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Target Enzyme Inhibition by Novel Thion Analogues of Monocrotophos: An Acute *In Vivo* Study in the Rat

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Organophosphorus insecticides are well known anticholinesterases, interferring with synaptic transmission of nerve impulses (Siddiqui et al. 1989; Chambers et al. 1989), whereas organochlorine pesticides affect membrane bound ATPases impacting conduction of nerve impulses (Jinna et al. 1989). However, inhibition of Na^+-K^+-, Mg^{2+}-and Ca^{2+}-ATPases by organophosphorus compounds has also been reported (Brown and Sharma, 1976; Anjum and Siddiqui, 1990). A number of monocrotophos (MCP) analogues synthesized at Indian Institute of Chemical Technology (IICT), Hyderabad were tested for their bioefficacy. Two of them (RPR-II and RPR-V) (Fig. 1) were found to be less toxic to non-target organisms and equally potent against target pests when compared with MCP (Qadri et al. 1986). Studies on the biochemical toxicology of these two compounds showed them to be less neurotoxic than MCP based on their potential to inhibit brain ChE in the rat (Siddiqui et al. 1991). These novel compounds depleted glutathione and caused inhibition of glutathione-S-transferase in hepatic and extra hepatic tissues of the rat (Siddiqui et al. 1990). Recently, we have shown a strong correlation between spectral binding of the two MCP analogues to hepatic microsomal cytochrome P-450 and their LD_{50}s in the rat (Siddiqui et al. 1992). In the present study, we investigated the ability of these two analogues to inhibit various brain ATPases (Na^+-K^+-, Mg^{2+}- and Ca^{2+}-ATPases) in addition to the well known target enzyme of organophosphorus compounds, cholinesterase. A comparative sensitivity of various ATPases in the brain and red blood cell (RBC) acetylcholinesterase to these novel insecticides and also the sexual dimorphism, if any, were investigated.

MATERIALS AND METHODS

A technical grade sample of MCP was obtained through the courtesy of M/s NOCIL, Bombay, India. RPR-II and RPR-V were synthesized according to the procedure of Jones and Badesha (1981). As seen from the structures (Fig. 1), these compounds can be conveniently made from diethyl thiophosphoryl chloride and the respective 1,3-dicarbonyl compounds. ATP was procured from SIGMA Chemical Co., St. Louis, USA. Ouabain and EGTA were purchased from MERCK,

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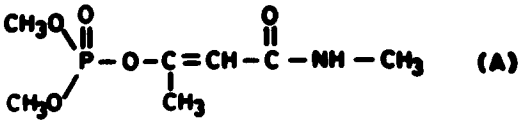
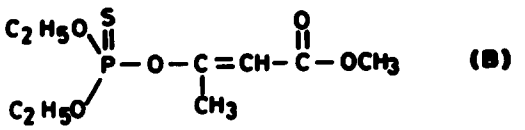


(A)



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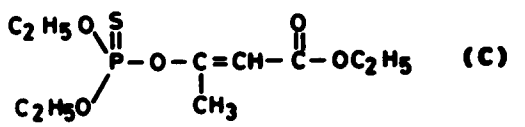

(C)

Figure 1. Structures of (A) phosphoric acid dimethyl (1-methyl-3-(methyl-amino)-3-oxo-n-propenyl) ester or Monocrotophos (MCP) (B) 2-butenoic acid-3-(diethoxyphosphinothionyl) methyl ester (RPR-II) and (C) 2-butenoic acid-3-(diethoxyphosphinothionyl) ethyl ester (RPR-V)

Frankfurt, Germany. All remaining chemicals were of Analar grade.

Adult male and female albino Wistar rats weighing between 150 and 180 g were obtained from National Institute of Nutrition, Hyderabad, India. They were acclimatized for a week prior to the experiment and then divided into four groups containing six rats of each sex. Each group was housed separately. Three groups were treated by oral intubation with 0.96, 1.23 and 3.0 mg/kg/bw of MCP, RPR-II and RPR-V, respectively. Coconut oil served as the vehicle. These doses corresponded to 1/10th of their respective LD_{50} values. The fourth group served as the control group and received coconut oil only. The control and experimental rats were maintained at 25 ± 2°C. Water and feed were provided ad libitum. Twenty four hrs after dosing, the rats were killed by decapitation and blood was collected directly in preheparinised vials to determine RBC ChE following the method of Ellman et al. (1961) as modified by Chambers and Chambers (1989). Simultaneously, brains of control and treated rats were removed and immediately placed on ice. The homogenisation and subsequent fractionation were done according to the method of Koch (1969a) as described by Jinna et al. (1989). Centrifugation of the post-nuclear fraction at 13,000 g for 20 min resulted in the P_2 fraction, which consisted of nerve endings and microchondria that were used for the assay of various ATPases. The Na^+-K^+-, Mg^{2+}-, and Ca^{2+}-ATPase activities were determined separately using appropriate inhibitors following the method of Jinna et al. (1989). Protein was determined as described by Lowry et al. (1951) using bovine serum albumin as the standard.

