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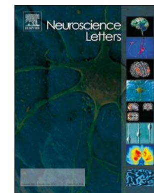

Review article

Toxins as tools: Fingerprinting neuronal pharmacology

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Toxins have been used as tools for decades to study the structure and function of neuronal ion channels and receptors. The biological origin of these toxins varies from single cell organisms, including bacteria and algae, to complex multicellular organisms, including a wide variety of plants and venomous animals. Toxins are a structurally and functionally diverse group of compounds that often modulate neuronal function by interacting with an ion channel or receptor. Many of these toxins display high affinity and exquisite selectivity, making them valuable tools to probe the structure and function of neuronal ion channels and receptors. This review article provides an overview of the experimental techniques used to assess the effects that toxins have on neuronal function, as well as discussion on toxins that have been used as tools, with a focus on toxins that target voltage-gated and ligand-gated ion channels.

1. Introduction

In this review, we use the term 'toxin' broadly to include any small molecule or peptide of biological origin that has activity on neuronal ion channels or receptors, regardless of the route of exposure. This includes 'poisons', which are toxins that are ingested or absorbed, and 'venoms', which are toxins that are injected. It should be noted that all compounds can be considered toxic, and that it is the dose that determines toxicity, a concept first devised by physician Paracelsus in the 16th century. Therefore, neurotoxins are generally distinguished by their ability to bind to a neuronal ion channel or receptor with relatively high affinity (in the nanomolar to micromolar range).

The biological origins of toxins discussed in this review are diverse, ranging from single cell organisms, such as bacteria and algae, to multicellular organisms, which includes plants and animals. These toxins may be produced to deter predators, such as capsaicin produced by chilli peppers, or used to capture prey, as is the case for most venom-producing animals such as spiders, snakes, scorpions and cone snails. It is therefore not surprising that toxins that target the nervous system have independently evolved many times over in numerous plant and animal species. They modulate neuronal functions in many ways, generally leading to the disruption of normal electrical activity or chemical messenger signalling. Many of these toxins display high affinity and selectivity for a particular molecular target, making them useful pharmacological tools to probe neuronal ion channel and receptor function. However, it should be noted that even toxins lacking exquisite selectivity are still useful pharmacological tools, with numerous examples of toxins in the literature used to discover novel ion channel subtypes, improve our understanding of ion channel structure-function relationships, and identify novel allosteric binding sites, all of which provide essential insights for the rational design of more selective probes in the future. In addition, toxins can be used to identify novel therapeutic targets, with the most notable example being the ω-conotoxins establishing $Ca_V2.2$ as a novel therapeutic target for the treatment of neuropathic pain.

The aim of this review is to provide an overview of the techniques commonly used to assess the effects that toxins have on neuronal function (Fig. 1), to provide notable examples of toxins that have been used in the literature as tools to fingerprint neuronal pharmacology, and to discuss the limitations of using toxins as tools.

2. Techniques for assessing neuronal function

2.1. Patch-clamp electrophysiology

One of the most common experimental techniques for assessing neuronal function is electrophysiology. In these experiments the electrical properties of neurons, associated with the flow of ions, are probed using micropipettes [125]. The principles of electrophysiology are used to study both intracellular and extracellular properties of cells. Furthermore, emerging technology such as planar patch clamp and multi-electrode array platforms continue to enhance our understanding of neuronal function in a high-throughput manner [130,165]. In practice, neurons isolated in culture, in *ex vivo* slices (eg. brain, spinal cord) or *in*

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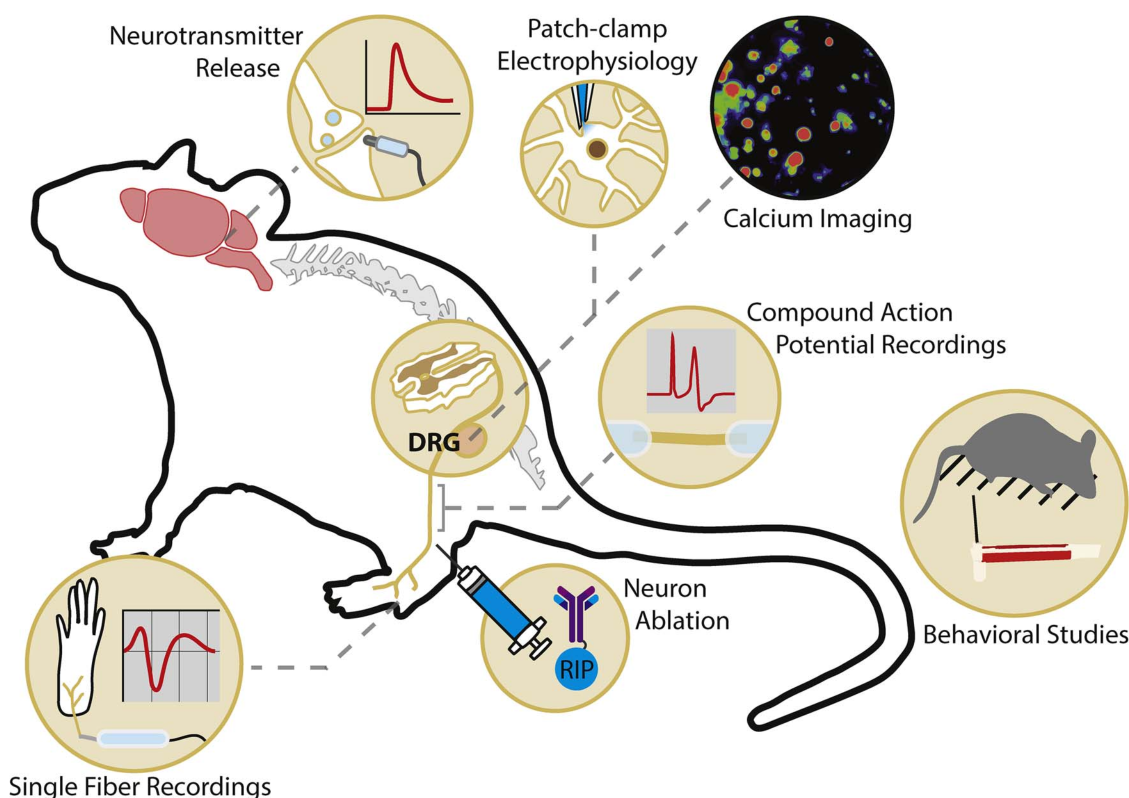

