Bull. Environ. Contam. Toxicol. (1990) 44:721–728

Bulletin of Environmental Contamination and Toxicology

Heterogeneity in Response of Different Areas of Rabbit Brain to Malathion

T. S. Vijayakumar and V. R. Selvarajan

Department of Zoology, University of Madras, Guindy Campus, Madras 600 025, India

Neurotoxicants acting on the central nervous system affect the different regions of the brain unequally. This unequal sensitivity of different brain areas are attributed to the unique biochemistry of the cells, differences in degrees of vascularisation and differential functional demands on cells (Norton, 1980). Studies on the selective damage of the brain may throw light not only on the toxicant action but also on the functional organisation of the tissue. Very few authors have studied responses of different regions of brain to toxicants. But the heterogenous response among sub-divisions of major regions of the brain has not been studied so far.

Xenobiotics raising much concern in the pollution point of view are the insecticides and among them organophosphates are unmatched in extensive use (Lienske, 1980). Apart from affecting various physiological and biochemical parameters like EEG, ECG, proteins, lipids; enzymes like ATPases, SDH, LDH etc, the primary action of organophosphorus insecticides is inhibition of acetylcholinesterase (AChE) at cholinergic nerve terminals, which has been extensively reported (Adrian et al, 1947; Holmstdet, 1959; Usdin, 1970; Davis & Richardson, 1980 and Ecobichon, 1983). Malathion is one of the most widely used and studied OP compound, whose toxicity is mainly due to AChE inhibition, following its conversion to malaoxon in the liver (Namba, 1971). As such the activity of the enzyme serves as an excellent indicator of the impact of the insecticide on the various regions of the brain. The present communication summarises the observations made on the effect of malathion on the AChE activity in different regions as well as sub-divisions of the same region of rabbit brain; with special reference to the sensitivity, recovery capacity and recovery rate of the individual regions studied.

Send reprint requests to T.S. Vijayakumar at the above address.

MATERIALS & METHODS

Malathion (Technical grade) was obtained from Cynamide India, Ltd., Bombay. Male albino rabbits (Oryctolagus cuniculus) of the New Zealand cross variety, weighing 800-1000g were obtained from The Tamil Nadu Agricultural and Livestock Research Institute, Katupakkam. Experimental animals were given sublethal dose (188 mg/kg body weight) of malathion in acetone and controls were given only acetone, orally. The animals were decapitated on 1, 5, 10, 20 and 30 days after administration. The brain was divided into six regions of which three were further sub divided (Fikova & Marsala, 1952) . The twelve regions studied were - Olfactory bulb (OLB); Pons (PO); Medulla Oblongata (MO); four sub divisions of the Cerebral hemispheres - Right Frontal (CBRF), Left Frontal (CBLF), Right Temporal (CBRT) and Left Temporal (CBLT); two sub divisions of the Optic lobes - Anterior (OPLA) and Posterior (OPLP); and three sub-divisions of the Cerebellum - Vermis (CLV), Flocculus (CLF) and Lateralis (CLL). The brain tissue was homogenised in 0.1M phosphate buffer (pH 8.0) and centrifuged at 1500 rpm for 5 minutes. The supernatant was reacted with Acetyl Thiocholine Iodide in the presence of 5-5-dithiobis-2-nitrobenzoate (DTNB) and the formation of the yellow anion of 5-thio-2-nitro benzoic acid was measured spectrophotometrically following Ellman et al (1961). Analysis of variance and Student's Newman Kaul's test were used to compare AChE activity in different regions and levels of AChE inhibition, respectively (Zar, 1974).

RESULTS & DISCUSSION.

Significant decrease in AChE activity was seen in all the regions studied, following malathion administration (Table 1). Maximum decrease was seen on the 5th day following which the regions tend to recover in varied capacities. Inhibition data for AChE activity of all the regions studied, expressed as percentage of reduction of enzyme activity when compared with mean normal activity on 1, 5, 10, 20 and 30 days following administration, are summarised in figure 1.

