Cholinesterase inhibition in the bulb mite
*Rhizoglyphus echinopus* (Acari: Acaridae) in relation to the acaricidal action of
organophosphates and carbamates

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ABSTRACT


The 5000-g supernatant fraction of whole-bulb-mite homogenates was shown to possess a cholinesterase (ChE) that hydrolyzed acylcholine esters in the order acetyl > propionyl > butyryl. Acetyl-β-methylcholine, but not benzoylcholine, also was hydrolyzed as were acetyltiocholine and acetyl-β-methylthiocholine. No inhibition by excess substrate was observed at cholinester concentrations as high as 30mM. Cholinesterase activity was markedly insensitive to eserine and to certain other carbamates and organophosphates. Only organophosphates of the dimethylphosphate type generally were active ChE inhibitors. It was concluded that the inability of carbamates such as eserine, and organophosphates such as those with alkyl groups larger than dimethyl, to inhibit the bulb-mite ChE was probably a consequence of the nature of the esteratic site. The data suggested that ChE inhibition was likely involved in the toxicity to bulb mites of some of the toxic carbamates and organophosphates, but that it might not be the only mechanism involved, at least with several of the compounds.

INTRODUCTION

In a recent study of the toxicity to bulb mites (*Rhizoglyphus echinopus* (Fumouze and Robin)) of acaricides from many different chemical classes, only some organophosphates (OP's) and carbamates possessed 72-h *LC₅₀* values of less than 1000 ppm (Knowles et al., 1988). The most toxic OP and carbamate were dimethoate and formetanate, with *LC₅₀* values of only 344...
and 408 ppm, respectively. Thus, bulb mites were not especially susceptible even to members of these two classes of acaricide. Because OP's and carbamates are thought to exert their toxic action by inhibiting cholinesterase (ChE) (O’Brien, 1967) and because a modified or insensitive ChE has been shown to be a major factor in the differential toxicity of some OP and carbamate acaricides to resistant and susceptible mites (Smissaert, 1964; Voss and Matsumura, 1964; Ballantyne and Harrison, 1967; Zahavi and Tahori, 1970; Blank and Osborne, 1979; Van de Baan et al., 1985; Anber and Overmeer, 1988), it was of interest to examine the properties of bulb mite ChE and to study the effects of selected OP’s and carbamates on ChE activity.

MATERIALS AND METHODS

Mites

Bulb mites were reared at high humidity in the dark at 26 ± 1°C in petri dishes containing a wheat-germ-based medium designed for acarid mites (Bot and Meyer, 1967).

ChE assay and inhibition

Bulb mites were homogenized in 0.134M phosphate buffer (pH 7.2), and the homogenate was centrifuged at 5000 g for 10 min at 4°C. The supernatant was used in these studies. The 0.3-ml incubation mixture consisted of enzyme preparation (0.25 ml), substrate (0.03 ml), and buffer (0.02 ml). The concentrations of enzyme preparation and substrate during the incubation were 100 mg ml⁻¹ (wet weight equivalent) and 10mM, respectively, except in those experiments where the effects of homogenate and substrate concentrations were studied. The mixture was incubated for 60 min in a water bath at 37.5°C, and the residual cholinester was determined at 540 nm by the colorimetric method of Hestrin (1949) as described by Herne and Brown (1969). Substrates evaluated with this method included acetylcholine iodide (ACh), propionylcholine iodide (PrCh), butyrylcholine iodide (BuCh), acetyl-β-methylcholine bromide (MeCh), and benzoylcholine chloride (BzCh). Standard curves for substrates were prepared in presence of boiled homogenate. In activity/substrate-concentration relationship studies, it was necessary to dilute the mixture containing the highest substrate concentration (30mM) 3-fold with 0.074M ferric chloride solution prior to reading the absorbance.

In some experiments, the method of Ellman et al. (1961) was used to assay bulb-mite ChE activity. The 1.5-ml incubation mixture consisted of enzyme

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1All supplied by Sigma, St. Louis, MO.