Effect of Chlordecone on Rate Brain Parts Ach Levels in Vivo

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Abstract - Chlordecone belongs to Organochlorine group of pesticides and its use in agriculture is known to effect the normal functioning of non-target forms. We investigated the *IN VIVO* effect of Chlordecone on ACh levels of various brain parts of rat. Rats receiving a sub-lethal and lethal doses of Chlordecone showed increased the ACh content of all brain parts and the changes were found to be statistically significant over the control (P<0.001). The results indicate that Chlordecone treatment is favoring ACh accumulation in various regions of the brain.

I.INTRODUCTION

In the years which followed natural was good and concern was voiced from some quarters that the potential risks of pesticide out-weighed the benefits. This View has not diminished but had been tempered in recent years with the emergence of other concerns allied to food production and the realization that food like life is not risk free.

Recently, we've all been aware of the powerful forces at work that have impacted on our everyday lives and the livelihoods of those in agriculture. Those forces have been instrumental in setting the scence for the legislative frame work and consumer perception moveing forward. Many of us and in particular our farmers have been on the (BSE) roller coaster, experiencing constantly changing advice and confusion. The safety of genetically, modified crops have been under the spotlight with debate from all quarters ranging from pressure groups, through manufacturers and retailers to regulators across Europe .

A. Mode of action:

The toxicity of organochorides involves the functional disruption of sensory and motor nerve fibres and the motor cortex (Narahashi and Yamaski, 1968). The disruption of the neural mechanism is due to the penetration of organochlorides into the axon membrane altering the permeability to Na^+ and K^+ , subsequently inhibiting Na^+ -K^+ and $Mg^{+\ +}$ adenosine triphosphate activities in the nerve endings (Corbett, 1974).

Organochlorine compounds found to disrupt energy systems by inhibiting Mg^{2+} ATPase and aerobic segmental enzymes of brain (Cut Komp and Koch, 1981). Accumulation of ACh content in synaptic region was also noted (Omer et al., 1971). Inhibition of AChE and accumulation of ACh content by OCI compound shows the neurotoxic action (Omer et al., 1971).

B. Neurotoxic effects of chlordecone:

Chlordecone, better known as Kepone®, belonging to OCI group of compounds, is a colourless, odorless crystalline solid, It is a polycyclic chlorinate compound with a caged structure similar to mirex (Desaiah, 1980).

Several affected workers showed toxic symptoms involving primarily the nervous system, as evidenced by tremors, ataxia, slurred speech, mental changes, headache, irritability and exaggerated startle response (Desaiah, 1982; 1985). In addition to the neurotoxic symptoms, the chlordecone intoxicated workers also showed hepatosplenomegaly and presumed sterility (Guzelian, 1982).

The general biochemical toxic effects of chlordecone are summarized as below. The susceptibility of various animal species differ considerably although there is not much sex difference (Sherman and Ross, 1961; Gaines,

1969; Larson et al., 1979). In most studies, animals exposed to chlordeone show weight loss (Guzelian, 1982). Chlordecone was a neurotoxin, estrogen, hepatotoxin and potentiator of halomethane toxicity.

II. MATERIALS AND METHODS

Albino rats of the weight range 150 ± 5g were used for the present study. Rats were maintained at constant temperature of 25□C ± 5 □C, humidity 60-70% they were fed with ad libitum commercial diet supplied by Sri Kamadhenu Agencies, Bangalore, India. 24 hrs prior to experiment they were tested with free access to water. They were fasted with free access to water. Theywere divided into groups of 10 each, maintained in separate cages and were used either for LD50 study or chlordecone treatment. For sub-lethal and lethal does of chlordecone treatment the numbers of rats used were seven ech for every treatment.

After determination of LD□□ dose of chlordecone to rat per 48 hours, a rat colony was divided into three groups of seven each. I group acted as control one, the II group was gavaged with a sub-lethal chlordecone dose of 29.71 mg/kg and the III with a lethal dose chlordecone of 89.13 mg/kg. after 48 hrs chlordecone treatment of rats, the control and experimentalgroup of rats individually were anaesthetized with Katamine (7mg/Kg).

From the whole brains dissected, the various brain parts like cerebellum, hypothalamus, midbrain, striatum, hippocampus, cerebral cortex and medulla oblongate were separated and individually frozen in liquid nitrogen and were stored at-80^{o}C till used.

III.METHODS

Determination of Acetylcholine Content (ACh)

ACh content in control and experimental samples was estimated by the method of Hestrin as described by Augustinsson (1957). The tissues (different parts of rat brain regions) individually were isolated, sliced, weighed and were transferred into clean test tubes. The tubes were kept in boiling water bath for 5 minutes to inactivate the acetylcholinesterase (AChE) enzyme activity and to release bound ACh as described by Vasantha et al., (1975) The tubes were cooled and the contents were homogenized in 2.0ml of distilled water, 2.0 ml of alkaline hydroxylamine hydrochloride and 1.0 ml of hydrochloric acid (1:1-HCl : H□O) were added to the homogenates. The contents were centrifuged at 2000 g/10 min and 1.0 ml of ferric chloride (FeCl□) was added to the supernatant. The optical density of the sample was measured at 540 nm in a spectrophotometer using the reagent blank ACh content was expressed as µmoles of ACh /gm wet wt of tissue.