Fig. 1. Overview of the experimental techniques commonly used to assess toxin effects on neuronal function.

vivo are probed with a small pipette filled with a solution similar to the intracellular contents [55,188]. In general, for whole-cell patch clamping after generation of a “gigaseal” between the cell membrane and the patch pipette, the cell membrane is breached allowing access between the intracellular electrolyte and the recording electrode. Resulting information is then compared to a reference electrode in the extracellular solution. In this configuration, neurons can be “clamped” for voltage or current, measuring the trans-membrane ionic current or membrane voltage, respectively. Many variations on this protocol have been devised including perforated patch, inside-out, and outside-in, however the principles of micropipette, reference and recording electrode set-up remain largely the same [81]. The use of different intracellular and extracellular solution combinations allows the experimenter to isolate particular membrane-spanning channels, and the use of temperature control devices whilst recording increases the scope of electrophysiology, contributing to the discovery of temperature sensitive channels [69]. Electrophysiology has and continues to be the gold standard for assessing the pharmacological effects of toxins on ion channels [137,142]. Toxins are perfused or added to the intracellular or extracellular recording solutions and the subsequent effect on ion channel biophysical properties are studied.

2.2. Calcium imaging

Calcium imaging remains a robust tool for assessing neuronal function. The influx of calcium via a number of different membrane bound channels tightly controls many intracellular processes [124]. Calcium imaging requires calcium indicators, including Fura-2 and Fluo-4, which exhibit changes in fluorescence upon the binding of calcium ions [174]. Neurons, *in vitro*, *ex vivo* or *in vivo*, with an indicator present are excited at appropriate wavelengths and captured by fluorescence microscopy using a charge coupled device (CCD) camera in real time [76]. Using this technique, toxin molecules can be perfused directly onto neurons to assess effects on Ca^{2+} permeability. Toxins may act directly on Ca^{2+} permeable channels or cause perturbations in intracellular Ca^{2+} levels via second messenger systems. Crucially, calcium imaging gives the user the ability to visualise more than one neuron at a time, and thus neuronal subpopulations can be identified based on functional response. Indeed toxins have been used in this manner to assess subpopulation-specific responses in a high-content approach known as “constellation pharmacology” [169]. This technique uses the selective nature of toxins to identify constellations of receptor and channel types functionally expressed on single or populations of neurons [43].

2.3. Single-fiber recordings

The rodent saphenous skin-nerve preparation is a valuable technique used to study responses of individual primary sensory neurons to mechanical, thermal and/or chemical stimuli [50,148]. In this method, the saphenous nerve along with the skin of the dorsal hind paw and lower leg is removed and placed in an organ bath chamber. The proximal end of the saphenous nerve is placed in a separate recording chamber and immersed in paraffin oil. To obtain a single-fiber recording, the nerve is desheathed and teased apart until mechanical probing identifies a single receptive field on the skin. The fiber is then classified based on conduction velocity (C-fiber < 1 m/s, A-fiber 1.6–12 m/s), mechanical von Frey threshold (low threshold 1–5.7 mN, high threshold 5.7-128 mN), and responsiveness to cold and heat [195]. The technique can be used to study the role of specific ion channels or receptors on neuronal excitability using transgenic mice or selective compounds, including toxins. To test the effect of a toxin, the receptive field on the skin is isolated with a ring to allow continual perfusion of the toxin to the peripheral nerve terminal. Spontaneous activity and responses to mechanical, cold and/or heat stimuli can then be compared before and after the addition of a toxin. A similar methodology is employed in transgenic mice, however due to the heterogeneity of primary sensory neurons; a much larger sample size is needed.

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