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Research paper

Effects of Deltamethrin on crayfish motor axon activity and neuromuscular transmission

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HIGHLIGHTS

- Effects of Deltamethrin (DM) on neuromuscular junction are studied in crayfish.
- DM leads to depolarization block by a ~20 mV step depolarization in some motor axons.
- DM shifts action potential (AP) initiation site in some axons.
- The shift could explain a reduction in synaptic delay observed in some synapses.
- The depolarization block occurs before AP duration or frequency is changed.

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ABSTRACT

Deltamethrin (DM) is a widely used pesticide known to target sodium channels. Although this compound has been studied extensively at molecular and behavioral levels, the detailed action of DM on cellular and synaptic function is less well documented. In this report, we show that DM at nanomolar concentrations can silence tonic motor output of the crayfish ventral superficial flexor (VSF) within ~10 min. Action potential (AP) amplitude was consistently reduced before silencing occurred, whereas AP duration and AP firing frequency did not change. In some synapses EPSP amplitude and synaptic delay were modified by DM, but the direction of change was not consistent. In order to better understand these diverse effects, intracellular recordings from motor axons of the crayfish opener were used for a detailed analysis. DM caused an initial, slow depolarization of resting membrane potential (V_m), which was accompanied by reduced AP amplitude but not AP duration. Resting V_m then underwent a step depolarization of ~20 mV, which we propose corresponds to the onset of the depolarization block. In addition, DM shifted the AP initiation site in some opener axons during prolonged firing. This shift occurred concomitantly with a reduction in synaptic delay. A similar reduction in synaptic delay was also detected at some VSF axons, and can be attributed to the same mechanism. Results reported here suggest that DM at low concentrations result in: (i) depolarization block of motor axons before changes in network output can be detected, (ii) variable effects on synaptic transmission, with this variability presumably due to the diverse morphology and excitability of motor axons.

1. Introduction

Pyrethroids are a family of synthetic pesticides widely used in agricultural and domestic settings. The clinical and environmental impacts of this family of compounds have been studied in detail [14,19,26]. Pyrethroids are considered safe because the dosages that induce detectable symptoms in mammals are about two thousand times higher than those capable of disabling insects [13,20]. Studies of these compounds at a molecular level suggest that they have a specific binding site on sodium channels and can effectively slow sodium channel inactivation and deactivation [4]. Despite the accumulation of extensive data, pyrethroid investigation remains an ongoing process because: (1) resistance to pyrethroids has emerged in some target populations [22] and (2) new findings have suggested a possible link between these compounds and autism in humans and development of the dopaminergic system in vertebrates [17,18].

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Deltamethrin (DM) is one of the commonly used type II pyrethroids, which differ from type I pyrethroids in molecular structure and in the symptoms they induce in rodents [2]. DM and other type II pyrethroids have been shown to create a persistent sodium current at resting V_m in crayfish giant axons [15]. Depolarization caused by the sodium current eventually leads to depolarization block of AP generation. While the use of giant axons has provided insights to the mechanisms of pyrethroid action, these axons are unlike typical neurons, which have complex morphology and non-uniform distribution of channel densities among subcellular compartments. The effects of pyrethroids on neuronal network, AP initiation in whole neurons and synaptic transmission have not been investigated in detail.

In this report, the crayfish ventral superficial flexor (VSF) and opener neuromuscular junction (NMJ) preparations are used to investigate the effects of pyrethroids at both network and cellular levels. The crayfish VSF system is composed of the ventral branch of third motor nerve, which contains six motor axons, and the muscle fibers of the VSF. The motor axons release glutamate or GABA. It has been suggested that the function of this nerve muscle system is to control posture and terrestrial walking [24,25]. The VSF is robust and can remain active for hours after the abdominal section of the ventral nerve cord is separated from the rostral ganglia and after sensory inputs are severed [24,25]. This autonomous activity is assumed to be driven by a central pattern generator network. Thus, this preparation can be used to examine the impact of pyrethroids on network activity that generates motor output by monitoring motor axon firing frequency. Furthermore, action potentials recorded with suction electrodes can inform us of any changes in action potential amplitude, duration and conduction velocity [6]. Finally, the ability to measure synaptic responses evoked by motor axons can inform us of the potential impact of pyrethroids on synaptic transmission.