The recovery capacity and recovery rate of the different regions studied, showed variations. Complete recovery of AChE activity indicated by statistically insignificant change ($P > 0.05$) was seen on 20th day in OLB, PO and MO; on 30th day in CBRT and CBLT. OPLA and OPLP showed statistically significant changes only on the 5th day whereas CBRF, CBLF,CLL,CLF and CLV showed significant changes even on the 30th day. As the recovery capacity of the regions varied, so did the recovery rate. The recovery rate of the different

Table - 1 Acetylcholinesterase activity in different regions of the brain of rabbit following malathion administration (μ moles of acetyl thiocholine iodide hydrolysed/mg tissue/minute) (mean of six values).

Days		1	5	10	20	30
Regions						
Olfactory bulb	C	0.067	0.066	0.065	0.068*	0.066*
	E	0.055	0.041	0.049	0.063*	0.063*
Cerebral right frontal	C	0.205	0.283	0.281	0.278	0.280
	E	0.179	0.132	0.179	0.213	0.253
Cerebral right temporal	C	0.223	0.219	0.227	0.222	0.223*
	E	0.163	0.128	0.169	0.197	0.217*
Cerebral left frontal	C	0.287	0.286	0.290	0.286	0.284
	E	0.208	0.116	0.199	0.231	0.264
Cerebral left temporal	C	0.289	0.290	0.293	0.290	0.289*
	E	0.201	0.148	0.213	0.260	0.280*
Optic lobe anterior	C	0.051*	0.054	0.052*	0.053*	0.053*
	E	0.042*	0.033	0.042*	0.049*	0.051*
Optic lobe posterior	C	0.040*	0.042	0.044*	0.042*	0.041*
	E	0.029*	0.027	0.033*	0.038*	0.040*
Cerebellum lateralis	C	0.379	0.379	0.381	0.387	0.385
	E	0.272	0.164	0.262	0.328	0.351
Cerebellum flocculus	C	0.390	0.397	0.394	0.389	0.393
	E	0.322	0.201	0.300	0.342	0.367
Cerebellum vermis	C	0.343	0.346	0.339	0.343	0.344
	E	0.240	0.166	0.256	0.281	0.325
Pons	C	0.087	0.089	0.093	0.086*	0.091*
	E	0.074	0.059	0.079	0.083*	0.090*
Medulla oblongata	C	0.115	0.114	0.121	0.112*	0.117*
	E	0.100	0.083	0.110	0.110*	0.118*

C - Control E - Experimental
* indicates statistically insignificant changes ($p > 0.05$).

