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Classic toxin review

Alpha neurotoxins

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ABSTRACT

α-Neurotoxins have been isolated from hydrophid, elapid and, more recently, colubrid snake venoms. Also referred to as postsynaptic neurotoxins or 'curare mimetic' neurotoxins, they play an important role in the capture and/or killing of prey by binding to the nicotinic acetylcholine receptor on the skeletal muscle disrupting neurotransmission. They are also thought to cause respiratory paralysis in envenomed humans. This review will discuss the historical background into the discovery, isolation, structure and mechanism of action of the α-neurotoxins, including targets and cellular outcomes, and then will examine the potential uses of α-neurotoxins as pharmacological tools and/or as drug leads.

1. Introduction and nomenclature

Neurotoxins were first isolated from snake venom approximately 50 years ago. Chang and Lee (1963) utilised zone electrophoresis on starch to isolate α-bungarotoxin, β-bungarotoxin and γ-bungarotoxin from the venom of the many-banded krait (*Bungarus multicinctus*). These neurotoxins were subsequently examined for pharmacological activity in a range of *in vitro* preparations and in mice to determine lethality (i.e. LD_{50} values). Using the chick biventer cervicis nerve-muscle (CBCNM) preparation, Chang and Lee (1963) showed that α-bungarotoxin inhibited nerve-mediated (i.e. indirect) twitches and abolished contractile responses to acetylcholine, indicative of a postsynaptic mode of action. In contrast, β-bungarotoxin and γ-bungarotoxin showed presynaptic neurotoxicity. The discovery of α-bungarotoxin lead to the characterisation of the nicotinic acetylcholine receptor (Changeux et al., 1970), which is now one of the most well characterised ionotropic receptors (Nirthanan and Gwee, 2004).

α-Bungarotoxin, β-bungarotoxin and γ-bungarotoxin were originally named based on how they separated under electrophoresis conditions (Chang, 1999). However, the group of snake neurotoxins that have postsynaptic blocking actions at the skeletal muscle end-plate are now widely referred to as α-neurotoxins although the term curare-mimetic toxins has also been used. As with many toxin classes, the naming of α-neurotoxins has been inconsistent and confusing, and is often based on a combination of the word "toxin" and a derivative of either the genus or species of the snake from which the toxin was isolated (see Tables 1–3). However, a more "rational nomenclature system", such as that proposed by King et al. (2008), will hopefully be followed in the future. This system uses the prefix 'α' to indicate that the toxin has antagonist activity at nicotinic acetylcholine receptors. The family of the snake is then included i.e. elapitoxin for a toxin from the Elapidae family. Following this the genus and species is shown using single letters i.e. Aa for *Acanthophis antarcticus*. Finally a number system is used to indicate whether the isolated toxin is a short-chain, long-chain or κ-neurotoxin. The number '1' denotes that the toxin is a short-chain α-neurotoxin; while numbers '2' and '3' indicate that the toxin is either a long-chain α-neurotoxin or a κ-neurotoxin respectively (Blacklow et al., 2011; King et al., 2008). In addition any isoforms the exist of the toxin would then be denoted with letters (i.e. a,b,c) (King et al., 2008).

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2. Sources and isolation

α-Neurotoxins have been long known to be important constituents of elapid and hydrophid venoms but more recently have also been discovered in the venom of colubrid snakes previously identified as 'harmless' (Lumsden et al., 2005, 2007; Pawlak et al., 2006, Tables 1–3). Snake α-neurotoxins have been isolated and characterised using a range of techniques.

2.1. Chromatography

Typically a combination of chromatographic techniques (e.g. size-exclusion high performance liquid chromatography (HPLC) followed by reverse-phase HPLC), or a single process (e.g. reverse-phase HPLC) with multiple 'runs' have been used to isolate and purify α-neurotoxins from snake venoms. Given the close structural (i.e. molecular weight and amino acid composition) and, hence, chemical (i.e. hydrophobic/hydrophilic nature) composition of many α-neurotoxins, it is often quite easy to predict their elution time when using the same or similar HPLC protocol (i.e. solvents and gradient conditions). Typically, as shown by a number of studies by our laboratory, short-chain α-neurotoxins elute around 15–17 min under reverse phase HPLC conditions (Kornhauser et al., 2010; Kuruppu et al., 2005; Tan et al., 2006). Conversely ion-exchange chromatography in combination with gel permeation chromatography is more commonly used for the isolation of long-chain α-neurotoxins from snake venoms, although reverse-phase chromatography was used for the isolation of mikatoxin (Nirthanan et al., 2002).

