Block of voltage- and ligand-gated ion channels by toxins using automated patch clamp – a novel tool to detect TTX in food samples?

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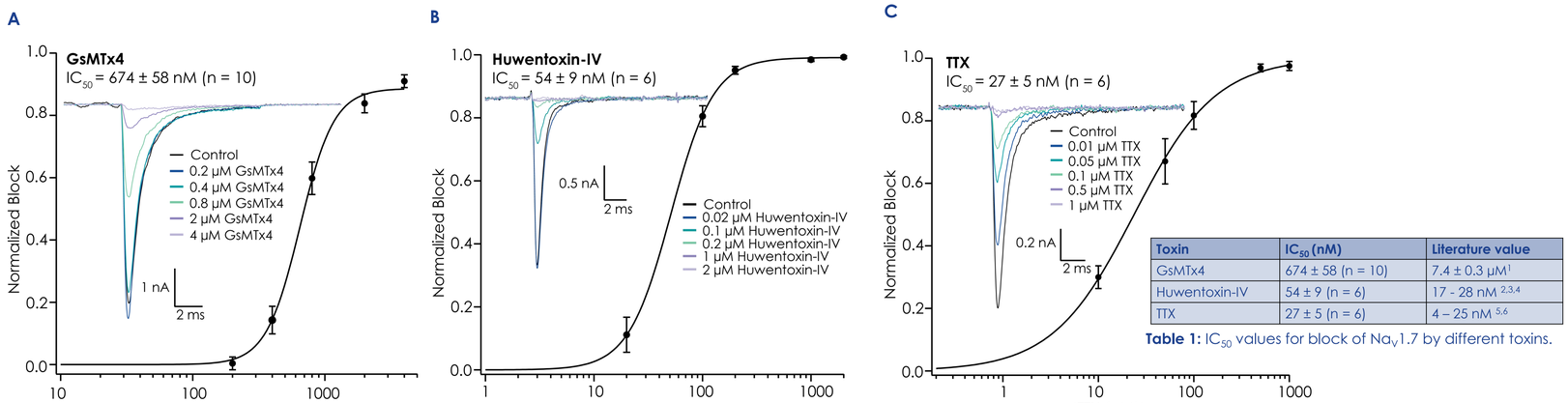
1 Introduction

Toxins from the natural world have been shown to block ion channels, and can be highly specific for particular subtypes. On the other hand, some toxins can be rather promiscuous and modulate the activity of several different ion channel families. We have used a medium throughput automated patch clamp (APC) system (Patchliner) to investigate the actions of different toxins including GsMTx4 and huwentoxin-IV on $Na_V1.7$ and α-conotoxin on nicotinic receptors. What is more, tetrodotoxin (TTX), which is present in some pufferfish species and shellfish, blocks Na_V channels with nM potency. TTX is responsible for thousands of intoxications each year in Japan and China, and there are 13 species of pufferfish found in the Mediterranean Sea. Traditional methods for detecting TTX in food can be low in specificity, high in cost and require trained personnel. We have used Neuro-2a cells endogenously expressing Na_V on the Patchliner to detect TTX in fish samples and conclude that this may be an applicable technique to test samples for contamination with deadly toxins targeting Na_V channels.

2 Block of $Na_V1.7$ by GsMTx4, Huwentoxin-IV and TTX

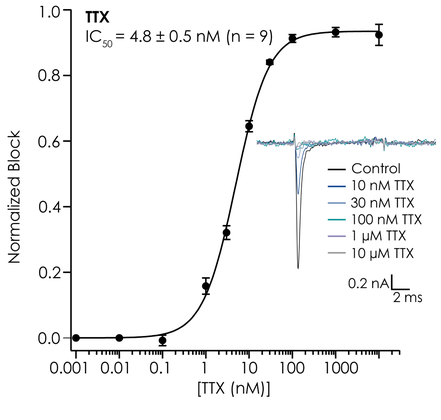


Toxin	IC_{50} (nM)	Literature value
GsMTx4	674 ± 58 (n = 10)	7.4 ± 0.3 µM[1]
Huwentoxin-IV	54 ± 9 (n = 6)	17 - 28 nM [2,3,4]
TTX	27 ± 5 (n = 6)	4 – 25 nM [5,6]

Table 1: IC_{50} values for block of $Na_V1.7$ by different toxins.

