# Inhibition of calmodulin-stimulated cyclic nucleotide phosphodiesterase by the insecticide DDT

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*Cyclic nucleotide phosphodiesterase* *Cyclic AMP* *Calmodulin* *Calcium* *DDT* *DDE*

## 1. INTRODUCTION

Calmodulin plays a key role in many calcium-mediated cellular processes (reviewed in [1–4]). Four $Ca^{2+}$ bind to each molecule of calmodulin with dissociation constants in the $\mu$M range. The calmodulin–$Ca^{2+}$ complex subsequently interacts with and stimulates phosphodiesterase [5], protein kinase [6], calcium-dependent ATPase [7], brain adenylate cyclase [8] and other enzymes. Activation can be inhibited by substances which bind to calmodulin such as antipsychotic drugs [9]. Benzodiazepines, however, inhibit calmodulin-dependent protein kinase by interacting with the membrane-bound kinase system itself [10].

The biochemical mechanism(s) of action of DDT and other chlorinated hydrocarbon insecticides is still little known. However, disruption of nerve cell functions [11] and egg-shell thinning observed in certain contaminated bird populations [12] suggest an effect on processes involving calcium [13]. Therefore the effect of DDT on calmodulin-mediated enzyme stimulation was studied. Here, I show that DDT at concentrations which are found in living organisms competitively inhibits calmodulin-mediated activation of phosphodiesterase.

*Abbreviations*: cAMP, adenosine 3':5'-cyclic monophosphate; DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane; DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene; DMSO, dimethylsulfoxide; EGTA, ethyleneglycol-bis-($\beta$-aminoethyl-ether)*N,N'*-tetra-acetic acid, SDS, sodium dodecyl sulfate

## 2. MATERIALS AND METHODS

p,p'DDT was obtained from Fluka (Buchs), DDE from Ciba-Geigy (Basel) and $\gamma$-hexachlorocyclohexane from Kodak. 5'-Nucleotidase from snake venom was from Sigma (N-5880), Dowex 1-X2 (200–400 mesh) from Bio-Rad, *t*-flupenthixol from H. Lundbeck and Co. (Copenhagen) and c[8-$^3$H]AMP from the Radiochemical Centre (Amersham).

Calmodulin was purified from calf brain as in [14] and was pure as judged by SDS–polyacrylamide gel electrophoresis. Calmodulin-dependent 3',5'-cAMP phosphodiesterase was prepared from calf brain as in [15]. Phosphodiesterase reaction mixtures contained 10 mM Tris–HCl (pH 7.6), 1 mM $MgCl_2$, 0.4 mM DTT, 0.3 mM $CaCl_2$, 0.1 mM EGTA, 0.33 U/ml of 5'-nucleotidase, 20 $\mu$M c[8-$^3$H]AMP (30 000 cpm/nmol), 5 $\mu$l enzyme and calmodulin as indicated in a total volume of 600 $\mu$l. Insecticides were dissolved in DMSO and added to give a final concentration of DMSO of 1%. Control incubations contained DMSO alone. Assays were performed for 15 min at 30°C and [8-$^3$H]adenosine was isolated by adsorption of the non-reacted substrate to Dowex 1-X2.

## 3. RESULTS AND DISCUSSION

An 11-fold stimulation of calf brain phosphodiesterase activity was observed at $\geq$ 40 nM calmodulin with half-maximal stimulation at 8 nM (not shown). When DDT was included in incubations containing 10 nM calmodulin significant inhibition of the enzyme was observed at $10^{-8}$ M (fig.1). A plateau was reached at ~ 55% inhibition and



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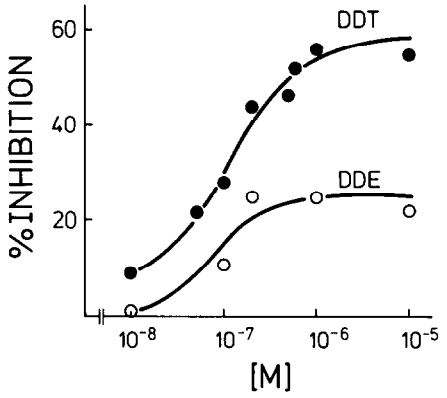


Fig.1. Inhibition of calmodulin-stimulated phosphodiesterase by DDT and by DDE. Assays were performed as in section 2. Calmodulin was 10 nM. Control values were 22 pmol/assay × min in the absence and 127 pmol/assay × min in the presence of calmodulin. Each point represents the mean of duplicate determinations; variation was < 5% of the mean. Concentrations of insecticides given are concentrations added to the incubation mixtures. Actual concentrations might be lower (see text).

