BRIEF COMMUNICATIONS

THE RECEPTOR FOR TETRODOTOXIN AND SAXITOXIN

A Structural Hypothesis

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Tetrodotoxin (TTX) and saxitoxin (STX) block sodium channels of nerve and muscle membranes at nanomolar concentrations (2, 7). The structure of TTX is known (Fig. 1), and a new structure of STX (Fig. 2) has just been determined by X-ray crystallography (13). This note describes a hypothesis for the toxin binding site based on the suggestions of several laboratories and inspired by the new STX structure.

The toxins act only from outside and not when perfused inside axons (10). Equilibrium and rate kinetic experiments show that toxin binding involves a reversible one-to-one interaction with an external receptor to block channels (see references in 14). In 1965, Kao and Nishiyama (8) proposed that a guanidinium group found in both toxin molecules forms the blocking complex by entering the Na channel and becoming stuck there because the rest of the molecule is too wide to pass. Their hypothesis offers a simple mechanical explanation for the block and is plausible since free guanidinium ions can pass through sodium channels. In 1967, Camougis et al. (1) suggested that TTX might also form an intermolecular hemilactal bond with its receptor, an idea that follows from the reversible intramolecular lactone-hemilactal transformation involving C_5 and C_{10} (Fig. 1) in free solution (2, 7). An —OH group belonging to the receptor would attack the lactone form at C_{10} to form an intermolecular hemilactal. Such bond formation would help to explain the firm binding of TTX with its receptor and the relative slowness (ca. 70 s time constant at 20° C) of the unbinding reaction (14).

Hille (5) extended the Kao-Nishiyama (8) hypothesis, proposing that the part of the channel where the toxins finally stick is the narrow ionic "selectivity filter." The proposed structure of this filter included a 3 by 5 Å constriction of the pore formed by a ring of six oxygen atoms. One group in the ring was supposed to be an ionized carboxylic acid that attracts cations. Indeed new evidence shows that TTX and STX

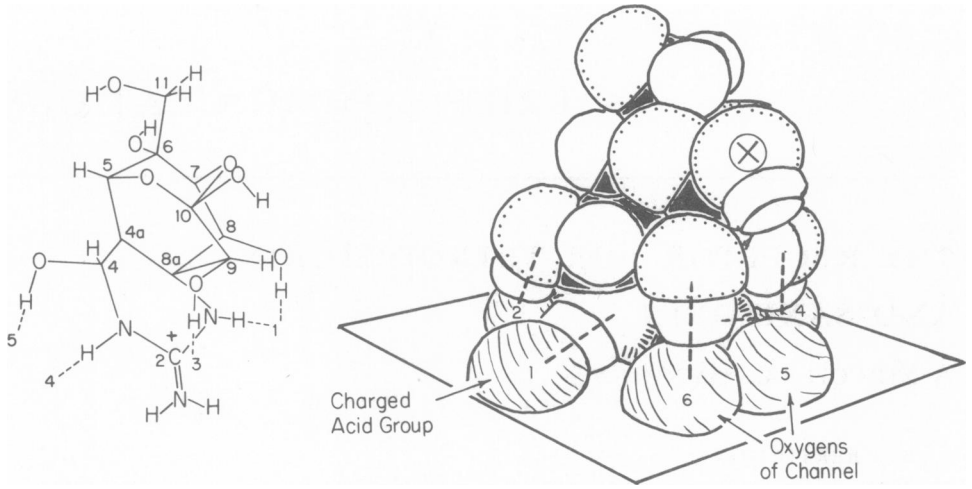

FIGURE 1 Tetrodotoxin complexed with the selectivity filter. *Right:* Perspective drawing of a CPK model of TTX forming hydrogen bonds (dashed lines) to the oxygen atoms (numbered and shaded) of the postulated selectivity filter (5). Atoms of toxin: carbons black, hydrogens white, oxygens dotted margin, nitrogens dashed margin. *Left:* Stick drawing of TTX oriented exactly as in the CPK model showing numbering of carbons (after reference 6).

binding are inhibited by just those cations that are thought to bind to the charge of the selectivity filter (3). Fig. 1 shows the structure of TTX and a drawing of the postulated TTX-selectivity-filter complex. In the complex the guanidinium group around C_2 is buried in the hole formed by the six shaded oxygen atoms of the pore. Other groups of the toxin molecule rest against the mouth of the pore, preventing the toxin from proceeding further. Five hydrogen bonds and the electrostatic attraction between the guanidinium group and the negative charge of the selectivity filter stabilize the complex.

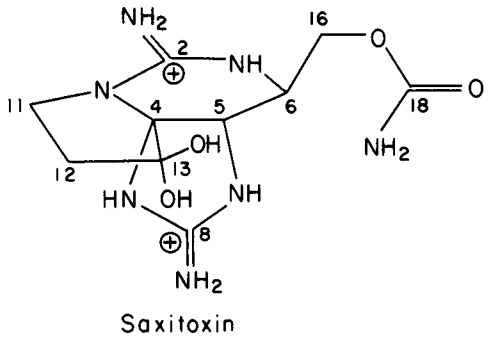

FIGURE 2 New molecular structure of saxitoxin showing numbering of carbon atoms (13). Orientation of molecule and folding of flexible carbamate —$CH_2OC(O)NH_2$ substituent drawn to correspond roughly with Fig. 3.

