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# THE EFFECT OF DDT AND THE PYRETHROIDS CISMETHRIN AND DECAMETHRIN ON THE ACETYL CHOLINE AND CYCLIC NUCLEOTIDE CONTENT OF RAT BRAIN

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## INTRODUCTION

The nucleotides cyclic AMP (cAMP) and cyclic GMP (cGMP) are believed to act as second messengers in nervous tissue (1 and 2). Various agents which cause hyperactivity, tremors or convulsions in rats increase the cGMP content of the brain (particularly in the cerebellum) without affecting cAMP (3-8). There is no clear common mode of action: thus oxotremorine is known also to elevate the acetyl choline (ACh) content of brain (3) while Harmaline is an amine oxidase inhibitor (4) and picrotoxin is a GABA receptor antagonist (5). Moreover intraventricular injection of the putative excitatory transmitters glycine and glutamate also elevate the cGMP levels while the depressant transmitter GABA lowers the level (6). In insects studies with the organochlorine and organophosphorus insecticides DDT and malathion have shown that they also cause increased levels of cGMP but not cAMP (9 and 10). Pyrethroids are now of considerable interest for potential use as insecticides (16) and the following study was carried out as part of an investigation into the mammalian toxicity of these compounds. The effects of toxic doses of pyrethroids and of DDT include convulsive tremors. Some pyrethroids (such as Decamethrin, NRDC 161, [S]-β-cyano-3-phenoxybenzyl cis-(1R, 3R)-2,2-dimethyl-3-(2,2-dibromovinyl) cyclopropane carboxylate) also cause profuse salivation (17) while others (such as Cismethrin, NRDC 119, 5-benzyl-3-furylmethyl (1.R.)-cis-chrysanthemate) do not. Therefore the effect of DDT and of the above pyrethroids on the acetylcholine, cAMP and cGMP content of the cerebellum and the rest of the brain was examined after oral administration of the insecticides to rats at doses which induced convulsive tremor.

## MATERIALS AND METHODS

DDT (purified, 99%) was from Shell Research Ltd., Sittingbourne, Kent; Cismethrin was made and purified by Dr. M. Elliot, Rothamsted Experimental Station, Herts. and Decamethrin was a purified sample from Roussel Uclaf, 93230 Romainville, France. Female rats

(Lac:P strain) 180 ± 10 gm were starved for 16 hrs overnight during which time they lost 10 gm weight. They were then dosed orally with the test compound (DDT 180 mg/kg; Cismethrin 100 mg/kg; Decamethrin 50 mg/kg) in arachis oil or with oil alone (all at 0.2 ml/100 g body weight). Tremors commenced about three hours after the dose of DDT (approx. $LD_{50}$) and had reached moderate intensity when the animals were killed one hour later. Experience suggested that death would not have occurred until several hours later. The doses of pyrethroids were estimated to be about 10% above $LD_{50}$ for these samples. Convulsive tremors commenced one hour after the dose of Cismethrin and the animals were prostrate when killed fifty minutes later. Salivation commenced one hour after the dose of Decamethrin and typical "writhing" convulsions were seen thirty minutes later; the animals were semi-prostrate when killed ten minutes later. Rats were killed by microwave inactivation focussed to the head (11) using an Elnode microwave unit (2.5 KW for 3 sec.). The heads were then immersed in liquid nitrogen for 10 sec. Brains were removed from the skull after a medullary section and then the cerebellum was separated from the rest of the brain in order that either the acetylcholine or cAMP and cGMP content of the separated portions could be determined.

Measurement of Acetylcholine: Acetylcholine was extracted from the samples of divided brain by homogenising in cold perchloric acid (2 ml of 0.2 M); the homogenate was centrifuged at 1000 g for 10 mins and the supernatant stored at 2° until assay. When required, the extract was adjusted to pH 6.8 with NaOH (1-N) and assayed by the contraction of leech muscle procedure (12) using a Stratham UC3 isometric transducer and Devices pen recorder. Extract samples were assayed with small pieces (7 x 1 mm) of the dorsal muscle of the medicinal leech in 1 ml of a modified Locke solution comprising NaCl (86 mM), KCl (3.1 mM), $CaCl_2$ (1.2 mM), $NaHCO_3$ (1.3 mM), glucose (6.2 mM), eserine (0.015 mM) pH 6.8 that had been bubbled with 95% $O_2$/5% $CO_2$: solutions were changed every 10 min. The response to extract was also tested after treatment of the muscle with d-tubocurarine (1 μg/ml). Standards of acetylcholine perchlorate (BDH, U.K.) were determined in the presence of boiled extract which was prepared by adjusting the pH of normal extract to 10-11 with NaOH (1-N), boiling for one min and readjusting pH to 6.8 with HCl (1-N). Results were calculated as nmol acetylcholine/g fresh tissue from a comparison of the mean contraction values obtained from standard and test extract solutions.