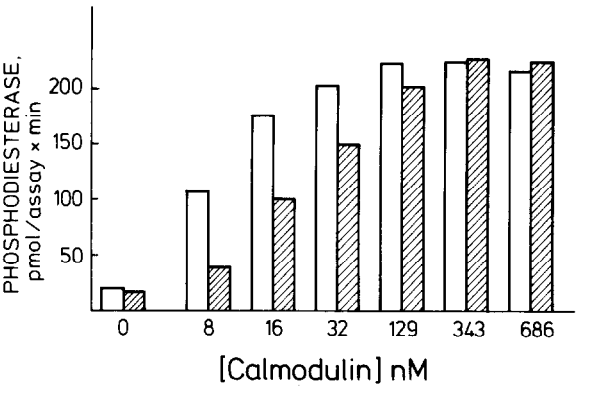


Fig.2. Effect of increasing concentrations of calmodulin on the inhibitory action of DDT: open bars, no DDT; hatched bars, $5 \times 10^{-6}$ M DDT. Basal activity (20 pmol/assay × min) has been subtracted from activity obtained in the presence of calmodulin to give the values shown. The means of duplicates are shown; variation was < 5% of the mean.

$5 \times 10^{-6}$ M DDT. Inhibition of the enzyme without added calmodulin (but with $Ca^{2+}$) was < 10% at all concentrations tested (not shown).

DDE is the most common metabolite of DDT found in organisms. Its insecticidal activity is much reduced compared to DDT. It also inhibits the calmodulin-mediated stimulation of phosphodiesterase (fig.1), but to a lesser degree. γ-Hexachlorocyclohexane, an unrelated chlorinated hydrocarbon insecticide, has no inhibitory effect at $10^{-5}$ M (not shown).

To establish whether DDT interacts with the enzyme directly, [calmodulin] was varied while [DDT] was held constant at $5 \times 10^{-6}$ M. Fig.2 shows that increasing the concentration of calmodulin overcomes the inhibitory effect of DDT. This observation indicates that DDT interacts with calmodulin itself or with a calmodulin-binding site on the enzyme.

Although the mechanism of action of DDT is not known, it has been shown to inhibit $Na^+,K^+$-ATPase [16–20], $Ca^{2+},Mg^{2+}$-ATPase [21,22] and carboanhydrase [23]. Whereas the effect on the latter enzyme only occurs at relatively high concentrations and seems to be due to precipitation of the enzyme by undissolved DDT [24], ATPases are inhibited at concentrations similar to those used here. In [16,17,19,21] a plateau was noticed at ~ 40–60% inhibition. One explanation for this observation, particularly in the case of ATPases, is the occurrence of multiple enzyme species. An alternative explanation, however, might be the limited solubility of the highly lipophilic compound DDT in aqueous solutions [24]. With regard to this study, the latter hypothesis is more likely for the following reasons:

(i) Increasing the concentration of DMSO increases the proportion of DDT dissolved. Although DMSO alone did affect phosphodiesterase, an enhancement of the inhibitory effect of $10^{-5}$ M DDT was obtained from 40.2% at 0.5% DMSO to 58.8% at 4% DMSO;

(ii) There is no evidence for different calmodulin-stimulated phosphodiesterase species and *t*-flupenthixol, an antipsychotic drug known to inhibit calmodulin-mediated effects, completely inhibited the stimulation of phosphodiesterase by calmodulin (not shown).

High levels of DDT reaching hundreds of ppm wet weight can still be found in animals, especially species representing the end of a food chain [25]. These concentrations are far above the ones which

are shown here to be inhibitory with regard to calmodulin-stimulated phosphodiesterase. Inhibition of calmodulin-mediated mechanisms might therefore be one important side effect of DDT.

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