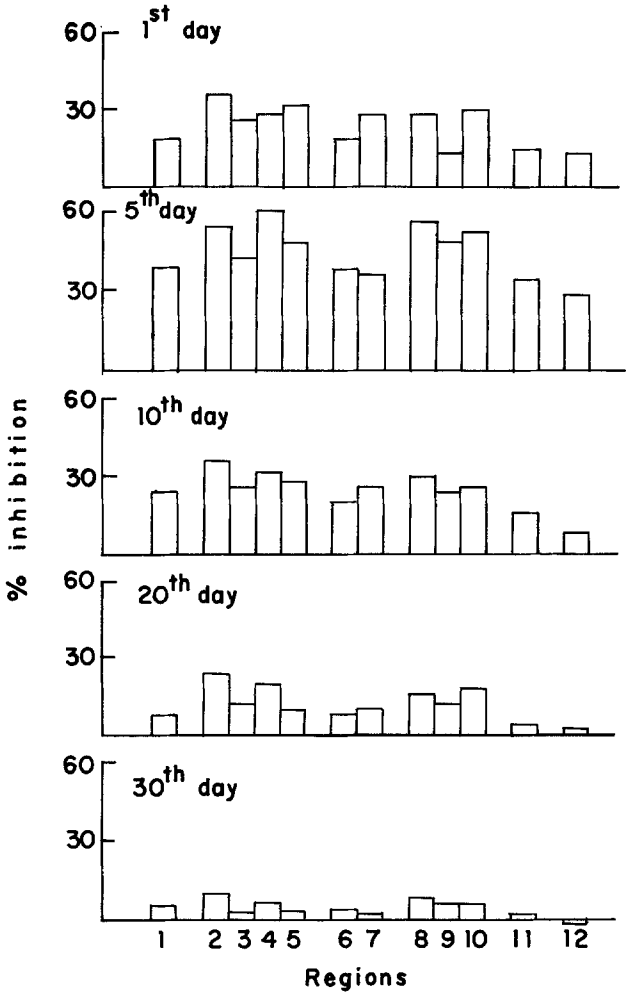

Figure. 1 Percent decrease in Acetylcholinesterase activity, on different days following malathion administration, showing the varied sensitivity of the different regions.

1. Olfactory bulb (OLB) 2. Cerebral right frontal (CBRF) 3. Cerebral right temporal (CBRT) 4. Cerebral left frontal (CBLF) 5. Cerebral left temporal (CBLT) 6. Optic lobe anterior (OPLA) 7. Optic lobe posterior (OPLP) 8. Cerebellum lateralis (CLL) 9. Cerebellum flocculus (CLF) 10. Cerebellum vermis (CLV) 11. Pons (PO) 12. Medulla oblongata (MO).

regions is shown in figure 2. OLB, OPLP, CBRF and CBRT showed almost uniform recovery upto 30 days. CBLF,CLL, CLV, CLF, OPLA, PO and MO showed rapid recovery from 5th to 10th day whereafter the process slowed down. CBLT showed rapid recovery from 5th to 20th day which slowed thereafter.

The accepted mode of action of organophosphorus insecticides is to phosphorylate Cholinesterases (ChE). However if an animal survives OP poisoning, ChE activity levels will return to normal (O'Brien, 1967). Recovery of ChE is said to primarily occur by the synthesis of new ChE molecules and by dephosphorylation of inhibited ChE (Davison, 1955; Blaber, 1960; Gersen & Shaw, 1961). Despite being very slow, the dissociation of ChE-antiChE complex plays a significant role in the recovery process (Lauwreys and Buchet, 1971). Dephosphorylation being a nucleophilic displacement reaction on the phosphorus atom leaving the enzyme, the reaction is promoted by nucleophilic agents like hydroxylamines and its derivatives such as oximes and hydroxamic acids (the pyridine-4-aldoxine methiodide (4-PAM) derivatives are highly efficient in comparison to other oximes). Inspite of the possibility of reactivation, the inhibited enzyme gradually changes into a nonreactivable form on storage (this process is called aging) due to dealkylation of the dialkoxy phosphinyl enzyme (Oosterbaan, 1965), after which the phosphorylated enzyme resents the nucleophilic attack of oximes because of its negative charges and becomes more stable (Eto, 1974). Reiner (1971) states that it is the nature of the pesticide which dictates the stability of the phosphorylated enzyme. Inhibition caused by ethyl compounds is reported to be more stable than that by methyl compounds. Malathion is a methyl compound, and thus the quick recovery can be explained. All these factors accounting for recovery are common to all the regions. Yet heterogenous recovery is exhibited. While the optic lobes recover by 10th day itself, the cerebellum is yet to recover even on the 30th day. In the same region; cerebral hemispheres the temporals recover in 30 days whereas even 30 days is not sufficient for the frontal lobes. Inhibition dependent recovery is not true in all the cases. While CBLF and CBRF (showing 59.44% and 53.36% inhibition on 5th day, respectively) recover by 30 days, CLF and CLV (showing 49.37% and 52.02% respectively on the 5th day) are yet to recover on the 30th day. All the above mentioned facts suggest the varied individual recovery capacity of the regions. The difference in recovery capacity among the different regions may be due to differences in the synthesis or reactivation of AChE or in the enzymatic hydrolysis, a major pathway of inactivation of organophosphates.