IV.RESULTS AND DISCUSSION

RESULTS:

The data presented in table-1 shows the ACh content of various parts of control and chlordecone treated rat brain. A sub- lethal and lethal dose of chlordecone has significantly ($P<0.001$) enhanced the rat cerebellum, Hypothalamus, mid brain, striatum, hippocampus, cerebral cortex and medulla oblongata ACh content. Concerning to the percent changes LD□□ does chlordecone treated rat brain parts showed more percent increase of their ACh content over the control compared to ⅓ LD□□ dose chlordecone treated ones (fig-1).

The results of table-1 further depicts that in the control group of brain parts the tissue specific trend for ACh observed was more for striatum and was followed by medulla oblongata>hippocampus>hypothalamus>mid brain>cerebral cortex>Cerebellum.

Discussion:

Acetylation of the nitrogenous alcohol choline gives rise to ACh, now recognized as a neurotransmitter in all major groups of animals (Hoar, 1976). The biosynthesis is catalzed by choline acetylase and readily reversed by acetylcholinesterase (AChE).

The most widely recognised action sites of ACh arethe endings of vertebrate motar nerves, the endings of the autonomic preganglionics and the parasympathetic post ganglionics. ACh is concerned with various visceral functions in both invertebrates and vertebrates.

As given by Hoar (1976), Claude Bernard launched the history of transmitter pharmacology in 1857 when he discovered that nerve stimulation failed to excite muscles that were poisoned with a plant alkaloid curare, even through the muscle remained responsible to direct stimulation.

The physiologists at present have discovered a wide range of drugs that interact with ACh. Hemicholinium – 3 prevents ACh synthesis in motor nerves, botulinum toxin, one of the most potent poisons in the world, prevent its release. Several different substances termed AGONISTS, act like ACh on the post – synaptic membrane; well known examples are carbachol, succinylcholine, necamethenium and nictene.

Biochemical experiments have indicated that exogenously applied NO is able to influence the release of several neurotransmitters such as glutamate, GABA, dopamine, ACh, adrenalin (Garth waite and Boulton

1995. The definitive role of ACh in regulation of NOS functions is well documented by Sudhir et al., (1994). He revealed that ACh -induced vasodilation is mediated by the release of endothelium-derived relaxing factors (EDRF's) (Furchgott and Zawadski, 1980) one of which is NO (Palmer et al., 1987; Ignarro et al., 1987). Endothelium-dependent vasorelaxation influences coronary blood flow and vascular resistance through effects on both epicardial and resistance coronary arteries (Luscher et al., 1990). The role of ACh in NO release at least in the cardiovascular system is reported by many investigators (Furchgott, 1993; Vanhoutt; 1993, Busse et al., 1993; Sudhir et al., 1994). In view of the key role played by the neurotransmitter molecule like ACh in NO release, the author attempted to study the in vivo effect of chlordecone on ACh levels of various brain parts of rat.

A sub-lethal and lethal does of chlordecone over a period of 48 hours increased the ACh content of all brain parts studied in the present investigation (table-1) and the changes were found to be statistically significant over the control ($P<0.001$). The results indicate that chlordecone treatment if favouring ACh accumulation in various regions of the brain.

The toxicity of OCI compounds involve the functional disruption of sensory and motor nerve fibres (Narahashi and Yamaski, 1968). Accumulation of ACh content in synaptic region was noted by Omeret al., (1971). Inhibition of AChE and accumulation of ACh content by OCI compounds shows their neurotoxic effect (Omer et al., 1971). ACh has been identified in many areas of the brain, and in the opinion of some, it is the only component that fits the most stringent criteria for a CNS transmitter (Myers, 1974). ACh is released from the endings of cholinergic fibres to effect synaptic transmission. Under normal circumstances ACh is hydrolysed by AChE immediately. There is no accumulation of ester as well. So the normal function of ACh depends upon its rapid destruction by AChE Maslora(1981).

Cholinesterases are of two types, viz. a) true cholinesterases and b) pseudocholinesterases or nonspecific cholinesterases. They differ in substrate preference and specificity, kinetics, substrate hydrolysis and sensitivity to some inhibitors in different animal systems (Augustinsson, 1963; Perse, 1972; Silver, 1974).

The physiological role of pseudocholinesterases has not yet been clearly specified. The pseudocholinesterases are relatively less efficient than AChE at low concentrations of substrate but more efficient at higher concentrations. Butyrylcholinesterase (BuChE) is a less specialized enzyme than AChE. Unlike AChE, it lacks anionic site in a position that specifically adapts it to react with ACh. It does, however, slowly react with Ach. It also reacts with a wide range if esters (Ecobichon and Comean, 1973).