The experimental data were analysed by Student's "t" test to determine the significance of the changes from controls.

RESULTS AND DISCUSSION

The data on RBC cholinesterase inhibition and brain Na^+-K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase in male and female rats 24 hrs after exposure to MCP, RPR-II and RPR-V are given in Tables 1 to 3. It is clear that all 3 compounds caused a statistically significant inhibition of RBC ChE in female rats whereas in male rats ChE was significantly inhibited by MCP and RPR-II only (Table 1). In a separate study, RPR-V was found to be 8 times and 2 times less potent in inhibiting brain AchE than MCP and RPR-II, respectively (Siddiqui et al. 1991), supporting the present data. However, only MCP was found to cause a significant inhibition (40 percent) of rat brain AchE activity 24 hrs after treatment at 10 percent of its LD_{50} dose (Siddiqui et al. 1991). RPR-II and RPR-V caused significant inhibition of brain Na^+-K^+-ATPase in female rats (Table 2). None of the test compounds significantly inhibited Mg^+-ATPase in female rats although MCP caused a nonsignificant inhibition of 12 percent. In male rats brain Mg^{2+}-ATPase activity was significantly inhibited by MCP and RPR-II whereas Na^+-K^+-ATPase was significantly inhibited by RPR-V only (Table 2). Interestingly, the pattern of brain Ca^{2+}-ATPase inhibition by the 3 compounds (Table 3) was similar to that of RBC ChE inhibition (Table 1). However, the degree of Ca^{2+}-ATPase inhibition by these compounds was much higher than for RBC ChE. All 3 compounds significantly inhibited Ca^{2+}-ATPase in female rats whereas in male rats, only MCP and RPR-II inhibited the enzyme significantly.

Exposure to organochlorine pesticides has been shown to affect the enzymes involved in active ion transport across membranes in different laboratory animals (Koch, 1969[b]; Desaiah, 1982) while organophosphorus pesticides inhibit brain AchE thus disrupting synaptic transmission (Rahman et al. 1989; Siddiqui et al. 1988). Ca^{2+}- has been shown to be involved in various synaptic functions eg. neurotransmitter release and turnover, generation of Ca^{2+}-spikes, protein phosphorylation, and regulation of Ca^{2+} - dependent K^+ channels (Mehrotra et al. 1988). Our study demonstrated the inhibition of Na^+-K^+-, Mg^{2+}- and Ca^{2+}- ATPases in addition to RBC ChE caused by these compounds. It is thus possible that not only synaptic transmission, but also nerve conduction and subsequent release of acetylcholine from synaptic vehicles that is modulated by Ca^{2+} - might be affected by the three compounds. These findings are supported by the reported inhibition of Na^+-K^+- and Mg^{2+}-ATPases by the organophosphate parathion in the rat (Jaramillo-Juarez et al. 1989). We have also found inhibition of fish (Tilapia mossambica) brain Ca^{2+}-ATPase by MCP, dimethoate and diazinon (Anjum and Siddiqui, 1990). Riedel and Christenson (1979) also reported inhibition of Na^+-K^+-ATPase by malathion. The role of ATPases in the maintenance of ionic gradients across membranes is well established (Skou, 1957). The inhibition of Ca^{2+}-ATPase by these pesticides may disrupt ATP utilisation within the synaptic area and alter the energy metabolism of the nerve terminal by secondarily altering the activities of other enzymes for which ATP or ADP may be allosteric effectors (Brown and Sharma, 1976).