2.2. In vitro assays

Confirmation of function following the isolation of neurotoxins is important, but often neglected, and can be established by the use of *in vitro* skeletal muscle preparations. The chick biventer cervicis nerve-muscle preparation, given the presence of both focally- and multiply-innervated muscle fibres (Hodgson and Wickramaratna, 2002), has proven to be an excellent screening tool to distinguish between pre- (β-neurotoxins) and postsynaptic (α-neurotoxins) neurotoxins (Harvey et al., 1994; Kornhauser et al., 2010; Kuruppu et al., 2005; Tan et al., 2006). Focally-innervated muscle fibres mediate the transient 'twitch' in response to electrical stimulation of the somatic nerve, whereas the multiply-innervated muscle fibres mediate the more prolonged contractions produced by exogenous nicotinic receptor agonists. The presence of different types of muscle fibres offers advantages over other skeletal muscle preparations such as the mouse or rat phrenic nerve hemidiaphragm (MHD/RHD) which only contain focally-innervated muscle fibres (Marshall, 1969). Both β-neurotoxins and α-neurotoxins will inhibit nerve-mediated twitches in the chick biventer nerve-muscle preparation. However, only α-neurotoxins will inhibit contractile responses to exogenous nicotinic receptor agonists (e.g. acetylcholine and/or carbachol). Therefore, the chick preparation represents a simple and effective method for the characterisation of snake venom neurotoxins.

The pharmacological activity of α-neurotoxins in skeletal muscle preparations can be examined and compared by obtaining t_{90} and pA_2 values. The former is defined as the time taken to produce 90% inhibition of nerve-mediated twitches (i.e. t_{90}). This can be used to confirm concentration-dependency of the inhibitory effects of α-neurotoxins and also enable comparison between inhibitory times for different α-neurotoxins when utilised at the same concentration. The pA_2 value provides a quantitative measure of antagonist potency of the α-neurotoxins at the skeletal muscle nicotinic receptor (i.e. −log M concentration of antagonist which causes a two-fold shift in the concentration–response curve). The pA_2 can be determined by obtaining contractile responses to a nicotinic receptor agonist (e.g. carbachol) in the presence and absence of increasing concentrations of the α-neurotoxin. Depending on the nature of the interaction between the neurotoxin and the receptor (i.e. reversible, pseudo-irreversible or irreversible binding) the data is then analysed by Schild analysis (Arunlakshana and Schild, 1959) or using a modified method (Lew and Angus, 1995; Christopoulos et al., 1999).

3. Structure

Most α-neurotoxins from elapid snake venoms are three-fingered toxins (Nirthanan and Gwee, 2004). Three-fingered toxins are flat molecules, having a small globular hydrophobic core from which three adjacent loops emerge i.e. 'three fingers'. The hydrophobic core contains the four disulphide bridges/bonds which are cross-linked and across the three loops there are five antiparallel β-strands that together create a large β-pleated sheet (Nirthanan et al., 2003; Servent and Ménez, 2002; Tsetlin, 1999).

3.1. Elapid α-neurotoxins

A large number of α-neurotoxins, both short- and long-chain, have been isolated from elapid snakes (Tables 1 and 2). Elapid α-neurotoxins are generally smaller in molecular weight to many other components in elapid venoms, and range between 6 and 9 kDa (see Tables 1 and 2). They can be classified according to their amino acid sequence into type I short-chain neurotoxins and type II long-chain neurotoxins (Endo and Tamiya, 1991).

3.1.1. Short chain α-neurotoxins

Short-chain postsynaptic neurotoxins consist of between 60 and 62 amino acids residues, with four disulphide bridges (Endo and Tamiya, 1991). Table 1 compares a number of short-chain α-neurotoxins isolated from elapid venoms. Their molecular weights range from 6 to 7 kDa and generally inhibit nerve-mediated twitch responses in the chick biventer preparation within 30 min at 1 μM with considerable variation in their degree of reversibility (Table 1).

The partial/full N-terminal amino acid sequence of a number of short-chain α-neurotoxins isolated from elapid venoms has been determined (Table 4). Typically short-chain neurotoxins isolated from elapid snakes have very similar N-terminal sequences i.e. variations of MTCYNQQSSE, with key cysteine (C) residues at positions 3 and/or 4. This structure is