here, we provide a brief description of the properties of the TTX-S and TTX-R sodium channels found in rat DRG neurons (51). For TTX-S and TTX-R sodium channels, the predicted IC50 values for TTX block are 0.3 nM and 100 M, while for STX block, they are 0.5 nM and 10 M. The two varieties of sodium channels differ in their TTX sensitivity by a factor of 300,000. The dynamics of sodium current differ noticeably; in both the activation

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and inactivation phases, TTX-S sodium currents move far more quickly than TTX-R sodium currents. Additionally, there are significant variations in the activation and inactivation voltages. The potentials for TTX-S and TTX-R channels for 50% sodium current activation and 50% inactivation, respectively, are 26 mV and 15 mV, respectively.

It should be mentioned that the sensitivity to specific compounds in TTX-S and TTX-R sodium channels differs significantly. Lead and cadmium both reduced sodium currents and moved the activation potential toward a depolarizing state, although TTX-R sodium channels were more strongly affected by these effects than TTX-S sodium channels were. For TTX-S and TTX-R sodium channels, respectively, the lead-induced shifts in the potential for 50% conductance are 15 mV and 25 mV, and the lead-induced reductions in maximum conductance are 8% and 55%, respectively. The variations in potential for 50% conductance caused by cadmium are 25 mV and 21 mV. Here, we give a brief overview of the characteristics of the sodium channels TTX-S and TTX-R that are present in rat DRG neurons (51). The anticipated IC50 values for TTX block for TTX-S and TTX-R sodium channels are 0.3 nM and 100 M, and for STX block, they are 0.5 nM and 10 M. The TTX sensitivity of the two types of sodium channels varies by a factor of 300,000. The dynamics of sodium current vary substantially; TTX-S sodium currents move much more swiftly than TTX-R sodium currents during both the activation and inactivation phases. The activation and inactivation voltages also show substantial differences. The potentials for TTX-S and TTX-R channels are 26 mV and 15 mV, respectively, for 50% sodium current activation and 50% inactivation.

Another notable variation between the TTX-S and TTX-R sodium channels was found in their sensitivity to the modulation brought on by the synthetic pyrethroid insecticides tetramethrin and allethin (52, 53). An illustration of an experiment for tetramethrin modulation. Tetramethrin has a concentration-dependent effect on the production of a large and slow tail current after the termination of a depolarizing pulse, but has little to no effect on the peak sodium current. A method has been established to determine the percentage of sodium channels affected by pyrethroids. Pyrethroids control the individual sodium channel by extending the open period and by blocking the inactivation (54, 55). (53). The sodium channels TTX-S and TTX-R for their percentages of tetramethrin modification as a function of concentration. Tetramethrin sensitivity of TTX-R sodium channels is 30-100 times greater than that of TTX-S sodium channels.

Many sodium channel types, including TTX-R sodium channels, have been cloned. Although sodium channels are divided into several categories, certain intriguing structural characteristics are being revealed (25, 56–59). The, 1 and 2 subunits make up the type I, type II, type IIA, and type III sodium channels in the rat brain. These channels are all vulnerable to the blocking effects of TTX and STX (IC50s in the order of nanomolar). Adult skeletal muscle is made up of the and subunits, possesses sodium channels with the 1 or SkM subtypes, and is sensitive to nanomolar concentrations of TTX as well as the blocking effects

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of -conotoxin GIIIA. The heart and denervated skeletal muscle contain H1 or SkM2 sodium channels, which are resistant to TTX and STX with IC50 values in the range of 2 to 6 M. With IC50s in the range of nanomolar, PN1 sodium channels found in neuroendocrine and peripheral neurons are also responsive to TTX and STX. SNS or PN3 and SNS2 or PN5 sodium channels are present in the dorsal root and trigeminal ganglion neurons, respectively. While SNS2 sodium channels are resistant to TTX with IC50s in the order of 1 M, SNS sodium channels are insensitive to TTX and STX with IC50s in the order of 30 M or more.

Animal resistance to TTX may result from causes other than sodium channel TTX sensitivity. Hemigrapsus sanguineus, a shore crab, has a high level of TTX resistance. Its bodily fluid was tested for neutralising effects against TTX in order to understand the mechanism of TTX resistance (60). When bodily fluid and TTX were administered to mice, TTX's deadly activity was significantly diminished. However, the bodily fluid was unable to stop the paralytic shellfish poisons' fatal effects. The TTX-binding, high molecular weight compounds (2,000,000) present in the bodily fluid are in charge of the TTX-neutralizing activity. TTX is likewise not effective on the horseshoe crab, Carcinoscoprius rotundicauda. The haemolymph contains both proteinaceous and non-proteinaceous components that are necessary for TTX-neutralizing activity (61).