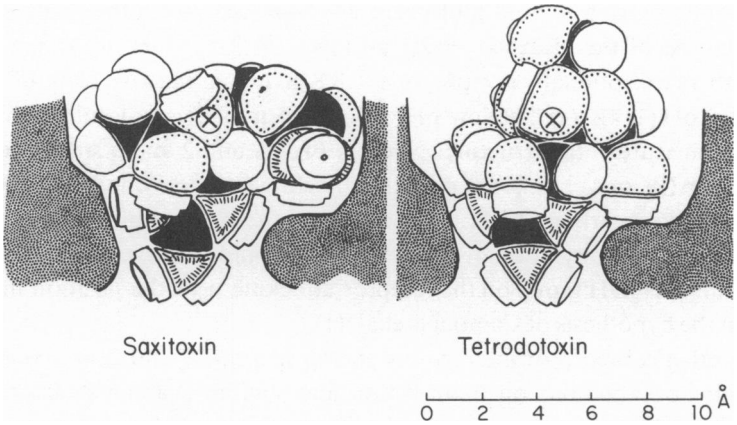

FIGURE 3 Saxitoxin and tetrodotoxin on their receptors. Drawn from CPK models with shading of toxin atoms same as in Fig. 1. The stippled areas represent the receptor in sagital section with narrow selectivity filter below. Most of the receptor is hydrogen bond accepting and there is a negative charge associated with the selectivity filter. A circled X has been drawn in the same position with respect to the receptor in the two cases. The X falls on an —OH group attached to an unusually electropositive carbon.

The hypothesis of Fig. 1 can now be extended on the basis of the new structure of STX (Fig. 2). The molecule is quite rigid except for a flexible $—CH_2OC(O)NH_2$ substituent. There are two guanidinium moieties, but only the one around C_8 protrudes sufficiently to be inserted into the postulated selectivity filter of the pore. When this is done, other groups of the toxin, including one —OH on C_{13} and the planar guanidinium group around C_2, rest against the pore, and both guanidinium charges come close to the negative charge of the pore. Fig. 3 is a drawing of the two hypothetical toxin complexes. The toxins were oriented to emphasize similarities, and then an outline of the hypothetical receptor was drawn to maximize contacts and hydrogen bonds to the toxins. To accommodate both toxins the antechambers of the pore must have a 9 by 10 Å cross section before narrowing to the 3+ by 5+ Å filter where the guanidinium group binds. Not all of the 9 by 10 Å area is needed by both toxins. Hence to explain known examples of selective toxin resistance (see references in 9) the geometry of this area could be adjusted to exclude either or both toxin molecules without altering the structure or selectivity of the selectivity filter. In a single pair of experiments with frog myelinated fibers at 12°C, I found the time constant for unblock from STX to be 41 s and that for TTX, 170 s. Block times were also shorter for STX. These faster rates with STX might be explained by the flexibility of the carbamate substituent on STX.

The new structure reveals that STX, like TTX, has an unusually electropositive carbon atom with several oxygen functions attached, in this case a hydrated ketone on C_{13}. When STX is chemically reduced one of the —OH groups on C_{13} is replaced by hydrogen and the biological activity is decreased to $<1\%$ (12). The importance of the structure around C_{13} is further suggested by the following properties common to both molecules: (*a*) The carbon atom lies in almost exactly the same position with respect to

the guanidinium group in both molecules, as can be seen from the X marks in Fig. 3 designating one of the attached —OH groups. (*b*) An —OH group on the carbon ionizes with an exceptionally low pK_a of 8.2–8.8 giving an inactive form of both molecules at high pH (1, 4). (*c*) The low pH cationic form of the molecules seems to be an equilibrium mixture of the structures given in Figs. 1 and 2 and a small proportion of carbonyl form (ketone or lactone) obtained by elimination of one of the oxygen functions on the special carbon (2, 7, 13). The rates of these reversible transformations are not known. The carbonyl form of both molecules could undergo a reversible addition reaction with an —OH group on the receptor attacking near the position marked X in Fig. 3 as in the hypothesis of Camougis et al. (1).

The hypothetical receptor interactions shown in Figs. 1 and 3 are consistent with many existing observations on toxin action and sodium channel structure, although some possible difficulties remain. The low potency of alkyl-, deoxy-, and anhydroderivatives of TTX involving —OH groups on C_4 and C_{10} (11) are readily explained through the loss of two of the five hydrogen bond donors in Fig. 1. However, the low activity of tetrodoaminotoxin (11) is not so easily explained since the replacement of the —OH at C_4 by an $—NH_2$ does not remove hydrogen bond donors. Conceivably the extra $—NH_2$ protonates at neutral pH and lowers the pK_a of the special —OH group on C_{10} which then dissociates to the inactive negative form. W. Ulbricht and H. H. Wagner (personal communication) have found that both the on and off rate constants for block by TTX are accelerated by lowering the pH to 5.3–6.0 as if the receptor interaction were an acid catalyzed reaction. Acid catalysis is expected if there is a nucleophilic attack by the receptor on a carbonyl group of the toxin.

Further testing of the hypothesis given here probably requires waiting for the synthesis of appropriate STX and TTX analogs and for more understanding of the equilibrium and rates of interconversion between ketone and hydrated ketone or lactone and hemilactal forms of the toxins.

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