Cholinesterases and ACh are found to differ in their concentrations in the different areas of rat brain. Striatum is the area rich in cholinergic neurons and the levels of AChE and ACh are high in this area (Stavinoha et al., 1976; Ehlert et al., 1980a; 1980b;) and lowest activity of AChE and ACh were in the area of cerebellum (Bennet et al., 1966). Studies on different brain areas where functional deficits should be readily associated and recognized are of importance for a fuller understanding of the toxic actions of insecticides (Shih, 1982). The functions of brain regions also significantly vary from each other. Inview of this, a study of the response of different brain regions to chlordecone toxicity with reference to ACh content would be worth while.

From the data presented in table-1, it can be infered that the control rat striatum showed highest content of its ACh and was followed by medulla oblongata > hippocampus > hypothalamus > midbrain > cerebral cortex > cerebellum and the trends obtained were in agreement with the reports of Bennet et al., (1966)

The percent elevation of ACh for any given brain region was more in lethal dose chlordecone treated brain regions, infering that higher the does of chlordecone higher the percent elevation of ACh (table-1).

The sum total of the cholinergic substances obtained by the assay presented for ACh determination be construed as the amount of ACh present or have accumulated in the respective tissues during chlordecone exposure could be due to lesser break down of ACh because of AChE is known to be inhibited upon OCI compounds exposure in animal models (Omer et al., 1971).

A reduction in muscarinic receptor density by chronic administration of OI like OP's is closely related to the degree of ChE inhibition (Sivam et al., 1983., Yamada et al., 1983a). The state of subsensitivity is the result of AChE inhibition and increased accumulation of ACh to which effector cells are exposed, causing the responsiveness of the cells to decrease (Foley and Mc Phillips 1973). It was reported that OI exposure result in decrease in the density of muscarinic receptors and this occurs when the brain AChE is inhibited (Russell et al., 1975). OI's further were shown to cause down-regulation or subsensitivity of cholinergic receptors resulting from elevated ACh concentration (Costa et al., 1982) as was observed in the present investigation. Number of environmental toxicants have been shown to impair ACh functioning including receptor mediated events in neuronal systems (Buckingham et al., 1997; Chao et al., 1997; Xu Hai-Bin et al., 1997; Katz et al., 1997; Guilhermino et al., 1998; Matsuda et al., 1998; Stefanidou et al., 1998; Tang et al., 1998; Ostby et al., 1999). Based on the above, the elevation in brain regions ACh levels in sub-lethal and lethal dose chlordecone treated rats (table-1) and blockade of receptor functions by OI's as cited above may inhibit/reduce the binding of ACh to its receptor sites (Van Helden et al., 1998; Slotkin 2009; Shelton, 2012) and thus in the current study one of the OI's studied chlordecone may impair the usual functioning of rat brain based ACh.

Fig.1: Percent change of sub-lethal and lethal

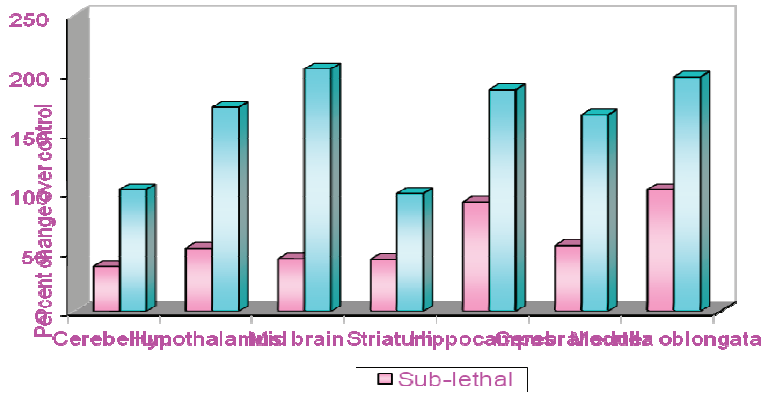

Table-1 :Effect of sub-lethal and lethal doses of Chlordecone on ACh levels in various parts of rat brain *in vivo*.

(Values expressed as µM of ACh/gm wet wt of tissue)

Name of the Brain part	Control	Sub-lethal Experimental	Lethal Experimental
Cerebellum SD PC t	0.482 "0.020	0.664 "0.033 37.75 P<0.001	0.978 "0.024 102.90 P<0.001
Hypothalamus SD PC t	0.602 "0.041	0.923 "0.027 53.32 P<0.001	1.64 "0.044 172.42 P<0.001
Mid brain SD PC t	0.584 "0.021	0.844 "0.032 44.52 P<0.001	1.78 "0.062 204.79 P<0.001
Striatum SD PC t	1.34 "0.071	1.93 "0.044 44.02 P<0.001	2.67 "0.057 99.25 P<0.001
Hippocampus SD PC t	0.821 "0.037	1.58 "0.062 92.44 P<0.001	2.36 "0.091 187.45 P<0.001
Cerebral cortex SD PC t	0.541 "0.046	0.841 "0.037 55.45 P<0.001	1.44 "0.042 166.17 P<0.001
Medulla oblongata SD PC t	0.917 "0.037	1.86 "0.036 102.83 P<0.001	2.73 "0.075 197.70 P<0.001

Each value is the mean " SD of 7 samples.

SD : Standard deviation

PC : Percent change over control ones

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