Site of TTX/STX Action and Biding

Based on binding tests, chemical compounds that affect the sodium channels can be divided into a number of classes (24). Site 1 of the sodium channels is bound by TTX, STX, and -conotoxin, blocking the channel. Veratridine, aconitine, batrachotoxin, and grayanotoxins bind to site 2 to prolong the sodium channel opening. Sea anemone toxin and -scorpion toxins (class 1), which likewise prolong sodium channel opening, bind to site 3. Classes 2 and 3 scorpion toxins bind to site 4, which modifies channel gating. Ciguatoxin and brevetoxins bind to site 5, extending the opening of the sodium channel. Pyrethroids bind to site 6 and modify the gating and prolong sodium channel opening.

Short segments SS1 and SS2 connecting transmembrane segments S5 and S6 of each domain provide binding site 1 for sodium channels for TTX and STX. (25). This region functions as a selectivity filter for different ions, and TTX and STX block the selectivity filter in the sodium channel's outer pore (62). All four domains have similar places for amino acid residues, the majority of which are negatively charged. These locations are thought to create outer and inner rings, which act as the binding sites for TTX and STX (25). Due to variations in amino acids, cardiac sodium channels are 200–1000 times less responsive to TTX/STX than TTX-S neuronal sodium channels. Unlike the cardiac sodium channels, which have cysteine in the equivalent place, the brain and skeletal muscle sodium channels have phenylalanine and tyrosine in the P-loop of domain I, respectively (63–67). The sodium channels of the dorsal root ganglia (PN3/SNS) include serine in domain I, which is supported by a significant increase in TTX affinity when serine is replaced with phenylalanine (51), making them even more resistant to TTX than the cardiac sodium channels (68).

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PHARMACOLOGY OF TETRODOTOXIN:

A neuroprotective agent could be created using a variety of methods. Membrane depolarization brought on by ischemia results in repeated discharges. These discharges at the nerve terminals allow calcium channels to open, which allows Ca2 to enter the area and release neurotransmitters. The released glutamate at the glutamatergic synapses opens the NMDA receptor/channel, allowing Ca2 ions to enter. The neuron dies as a result of the rise in intracellular Ca2 levels. Therefore, blocking presynaptic sodium channels, presynaptic calcium channels, and/or NMDA receptors may be used to prevent or reduce the ischemia damage.

It was discovered that TTX was efficient in reducing the ischemia damages brought on by artery occlusion in the rat hippocampus (70) as well as those brought on by veratridine exposure in cerebellar neurons and hippocampal neurons (71). After spinal cord damage, TTX microinjection in a focused area lessens tissue loss and neurological impairments. The injection of TTX into the injury site in rats after a standardised weight-drop contusion was successful in lessening the damage to the large-diameter axons (72).

In vitro, TTX is effective at preventing electrographic seizures (73). After localised injection of 50 M TTX in rat hippocampal slices, stimulation-evoked seizures were temporarily stopped, while responses to single stimuli were only marginally affected. Stratum radiatum and/or stratum lacunosum-moleculare in the CA2/3 region were almost usually the sites of TTX injections that prevented electrographic seizures. Low doses of TTX in the perfusion medium were useful in preventing electrographic seizures (5–20 nM). Additionally, rats who received TTX did not develop post-traumatic epileptogenesis (74). Thin sheets of Elvax polymer containing TTX implanted over lesions were successful in reducing evoked epileptiform potentials, which were seen in the damaged cortex as evoked epileptiform field potentials.

There have been several attempts to develop medications that can counteract the intoxication brought on by TTX and STX. In guinea pigs, 4-Aminopyridine, at 1-2 mg/kg (im), restored the toxin-induced diaphragmatic block, bradypnea, bradycardia, and reduced cerebral activity (75, 76).

In rabbits undergoing excimer laser keratectomy, TTX given topically to the eye proved successful in inducing analgesia without resulting in any systemic damage (77). In the rabbit cornea, it was demonstrated that topically given TTX at 1 or 10 mM is a long-acting anaesthetic (78). For extended local anaesthetic of the percutaneous sciatic nerve in rats, subcutaneous injections of TTX combined with epinephrine were successful; the median effective concentration of TTX was 11.5 M, and reversible blocks lasted for more than 13 hours (79).

In a mouse model of ischemia reperfusion damage, TTX has been demonstrated to have a renoprotective effect that greatly increases posttransplant graft dysfunction (80). Rats without

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nephrectomy were also able to have their kidneys protected by TTX against thermal ischemia (81). A monoclonal antibody has been created to block TTX. 1.5 mouse units of TTX were intraperitoneally given into the mice, and three minutes later, 100 g of the antibody immunoglobulin G (IgG) was injected into the tail vein. The survival rate was 100 percent (82). The monoclonal antibody could neutralise TTX in vitro and was highly specific for TTX, with no cross-reactions to TTX compounds or paralytic shellfish toxins (83). Investigations were conducted to determine whether TTX-specific monoclonal antibodies could provide passive protection against deadly TTX challenge (84). The monoclonal antibody, T20G10, had high specificity for TTX, little antihydrotetrodotoxin reactivity, and no tetrodonic acid reactivity. In an in vitro radio ligand receptor binding experiment, T20G10 selectively decreased TTX binding but had no impact on STX binding. Mice that received TTX orally and T20G10 did not die.

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