Table 1 : Effect of MCP, RPR-II and RPR-V on rat RBC cholinesterase activity 24 hrs after treatment

Compound	RBC Cholinesterase Activity[a]	
	Male	Female
Control	4.485 ± 0.112	4.302 ± 0.045
RPR-II	3.711 ± 0.039* (-17.26)	3.639 ± 0.032* (-14.42)
RPR-V	4.374 ± 0.067 (-2.48)	3.860 ± 0.029* (-10.28)
MCP	3.013 ± 0.013* (-12.76)	3.607 ± 0.056* (-16.16)

Data presented are mean ± SEM of six rats
Values in parentheses indicate percentage decrease from control mean
[a] μ moles hydrolysed/min/ml blood
* Significantly different from control mean ($p<0.05$)

Table 2 : Effect of MCP, RPR-II and RPR-V on rat brain Na^+-K^+ Mg^{2+}-ATPase 24 hrs after treatment

Compound	Na^+-K^+-AtPase[a]		Mg^{2+}-ATPase[a]	
	Male	Female	Male	Female
Control	1.256 ± 0.029	1.226 ± 0.018	2.445 ± 0.037	2.000 ± 0.065
RPR-II	1.171 ± 0.038 (-6.77)	0.907 ± 0.053* (-26.02)	1.663 ± 0.019* (-31.99)	1.891 ± 0.010 (-5.46)
RPR-V	1.130 ± 0.006* (-10.04)	1.054 ± 0.034* (-14.03)	2.359 ± 0.043 (-3.51)	1.868 ± 0.032 (-6.60)
MCP	1.077 ± 0.039 (-14.26)	1.110 ± 0.059 (-9.47)	2.101 ± 0.023* (-14.07)	1.752 ± 0.043 (-12.40)

Data presented are mean ± SEM of six rats
Values in parentheses indicate percentage decrease from control mean
[a] μ moles of Pi formed/hr/mg protein
* Significantly different from control mean ($p<0.05$)

Table 3 : Effect of MCP, RPR-II and RPR-V on rat brain Ca^{2+}-ATPase 24 hrs after treatment

Compound	Ca^{2+} - ATPase Activity[a]	
	Male	Female
Control	0.692 ± 0.015	0.615 ± 0.008
RPR-II	0.237 ± 0.025* (-65.76)	0.361 ± 0.027* (-41.31)
RPR-V	0.619 ± 0.054 (-10.55)	0.464 ± 0.015* (-24.56)
MCP	0.404 ± 0.043* (-41.62)	0.336 ± 0.009* (-45.37)

Data presented are mean ± SEM of six rats
Values in parentheses indicate percentage decrease from control mean
[a] μ moles of Pi formed/hr/mg protein
* Significantly different from control mean ($p<0.05$)

As evident from Tables 1 and 3, RPR-V did not cause significant inhibition of RBC ChE and Ca^{2+}-ATPase in male rats whereas these two enzymes were inhibited by RPR-V in female rats suggesting sexual dimorphism in the inhibition of the two enzymes by RPR-V. Sexual dimorphism was also apparent in the case of Na^{+}-K^{+}- and Mg^{2+}-ATPases inhibition by RPR-II. It has been reported that young rats do not show sex differences in the sensitivity to parathion (Benke and Murphy, 1975). In contrast, mature female rats showed higher sensitivity than male rats to parathion. No sexual dimorphism was reported by Jarmillo-Juarez et al. (1989) on the effect of parathion on renal ATPases. However, Szubartowska and Gromysz-Kalkowska (1992) reported the sexual dimorphism on fenitrothion effects in quails.

There are many reports describing the effects of pesticides either on ATPases or cholinesterases (Chambers et al. 1989; Koch, 1969b; Desaiah, 1982). However, a single study to determine their effects both on various ATPases and cholinesterases may be more important from a target enzyme pesticide interaction point of view and for a valid comparison of the relative sensitivities of the two types of enzymes to a pesticidal ligand. In this context, the present study indicated that MCP and two analogues besides inhibiting the well-known target of organophosphorus pesticides cholinesterase also inhibit various ATPases. Also, the fact that brain Ca^{2+}-ATPase is more sensitive to inhibition by the three organophosphorus compounds than RBC ChE, may be of value in biomarker-based evaluation of exposure to these novel insecticides. Inhibition of Ca^{2+}-ATPase might also upset the Ca^{2+}- dependent biochemical events in the organism.

