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Cholinesterases in spider mites susceptible and resistant to organophosphates

In a previous paper (SMISSAERT, 1964) evidence has been provided that the mechanism of resistance to organophosphates (OP) in the strain of spider mites (*Tetranychus urticae*) studied, is a decreased sensitivity of the site of action of OP-poisoning viz. the cholinesterase (ChE). The difference in inhibition rate of the ChE in the susceptible (S) and resistant (R) strain is caused by a single gene. Furthermore the rate of acetylcholine (ACh) hydrolysis by the ChE of the R-strain appeared to be considerably lower than that of the S-strain. These results have been essentially confirmed by Voss and MATSUMURA (1964-1965).

Our recent work concentrates on the question whether Ach hidrolysis is inhibited by excess of substrate, a phenomenon well established for nerve ChE of mammals and the ChE of several insects (true ChE type). Several authors (Voss, 1960; DAUTERMAN and MEHROTRA, 1963) reported experiments on the ChE of *T. urticae* showing no inhibition at high substrate concentrations and therefore in this respect resembling the pseudo-ChE of mammalian plasma. However, our experiments strongly suggest the existence of a ChE that is inhibited at substrate concentrations higher than $5 \cdot 10^{-3}$ M. The reason for this discrepancy may be twofold. In the first place the warburg manometric technique, used by these authors, is not suitable to show the decreased activity at high substrate concentration, since at the range of optimal concentrations (about 10^{-3} M) too low values are obtained (see also KRYSAN and CHADWICK, 1963). Apart from this less important technical difficulty we found that there is a second enzyme in the crude homogenates or supernatants which hydrolyses appreciable amounts of ACh at high substrate concentrations. In fact, the decrease in activity of the « true » ChE at high substrate concentrations is more or less masked by the increase in activity of the second enzyme. As

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yet practically nothing is known about the nature of this second enzyme. Its affinity to ACh and Acetylthiocholine (ASCh) must be considerably lower than that of the « true » ChE. A more quantitative study of these enzymes has to wait till their activities can be studied separately.

There are other experiments that indicate the existence of more than one ChE. When supernatants are subjected to electrophoresis in agar gels and ASCh ($1{,}7 \times 10^{-3}$ M) is used as a substrate, two bands show up in many experiments. A first band, inhibited by 10^{-5} M eserine added simultaneously with the substrate, probably represents the « true » ChE. Whether the second band, which is less distinct and not always visible under the conditions employed, represents the enzyme with low affinity to ACh mentioned before, is not yet known.

We may draw attention to the possibility that the second enzyme, in the quantitative as well as in the electrophoresis experiments, could be an artefact or represent a different state of the ChE. The first distinct band shows up in both strains at about the same place. The one obtained in strain S is absent after preincubation with 3×10^{-5} M paraoxon for 30 minutes whereas that of strain R is unaffected. This confirms the earlier results showing a difference in inhibition rate of the enzymes of the two strains.

So far, two general conclusions can be made. Firstly, spider mites do have a ChE which is inhibited by excess of substrate just as all animals in which ChE is more extensively studied. One wonders whether those arthropods of which ChE is reported to be not inhibited by high ACh concentrations, will also show this property on further examination. Secondlly, earlier results on mite ChE obtained with crude homogenates or supernatants at ACh concentrations higher than 10^{-3} are suspected to be due to the activity of more than one enzyme.

It seems important to stress that this work has been done with a R-strain obtained from HELLE (1965), who crossed a single major-gene for parathion resistance into the genome of the S-strain. Due to this procedure we are rather sure that all of the resistance is caused by the changed ChE. The use of such a strain with only the major resistance gene seems also advantageous for practical reasons. Upon selection in the field generally a major factor will be selected for, since minor factors do not provide enough resistance to promote the chance for survival significantly. If the major factor contributes enough resistance for survival, no selection for secondary resistance factors takes place. Therefore, besides having advantages in analysis of the cause of resistance, strains in which only a major factors is present are

to be preferred over laboratory strains in which an « unnatural » high degree of resistance is accumulated by excessively high selection pressure.

SUMMARY

The acetylcholine hydrolysis by cholinesterase of susceptible mites has been shown to be inhibited by excess of this substrate. Agar gel electrophoresis revealed two acetyl-thio-coline hydrolysing enzymes with different mobility. The one inhibited by excess of substrate has a lower susceptibility to organophosphates in a resistant strain.

RIASSUNTO

È stato dimostrato che l'idrolisi dell'acetilcolina ad opera della colinesterasi di Acari suscettibili viene inibita dall'eccesso di tale substrato. L'elettroforesi su gel di agar rivela, mediante differenti mobilità, che due sono gli enzimi idrolizzanti l'acetil-tio-colina. Quello inibito dall'eccesso di substrato, manifesta minore suscettibilità agli organo-fosfati in ceppi resistenti.

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