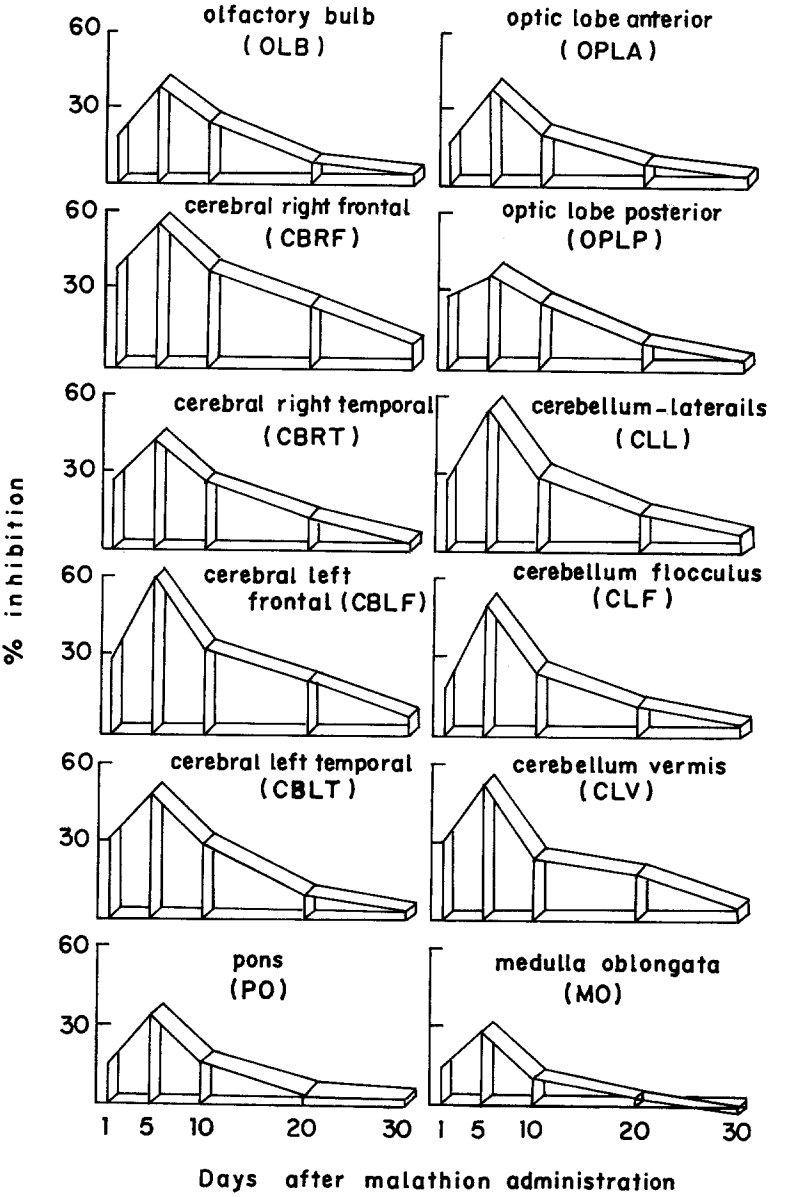

Figure .2 Recovery rate of Acetylcholinesterase activity (expressed as % change) for 30 days in regions and sub-regions of rabbit brain following malathion administration.

Finally coming to the differential sensitivity among the regions - heterogeneity is well established. While cerebellum and cerebral hemispheres show a very high percentage of inhibition, OLB, PO, MO and optic lobes are less affected. Similar heterogeneity is observed between subdivisions of same region. e.g., in the cerebral hemisphere, the frontals are more affected than the temporals and the right lobes are less affected than the left ones. The anterior optic lobe is more affected than the posterior one. In the cerebellum, the impact is as follows: CLL > CLV > CLF. Not only the sensitivity, but the recovery capacity and rate do vary. While the frontals recover completely by 30 days, the temporals are yet to recover by then. In cerebellum the most affected CLL recovers faster by 20 days than the less affected CLV.

Selective exposure due to- differences in ease of penetration to some areas through barriers; selective anoxia via differences in blood flow and metabolic requirements of some elements; selective sensitivity resulting from qualitative or quantitative biochemical differences in cell compartments - are attributed to the selective damage to one or more areas or compartments of the nervous system (Norton, 1980). The results of the present study, will no doubt, substantiate the above statement, proving that different areas or subdivisions of the same region of the brain also exhibit heterogeneity as in the case between different regions of the brain.