Measurement of cAMP and cGMP: Cyclic nucleotide content of the tissues was measured by the competitive binding technique with [$^3$H]-cAMP and [$^3$H]-cGMP and specific binding proteins using commercial assay kits obtained from Radiochemical Centre, Amersham, U.K. The brain tissue was homogenized in cold buffer containing Tris (50 mM) and EDTA (4 mM) at pH 7.5. A sample was taken for protein determination (13) and the remainder was heated for 10 min to coagulate the protein and then centrifuged at 13,000 x g (60 min): the supernatants were used for determination of cAMP and cGMP content. Results were calculated as

pmol cAMP or cGMP/mg protein for tissue samples from the same animals.

RESULTS AND DISCUSSION

Preliminary experiments showed that levels of cyclic nucleotide in rat brain were not significantly affected by starvation or by administration of arachis oil. However, administration of oil alone to unstarved rats had a marked effect on the brain ACh content: 2 hrs after dosing this was found to be in 2 rats 13.5 and 13.2 nmol/g compared with 28.4 ± 6.7 (S.D.; 4 rats) in normal undosed rats. Similar low levels were found in the brains of rats which received oil after overnight starvation. Our value for the normal rat agrees with other published data (14 and 15). In view of the marked effect of dosing with oil the lower levels (about 40-50% of oil-dosed controls in both regions of brain) seen after dosing with Decamethrin (Table 1) must be interpreted cautiously. However, it is of interest that this compound caused profuse salivation, which is a typical cholinergic response, but that the ACh content of brain was depressed rather than raised. In the case of insects poisoned with Malathion, Bodnaryk (10) observed that acetyl cholinesterase levels fell before the rise in cGMP occurred. Because of the observed symptoms, the effect of cholinergic antagonists on the response of rats to Decamethrin poisoning is being studied in these laboratories.

TABLE 1: Acetyl choline, Cyclic AMP and Cyclic GMP content of brain after oral dosing of rats with DDT, Cismethrin and Decamethrin

| Treatment | Whole Brain Minus Cerebellum | | |
|---|---|---|---|
| | Acetyl choline (nmol/gm) | Cyclic AMP (pmol/mg prot) | Cyclic GMP (pmol/mg prot) |
| Controls (Arachis oil 2 hr) | 12.8 ± 1.1 (5) | 5.59 ± 1.05 (4) | 0.57 ± 0.09 (4) |
| DDT (180 mg/kg 4 hr) | 10.9 ± 2.7 (5) | 6.26 ± 1.38 (4) | 1.39 ± 0.66 (4) |
| Cismethrin (100 mg/kg 2 hr) | 9.2 ± 2.8 (5) | 6.06 ± 0.79 (4) | 1.26 ± 0.25 (4) |
| Decamethrin (50 mg/kg 2 hr) | 6.0 ± 0.90 (4) | 6.34 ± 1.80 (4) | 1.64 ± 0.23 (4) |
| | Cerebellum | | |
| | Acetyl choline (nmol/gm) | Cyclic AMP (pmol/mg prot) | Cyclic GMP (pmol/mg prot) |
| Controls (Arachis oil 2 hr) | 3.9 ± 0.93 (5) | 5.10 ± 0.39 (4) | 4.07 ± 1.14 (4) |
| DDT (180 mg/kg 4 hr) | 5.2 ± 0.81 (5) | 4.02 ± 0.65 (4) | 21.76 ± 2.95 (4) |
| Cismethrin (100 mg/kg 2 hr) | 3.3 ± 1.45 (6) | 4.99 ± 0.66 (4) | 40.23 ± 4.24 (4) |
| Decamethrin (50 mg/kg 2 hr) | 1.6 ± 0.7 (4) | 4.58 ± 0.36 (4) | 32.28 ± 7.37 (4) |

Data is presented as the mean ± S.D. with the number of animals used given in parentheses. All rats were starved overnight before dosing.

Table 1 shows that toxic oral doses of DDT, Cismethrin and Decamethrin all produced marked increases in the levels of cGMP but not cAMP in the brain. The most marked increase in cGMP was in the cerebellum where a 5 to 10-fold change was observed compared with a 2-fold change in the whole brain minus the cerebellum.

The convulsive symptoms observed in rats dosed with the three pesticides appeared different in each case, yet all were associated with a marked rise in cGMP. In an attempt to distinguish cause and effect time-course studies are proposed to see if the rise precedes the onset of symptoms or can be induced at non-convulsive doses as has been reported for one of the toxic bicyclic phosphates (8).

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