Hypothesis

A hypothesis for the mechanism of sodium channel opening by batrachotoxin and related toxins

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The mechanism of action of one class of sodium channel opening agents (batrachotoxinin, veratridine, aconitine and grayanotoxin) is proposed to involve complexation of a triad of agent oxygen atoms with the ϵ-ammonium ion of a channel lysing side chain, holding open the mouth or exit of the ion channel. This idea complements the oxygen triad model derived by structural considerations (Masutani, T., Seyama, I., Narahashi, T. and Iwasa, J. (1981) J. Pharm. Exp. Therap. 217, 812) and extended by crystal structure comparisons (Codding, P.W. (1983) J. Am. Chem. Soc. 105, 3172). The mechanism is based on results for acetylcholine receptor ion channel gating, structure and function, using single group rotation (SGR) theory (cf. Kosower, E.M. (1983) Biochem. Biophys. Res. Commun. 111, 1022 and in press (1983); FEBS Lett. (1983) 155, 245; ibid. 157, 144; Biophys. J. (1983) 45, in press).

1. INTRODUCTION

Channel opening toxins can be divided into two classes: (A) alkaloids and terpenoids; (B) small polypeptides. Class A includes one of the most toxic small molecules known to man, the active principle of a South American frog exudate, batrachotoxin [1,2], as well as grayanotoxin I, aconitine (the toxic agent derived from the plant, monkshood) and veratridine. Class B contains the polypeptides derived from scorpion and sea anemone toxins. It is always of interest to search for possible common features in materials with similar biological actions.

Single group rotation (SGR) theory (an examination of the biological consequences of a minimal conformational change) led to a model for the binding of acetylcholine (ACh) to the acetylcholine receptor (AChR) and for the gating and structure of the AChR ion channel [3]. One of the important predictions of SGR theory applied to the AChR was that there would be a particular amino acid sequence for the ion-carrying portion of the ion channel: lys(1), glu(4), lys(8), glu(11)... along an α-helical segment of protein. After the complete amino acid sequences for the AChR were published [4–8], the predicted sequence was identified in the α-subunit of the AChR. The theory led to the selection of 7 ion channel elements among all of the subunits, and the construction of a structural model for the bilayer portion of the AChR, accounting for about 25% of the 2333 amino acids in the receptor and including a binding site, a gating mechanism and an ion channel [9–12]. SGR theory has been used to model a further 25% of the AChR [13]. It was proposed [3] that the motion of a lysine out of the channel should accompany activation ('gating') of the channel, and that similar features might be found in other channels, e.g., the sodium channel. It was thus logical to search for a mechanism by which channel opening molecules could assist in keeping lysine out of the ion channel.

2. THEORY AND DISCUSSION

We would like to propose that class A molecules open the channel by interacting with the ϵ-

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ammonium group of lysine at the entry or exit. A negative charge (glutamate or aspartate, the 'exo-channel anion') is expected to be present near the entry or exit to the channel as a binding site for the lysine ammonium ion. The proposal can be reformulated as a set of questions:

(i) Do toxin molecules contain groups capable of holding the ϵ-ammonium ion out of the ion channel?

(ii) Do the toxin molecules carry groups which might interact with the glu or asp 'exo-channel anion'?

To answer the first question, I turned to the finding in [14] and [15] that an ammonium ion is complex particularly well by a triad of oxygens in an appropriate geometric arrangement. I searched for and discovered this structural feature in the class A channel-opening toxins. For each type of class A molecule, crystal structures of closely related compounds show that a triad of oxygens is present. In addition, there are other groups in the toxin molecule (protonated nitrogen or hydroxyl) suitable for hydrogen-bonding to an anion.

The structure and oxygen triad for batrachotoxin (BTX) (1) are illustrated in fig.1 together with the crystal structure for a *p*-bromobenzoate [16]. It should be noted that BTX incorporates a 20α-2,4-dimethylpyrrole-3-carboxylate group but that the 20α-benzoate ester is just as potent physiologically as natural BTX [17]. A schematic model of how BTX or other oxygen triad toxins can favor open channels rather than closed channels is shown in fig.2. A preliminary account of our mechanistic analysis has been presented in [18]. Proximity of the combined BTX to the channel entry might inhibit approach of ions; a low conductance is found for the BTX-opened channel [19,20].

The oxygen triad idea was developed independently in [21] for grayanotoxin (GTX) derivatives. The crystal structure studies for GTX II [22] may be used as a guide to the relative positions of the oxygen-containing groups in GTX. The extensive set of derivatives studied in [21] allows us to accept their conclusion that the probable oxygen triad in GTX derivatives consists of the 3β, 5β and 6β OH groups.

The second question, concerning toxin groups which might interact with the exo-channel anion, can be answered in the affirmative. The 14 and 15

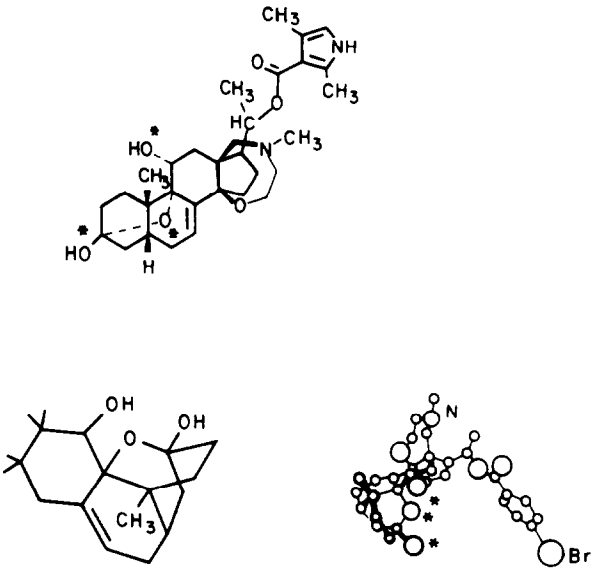


Fig.1. The structural formula for batrachotoxin 20α-2,4-dimethylpyrrole-3-carboxylate (BTX) (1) together with a crystal structure for batrachotoxin *p*-bromobenzoate. The triad oxygens are starred in both the molecular formula and the crystal structure. A partial structure of BTX at the lower left shows the triad oxygen clearly.