Acknowledgment. The authors gratefully acknowledge the University Grants Commission, New Delhi - for the financial assistance and Dr. Nausheen Dawood and S Durairaj for their encouragement and timely help.

REFERENCES

Adrian EE, Feldberg N, Kilby BA (1947) The Cholinesterase inhibiting action of fluoro phosphorates. Br J Pharmacol Chemother. 2 : 56 - 58.

Blaber LC, Creasey NH (1960) The mode of recovery of Cholinesterase activity in vivo after organophosphorus poisoning. Biochem.J. 77 : 597.

Davis CS, Richardson RJ (1980) Organophosphorus compounds. In: Spencer PS, Schaunburg HH (ed) Experimental and Clinical Neuorotoxicology. Williams & Williams. Baltimore, pp 527 - 544.

Davison AN (1955). Return of Cholinesterase activity in the rat after inhibition by organophosphorus compounds 2. A comparative study of true and pseudo cholinesterases. Biochem J 60 : 339.

Ecobichon DJ (1983) Organophosphorus Ester Insecticides IN : Ecobichon DJ, Joy RM (ed). Pesticides and

Neurological diseases. CRC Press Ltd., Boca Raton, pp 151 - 203.
Ellman GL, Courtney KD, Andres JR. V, Featherstone RM (1961) A new rapid colorimetric determination of acetylcholinesterase activity.Biochem pharmacol. 7:88.
Eto M (1974) Organophosphorus pesticide - Organic and biological chemistry. CRC Press Ltd., Cleveland.
Fikova, Marsala J (1952) Stereotaxic atlases for the cat, rabbit and rat brain In: Yau Bures, Petran U, Zachar F (ed.) Electrophysiological methods in Biological research. Publishing House of the Czekoslovakian Academy of Scienes, Prague, pp 426-443.
Gerson S, Shaw FN (1961) Psychiatric sequlae of chronic exposure to organophosphorus insecticides. Lancet 1 : 1371 - 1374.
Holmstedt B (1959) Pharmacology of Organophosphorus Cholinesterase inhibitors. Pharmacol Rev 11 : 567 - 688.
Lauwerys R, Buchet JP (1971) Studies on the mechanism of toxicants of the organophosphate pesticide Triamiphos. Eur J Pharmacol 16 : 361 - 366.
Lieske CN, Clarke JH, Meyer HG, Lowe JR (1980) Spontaneous and induced reactivation of AChE, inhibited by Three Organophosphinates. Pestic Biochem Physiol 13 : 205 - 212.
Namba T (1971) Cholinesterase inhibition by organophosphorus compounds and its clinical effects. Bull W.H.O. 44 : 289 - 307.
Norton S (1980) Toxic responses of the Central Nervous System In. Doull J, Klaesen CD, Amdur MO (ed) Toxicology - The Basic Science of Poisons. Macmillan, New York.
O'Brien RD (1960) Toxic phosphorus esters : Academic Press, New York.
O'Brien RD (1967) Insecticides : Action and Metabolism. Academic Press, New York. pp 33 - 87.
Oosterbaan RA, Jansz HS (1965) Cholinesterases, esterases and lipases. In : Florkin M and Stotz EH (ed) Comprehensive Biochemistry, Vol.16. Elsevier, Amsterdam, pp.1.
Reiner E (1971) Spontaneous reactivation of phosphorylated and carbamylated cholinesterases. Bull W.H.O. 44 : 109 - 112.
Usdin E (1970) Reactions of cholinesterases with substrates, inhibitors and reactivators. In : Karczmar AJ (ed) Anticholinesterase agents. International Encyclopaedia of Pharmacology and Therapeutics. Section 3. Vol 1. Pergamon, Oxford, pp 47 - 354.
Zar JH (1974) Biostatistical Analysis. Prentice Hall, London.
Received June 8, 1989; accepted August 2, 1989.