The VSF system is versatile for examining multiple aspects of pyrethroid function, and can be deployed in undergraduate teaching laboratories for group efforts toward data collection. However, the VSF preparation cannot provide details of subcellular events. For the latter purpose, presynaptic axons of the crayfish opener NMJ can be used. The axons in the opener preparation are accessible to two electrode current clamp at multiple locations [9,10], thus allowing for the examination of potential subcellular events induced by DM. Previous studies have shown that proximal and distal branches of the axons exhibit different sensitivity to Na^+ and K^+ channel blockers, suggesting differential distribution in the density of these two channels [9,10]. Thus, axons in the opener offer an opportunity to examine the intracellular consequences of pyrethroid poisoning on branching axons with non-uniform channel density.

Although ion channels in the crayfish are not well characterized at a molecular level, crayfish sodium channels exhibit similar kinetic and pharmacological properties to those of insects. Several important studies of pyrethroid action on sodium channels have been performed in the medial giant axons of the crayfish [15,16]. Conclusions from these studies were consistent with later studies using insects [22,23]. Thus, the crayfish VSF preparations could serve as a model system to investigate the action of pyrethroids on insects. This report examines the cellular effects of deltamethrin on crayfish VSF and opener NMJ systems.

2. Methods

2.1. Dissection and preparation

Crayfish, Procambarus clarkii, with a head-to-tail length of ~5 cm, were used in all experiments. Animals of both sexes were immobilized on ice and then decapitated by cutting behind the eye stocks. The abdominal section (tail) of the animal was then separated from the thorax using scissors. The tail was pinned dorsal side up in a 100 × 25 mm petri dish. The ventral nerve cord, the ventral branch of the third motor nerve and the VSF muscle fibers were exposed after removing the deep flexor muscles. In experiments performed by students in the teaching laboratory, the tails were pinned ventral side up and the third nerve and VSF muscle fibers were accessed by removing the cuticle between the sternites [1]. Dissection and recording were performed at room temperature. Opener neuromuscular junction dissection and recording have been detailed before [10]. The opener of the first walking leg was taken from animals that were later used for VSF experiments.

Crayfish saline contained (in mM): 195 NaCl, 5.4 KCl, 13.5 $CaCl_2$, 2.6 $MgCl_2$, and 10HEPES (pH 7.4). The saline was circulated by a peristaltic pump with a total system volume of 50 ml. The system included recording dish, tubing and a reservoir. Deltamethrin (Sigma-Aldrich D9315) was dissolved in DMSO (10 mM) and serially diluted before being added to the reservoir of the perfusion system and vigorously mixed with a transfer pipette. The final concentration of DMSO was typically less than 0.1% and had been checked to be inert. To ensure that DM was completely removed after each experiment, at the end of each recording session the entire system was flushed with distilled water followed by 100 ml of 95% ethanol.

2.2. Electrophysiology

Intracellular electrodes filled with 3 M KCl (~5 MΩ in resistance) were used for recording from muscle fibers. Sharp electrodes used in the opener axons were filled with 1 M Kmethansulphonate

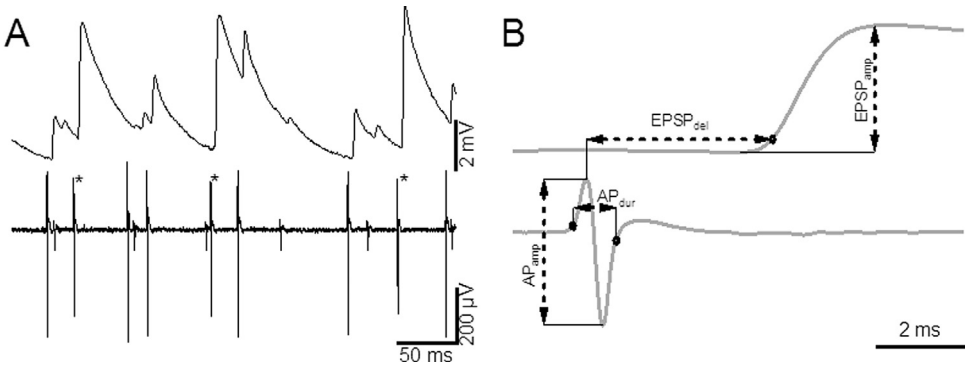

Fig. 1. Simultaneous recordings from the third motor nerve and a VSF muscle fiber. (A) Suction electrode recording from the third nerve (lower) shows APs with varying amplitudes arising from different axons. One of the axons (*) consistently evoked large EPSPs (upper). (B) Averaged AP and EPSP (n = 120), from the axon marked by * in A. The main parameters analyzed in this report are defined graphically. Traces in B share the vertical scales shown in A.