OH groups of GTX should be suitable for binding to a polar group, serving a function similar to that of the nitrogens in BTX and veratridine (V). The protonated nitrogens of BTX and V are excellent groups for binding to an exo-channel anion at the entry (or exit) to the channel.

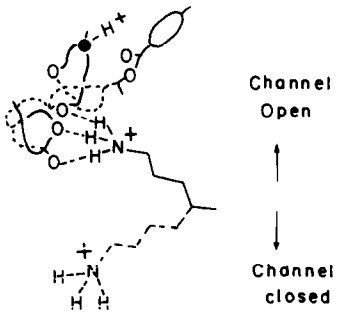
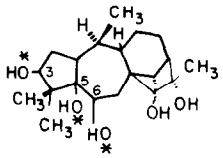


Fig.2. A schematic representation of the relationship between the channel ϵ-ammonium group of lysine and an oxygen triad toxin (in this case, BTX) associated with the protein near the mouth (or exit) of the channel.

The idea that an oxygen triad is important in the biological activity of class A toxins has been extended in [23,24], in which the crystal structures of V [23] and aconitine (A) [24] were determined. The positions of the nitrogens as well as the oxygen triads in BTX and V were matched and it was noted that the conformation of A may be changed on protonation as in the case of jesaconitine perchlorate [25,26]. The structural studies under way [23] on grayanotoxin derivatives should help to understand the structural features required for the activity of class A toxins. The formula for α-dihydroGTX II [2] and a possible structure for the ammonium ion complex are shown in fig.3. The structures of aconitine [3] and veratridine [4] are given in fig.4; the probable oxygen triad atoms are starred.

The oxygen–oxygen distances for the triads selected in [23,24] are shown in fig.5. Hydrogen bonding of an ammonium ion would presumably involve at least two of the oxygens in a triad. Given a range of N-H$^+$...O (hydrogen bond) distances between 2.50 and 3.00 A [15], a tetrahedral HNH angle of 109°28′ and straight hydrogen bonds, the O–O distances should be between 4.1 and 4.9 A. Only one of the distances in the oxygen triad is consistently within that range. The 'envelope of opportunity' (the volume within which the ϵ-ammonium ion may associate with the triad oxygens) must be differently shaped than that for the



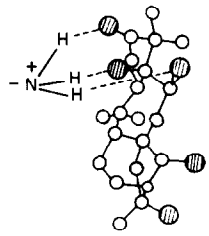
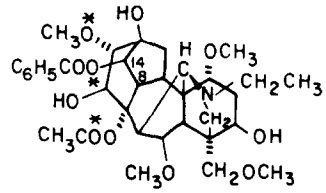


Fig.3. The formula for α-dihydroGTX II (2) along with a plausible structure for the ammonium complex of the toxin (ammonium ion–oxygen triad complex).



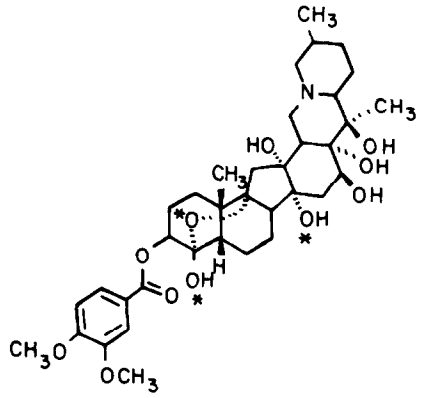


Fig.4. Structures of: (a) aconitine (3) and (b) veratridine (4) are shown with the oxygen triad atoms starred.

crown ethers (O–O distances of 4.82 A [15]) and would involve a bent hydrogen bond.

Strong support for the energetically favorable nature of ammonium ion complexation with a triad of oxygen atoms comes from the thermochemical studies of Meot-Ner [27] on gas-phase complexes of ammonium ions with ethers and 18-crown-6. The binding to the crown compound may be as much as 21 kcal/mol more than that to an ether reflecting the energetic advantage accrueing to multiple bonding. The implications for toxin action are profound and worthy of further ex-

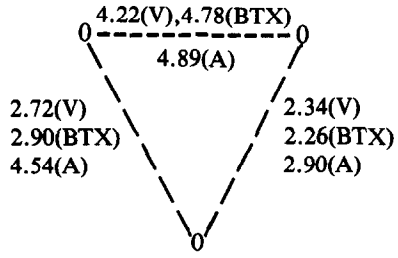


Fig.5. The oxygen–oxygen distances within oxygen triads of the toxins batrachotoxin (BTX), aconitine (A) and veratridine (V) [23].

ploration in view of the occurrence of oxygen triads in ionophores.

Class B toxins act at a site different from that of class A toxins as shown by synergy with the class A toxins, V [28] and BTX [29]. The lipid soluble class A toxins may act on the cytoplasmic side of the channel [30], whereas class B toxins probably act on the extracellular side of the channel.

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