Figure 1: Block of $Na_V1.7$ expressed in CHO cells by various toxins. A. Inhibition of $Na_V1.7$ by increasing concentrations of the spider toxin GsMTx-4. Shown is the concentration response curve for an average of 10 cells with traces from an example cell shown in the inset. GsMTx4 has been previously shown to block stretch-activated channels[7], including TRPC1[8], TRPC6[9] and mechano-activated channels such as Piezo1[10]. **B.** Inhibition of $Na_V1.7$ by increasing concentrations of the spider toxin huwentoxin-IV. Shown is the concentration response curve for an average of 6 cells with traces from an example cell shown in the inset. **C.** Inhibition of $Na_V1.7$ by increasing concentrations of the pufferfish toxin TTX. Shown is the concentration response curve for an average of 6 cells with the traces from an example cell shown in the inset. The IC_{50} values are shown in **Table 1** and were all in good agreement with values found in the literature[1-6] although we found GsMTx4 to be more potent on $Na_V1.7$ than previously reported[1]. Toxins kindly provided by Alomone Labs.

3 Block of Na_V in Neuro-2a cells by fish samples



Sample	TTX equivalent concentrations (mg TTX/kg tissue)
Muscle	1.59
Skin	3.32
Liver	13.8
Gonads	22.46

Table 2: Levels of TTX equivalent detected from different tissue extracts of the *L. sceleratus* pufferfish.

Figure 2: Block of Na_V expressed in Neuro-2a cells by TTX and TTX equivalent. A. Example concentration response curve for TTX blocking Na_V expressed in Neuro-2a cells. TTX blocked Na_V in Neuro-2a cells with an IC_{50} of 4.8 ± 0.5 nM (n = 9). The system (Neuro-2a cells on the Patchliner) was then used to quantify the TTX contents of several tissues of a *L. sceleratus* pufferfish specimen (**Table 2**). The system revealed the presence of TTX equivalent in all extracts with levels above the Japanese regulatory limit of 2 mg TTX equivalent/kg tissue, except for muscle. Gonads was the most toxic tissue followed by liver and skin. Data from ref. 11.

4 Block of nAChR α1β1γδ by α-conotoxin MI

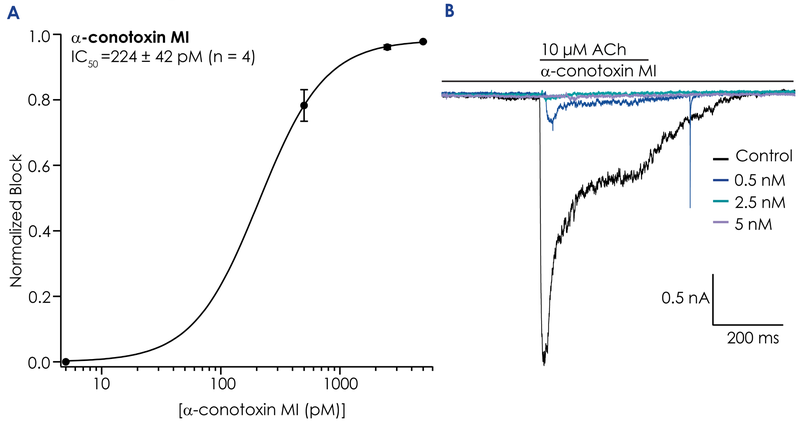


Figure 3: Block of nAChR α1β1γδ expressed in TE671 cells by α-conotoxin MI. A. Concentration response curve for block of nAChR by α-conotoxin. α-conotoxin blocked nAChR α1 responses with extremely high potency, with IC_{50} = 224 ± 42 pM (n = 4) in good agreement with the literature[12]. α-conotoxin was preincubated for 300 s before co-application with 10 µM ACh. When α-conotoxin was not pre-incubated the IC_{50} was 10X higher (2.5 ± 2.5 nM, n = 3; data not shown). **B.** Corresponding traces from an example well are shown in response to 10 µM ACh alone (control) and following pre-incubation in increasing concentrations of α-conotoxin. Toxin was kindly provided by Alomone Labs.

5 Conclusions

- $Na_V1.7$ was blocked by various toxins using automated patch clamp with IC_{50} values comparable with previous reports.
- Na_V currents recorded from Neuro-2a cells were blocked by nM concentrations of TTX.
- TTX equivalent toxins could be detected in tissue samples from fish and could be a useful tool for testing pufferfish samples present in Europe.
- AChR α1 was activated by ACh and blocked by α-conotoxin MI.

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