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Pesticide Biochemistry and Physiology 82 (2005) 1–15
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Biochemistry & Physiology
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Action of deltamethrin on N-type ($Ca_v2.2$) voltage-sensitive calcium channels in rat brain ☆

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Received 8 June 2004; accepted 9 November 2004
Available online 17 February 2005

Abstract

Isolated presynaptic nerve terminals prepared from whole rat brain were used to evaluate the action of deltamethrin on voltage-sensitive calcium channels by measuring calcium influx and endogenous glutamate release. Deltamethrin-enhanced K^+-stimulated calcium influx and subsequent Ca^{2+}-dependent glutamate release. The effect of deltamethrin was concentration-dependent, stereospecific, blocked by ω-conotoxin MVIIC but unaltered in the presence of tetrodotoxin. These results suggest that N-type voltage-sensitive calcium channels are a site of action at the presynaptic nerve terminal. Electrophysiological studies were carried out using rat brain $Ca_v2.2$ and β_3 subunits coexpressed in *Xenopus* oocytes to validate such action. Deltamethrin reduced barium peak current in a concentraion-dependent and stereospecific manner, increased the rate of activation, and prolonged the inactivation rate of this channel. These experiments support the conclusion that N-type voltage-sensitive calcium channel operation is altered by deltamethrin.

Keywords: Calcium channel; Deltamethrin; Electrophysiology; Neurotransmission; Presynaptic nerve terminal; Pyrethroids; Rat brain; Synaptosomes; *Xenopus laevis*

1. Introduction

Published studies originally designed to assess the acute neurotoxicity of pyrethroids in mammals following intravenous administration are incomplete in regards to the battery of currently registered pyrethroids [1]. It is well established that voltage-sensitive sodium channel isoforms are modified by pyrethroids. There is evidence,

☆ This work was supported by the Pyrethroid Working Group, a conglomerate of the major pyrethroid manufactures in the United States and is comprised of Aventis CropScience, Bayer Corp., DuPont Agricultural Products, FMC Corp., Valent USA Corp., and Syngenta.
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doi:10.1016/j.pestbp.2004.11.006

however, that suggests that other target sites, (voltage-sensitive calcium and chloride channels) may be involved with the development of the acute neurotoxic response [1]. Future mechanistic studies on each of these target sites are critical for the rational regulation of these insecticides under the Food Quality Protection Act criteria.

Pyrethroids are a class of synthetic insecticides derived from the naturally occurring botanical insecticide, pyrethrum. They are remarkably effective because they disrupt the insect nervous system at concentrations that result in little or no mammalian toxicity [2]. Because their toxicity depends on the three-dimensional configuration of the entire molecule, there is no common toxophore and their activities are highly stereospecific [1]. General symptoms of pyrethroid poisoning are neurotoxic and include hyperexcitation, convulsions, seizures, and paralysis [1]. Two main classes of pyrethroids have been characterized based on their observed symptomology in mammals. In general, tremor (T)-syndrome pyrethroids do not possess an α-cyano grouping in their alcohol moiety and choreoathetosis-salivation (CS)-syndrome pyrethroids generally do. CS-syndrome pyrethroids also elicit enhanced neurotransmitter release and increased cardiac contractions, both calcium (Ca^{2+})-dependent processes [3].

Acute in vivo toxicity studies indicated that the action of CS-syndrome pyrethroids on the nervous system was different than that of the T-syndrome pyrethroids. Deltamethrin (CS-syndrome pyrethroid) caused a 52% decrease in acetylcholine content of the cerebellum, where as DDT and cismethrin, a T-syndrome pyrethroid, caused no significant reduction [4]. CS-syndrome pyrethroids enhanced norepinephrine release from depolarized presynaptic nerve terminals isolated from rat brain while T-syndrome pyrethroids were much less potent and efficacious in evoking release [2]. Deltamethrin also has been reported to be a potent agonist of Ca^{2+}-dependent neurotransmitter release from presynaptic nerve terminals isolated from both vertebrate and invertebrate organisms [2,5–8]. Deltamethrin-enhanced neurotransmitter release was found to be stereospecific and highly dependent on external Ca^{2+} concentration [5]. In the presence of tetrodotoxin (TTX),[1] neurotransmitter release still was enhanced by deltamethrin but was blocked by the chlorinated phenylethylamine calcium channel blocker, D595 [9].

CS-syndrome pyrethroids were found to agonize the T-type ciliary calcium channels of *Paramecium tetraurelia*, a ciliate organism that does not possess a voltage-sensitive sodium channel, in a concentration-dependent and stereospecfic manner. Deltamethrin, at concentrations as low as 10 nM, resulted in increased Ca^{2+} influx, backward swimming (a Ca^{2+}-dependent avoidance behavior) and death by osmotic lysis [10]. Duce et al. [11] identified L- and T-type voltage-sensitive calcium channels by electrophysiological measurements in isolated housefly neuronal stoma. Only the T-type channel was affected by deltamethrin and resulted in a prolongation of the Ca^{2+} action potential due to a slowing of the inactivation kinetics and a shift in the I/V curve to more depolarizing voltages.

Pyrethroids have been shown to block distinct classes of voltage-sensitive calcium channels in a variety of non-neuronal mammalian systems. The T-syndrome pyrethroid, tetramethrin, blocks the low-voltage activated (T-type), calcium channel in mouse neuroblastoma cells (N1E-115) and rabbit sino-atrial cells, while producing little to no effect on high-voltage activated (L-type) voltage-sensitive calcium channels [12,13]. More recent electrophysiological studies using $Ca_v3.1$ (T-type), $Ca_v1.2$ (L-type), and $Ca_v2.1$ (P/Q-type) expressed in non-neuronal HEK cells indicated that all three channels were modified [14]. Bioallethrin, a T-syndrome pyrethroid, blocked all three calcium channels by causing a hyperpolarizing shift in the voltage-dependent inactivation and acceleration in the inactivation kinetics under steady-state depolarization [14]. The contribution of these effects is consistent with the channel block observed. Collectively, the above results strongly indicate that voltage-sensitive calcium channels are modi-

[1] *Abbreviations used:* BCA, bicinchoninic acid; BSA, bovine serum albumin; $[Ca^{2+}]_i$, internal free calcium concentrations; DMSO, dimethyl sulfoxide; EGTA, ethyleneglycol-bis-(β-aminoethylether) *N*,*N*,*N*,*N*′-tetraacetic acid; Hepes, *N*-[2-hydroxyethyl]piperazine-*N*′[2-ethanesulfonic acid]; R_{min}, minimal $[Ca^{2+}]_i$; R_{max}, maximal $[Ca^{2+}]_i$; TTX, tetrodotoxin.