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REFERENCES

Anjum F, Siddiqui MKJ (1990) In vitro sensitivity of brain Ca^{2+}-ATPase of fish T. mossambica to monocrotophos, diazinon, dimethoate and DDT. Ind J Exptl Biol 28:488-489

Benke GM, Murphy SD (1975) The influence of age on the toxicity and metabolism of methyl parathion and parathion in male and female rats. Toxicol Appl Pharmacol 31:254-269

Brown HR, Sharma RP (1976) Synaptosomal Adenosine Triphosphatase (ATPase) inhibition by organophosphates. Experientia 32:1540-1542

Chambers HW, Chambers JE (1989) An investigation of acetylcholinesterase inhibition and aging and choline acetyltransferase activity following a high level acute exposure to paraoxon. Pesticide Biochem Physiol 33:125-131

Chambers JE, Munson JR, Chambers HW (1989) Activation of the phosphorothionate insecticide parathion by rat brain in situ. Biochem Biophys Res Commun 165:327-333

Desaiah D (1982) Mechanism of chlordecone neurotoxicity : A review. Neurotoxicology 3:103-110

Ellman GL, Courtney KD, Anders V Jr, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88-95

Jaramillo-Juarez F, Posadas del Rio FA, Reyes JL, Rodriguez ML, Sanchez EI, Cuellar LH (1989) Effects of intrauterine exposure to parathion on the activity of renal ATPases in offspring. J Appl Toxicol 9:401-405

Jinna RR, Uzodinma JE, Desaiah D (1989) Age related changes in rat brain ATPases during treatment with chlordecone. J Toxicol Environ Hlth 27:199-208

Jones RA, Badesha SS (1981) Phase transfer catalysis : Phosphorylation of β-Dicarbonyl compounds. Syn Communs 11:557-560

Koch RB (1969a) Fractionation of olfactory tissue homogenates. Isolation of a concentrated plasma membrane fraction. J Neurochem 16:145-157

Koch RB (1969b) Inhibition of animal tissue ATPase activities by chorinated hydrocarbon pesticides. Chem Biol Interact 1:199-209

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin phenol reagent. J Biol Chem 193:265-275

Mehrotra BD, Reddy SR, Desaiah D (1988) Effect of subchronic dieldrin treatment on Calmodulin-regulated Ca^{+} pump activity in rat brain. J Toxicol Environ Hlth 25:461-469

Qadri SSH, Mustafa M, Grover P, Purohit P, Rahman MF, Neelkantan P, Rani R, Bhalerao UT (1986) Insecticidal activity of RPR series. Insect and pest control section, Newsletter and information circular on radiation techniques and their application to insect pests 38:15

Rahman MF, Siddiqui MKJ, Anjum F, Mustafa M (1989) Acute toxicity and antiacetylcholinesterase potential of biphenyl derivatives to some non-target organisms. Ind J Exptl Biol 27:138-140

Riedel B, Christenson G (1979) Effect of selected water toxicants and other chemicals upon Adenosine triphosphatase activity in vitro. Bull Environ Contam Toxicol 23:365-368

Siddiqui MKJ, Rahman MF, Mahboob M, Mustafa M (1988) Species differences in brain acetylcholinesterase and neuropathic target esterase response to monocrotophos. J Environ Sci Hlth Part B 23:291-299

Siddiqui MKJ, Rahman MF, Mustafa M (1989) Species selectivity in acetylcholinesterase inhibition by parathion and its oxygen analogue, paraoxon. Adv Bios 8:33-40

Siddiqui MKJ, Mahboob M, Mustafa M (1990) Hepatic & extra hepatic GSH depletion and GS-Transferase inhibition by monocrotophos and its two thiol analogues. Toxicology 64:271-279

Siddiqui MKJ, Rahman MF, Mustafa, M, Rahman MF, Bhalerao UT (1991) A comparative study of blood changes & brain AChE inhibition by monocrotophos and its analogues in rats. Ecotoxicol Environ Safety 21:283-289

Siddiqui MKJ, Mahboob M, Mustafa M (1992) Interaction of monocrotophos and its novel thione analogues with microsomal cyt P450 : in vitro & in vivo studies in rat. Toxicology (In press)

Skou JC (1957) The influence of some cations on an Adenosine triphosphatase from peripheral nerves. Biochim Biophys Acta 23:394-401

Szubartowska E, Gromysz-Kalkowska K (1992) Blood morphology in quails after poisoning with fenitrothion. Comp Biochem Physiol 101C:263-267

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