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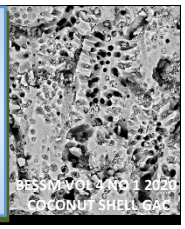

Acetylcholinesterase Enzyme (AChE) as a Biosensor and Biomarker for Pesticides: A Mini Review

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ABSTRACT

Due to the increase in pesticide usage the cost of food production has been drastically reduced worldwide. There are dangers related to the ever-increasing pesticide application especially to the non-target biota and to also to the environment at large. Pesticides bind with the active site of acetylcholinesterase (AChE) and inhibit the breakdown of acetylcholine and causes the blockage of synaptic transmission in cholinergic nerves. When AChE is inhibited, ChE accumulates and the nerve impulse cannot be stopped, leading to muscle contraction, paralysis and sometimes dead may occur. Pesticides and other chemicals that inhibit AChE activity can be able to cause abnormal behavioural patterns of the affected animals. The effects of AChE inhibition in vertebrate include vasodilation of blood vessels, slower heart rate, constriction of bronchioles and reduced secretion of mucus in the respiratory tract, intestinal cramps, secretion of saliva, sweat and tears, and constriction of eye pupil. The inhibition of the AChE activity will also definitely affect the optomotor behaviour of a fish which in turn will affect feeding capability, identification and avoidance of predators, and spatial orientation of the species. Carbamates, organophosphate and eserine are the major pesticides that inhibit the AChE activity of many animals. Cholinesterases including AChE have been considered as interesting biomarkers and biosensor for many years in the monitoring of environmental contamination. This is sensitive to selected organophosphate and carbamate pesticides and may be responding to low levels of contaminants in the environment, putatively by compounds other than or in addition to pesticides. In respect to the above AChE is regarded as a good Biosensor and biomarker in assessing pesticides and other chemical pollutants in the environment.

INTRODUCTION

Pesticides are extensively used in agriculture to increased yield and reduce the cost of food production. More than 300,000 metric tons of insecticides were sold to the agricultural sector world-wide [1]. Pesticides are among the most dangerous chemicals used to improve agricultural products against pests attack, but they are also the threat to non-target animals especially in the aquatic environment as a result of their toxic, persistence and ability to accumulate in to the animal tissues [2]. Aquatic community specifically fishes are continuously exposed to water pollutants such as pesticides and their derivatives via water runoff or rain [3]. The preponderance of the insecticides used were AChE inhibitors, 55% of them belonged to the group of organophosphates and 11% to carbamates [4]. Many of these chemicals eventually enter into aquatic environments through rivers, the atmosphere, agricultural run-off and industrial point sources and pose a potential threat to the indigenous biota [5]. The global annual pesticide usage is estimated to be 5.6 billion pounds [6], and biggest proportion of about 40% is herbicide [7,8].

Pesticides bind the active site of AChE and prevent breakdown of Ach resulting to blocking of synaptic transmission in cholinergic nerves [9]. If AChE is inhibited, acetylcholine (Ach) accumulates and nerve impulses cannot be stopped, causing prolonged muscle contraction, as a consequence paralysis occurs and death may result [10]. It is also known that pesticides and certain chemical compounds which inhibit AChE activity are known to disrupt the normal behavioural patterns in the affected animals [11]. The behavioural changes observed in the intoxicated animals like repeated opening and closing of opercula covering, hyper-extension of all fins, cock-screw swimming, S-jerks, coughing, burst-swimming can be directly related to the inhibition of peripheral and/or central nervous system due to inhibition of cholinesterase activity [12].

Effect of xenobiotics to acetylcholinesterase (AChE)

Due to the rapid increase of environmental pollutants in to aquatic environment, evaluation and understanding of the effects of such xenobiotics is paramount in order to safe and protect the aquatic biota [13]. Many xenobiotics especially insecticides are designed to inhibit serine esterase AChE. This inhibition permits the build-up of ACh and result in more intensive and prolonged activation of the receptor site [13]. Therefore, AChE inhibition in the peripheral nervous system could be a serious threat to the health and survival of the animal [14]. AChE is reported to be the most hydrolysed cholinesterase enzyme in the brain of *Clarias gariepinus* exposed to cadmium [15] and *Sparus aurata* exposed to organophosphate pesticide [16].

In fact, a few anticholinesterase compounds capable of causing morbidity or death in animals solely due to AChE inhibition in peripheral tissues with little or no inhibition to central nervous system [17,18]. The effects of AChE inhibition in vertebrate include vasodilation of blood vessels, slower heart rate, constriction of bronchioles and reduced secretion of mucus in the respiratory tract, intestinal cramps, secretion of saliva, sweat and tears, and constriction of eye pupils [19].

The inhibition of the AChE activity will also definitely affect the optomotor behaviour of a fish which in turn will affect feeding capability, identification and avoidance of predators, and spatial orientation of the species [12,20]. While in invertebrate (e.g. crustaceans) this accumulation of ACh by AChE inhibition induces the nerves poisoning such as restlessness, hyperactivity, tremors, convulsions, and paralysis[21,22]. Therefore, assessment of the inhibition of AChE activity in central nervous system and peripheral nervous system tissues are regarded as an integral part of the toxicity profile of an anti-AChE compound. These usually bring about the characteristic signs and symptoms which are grouped in Table 4.

Table 1. Effects of anticholinesterase compounds [23].

Receptors	Target organ	Symptoms and signs
Central	Central nervous system (CNS)	Giddiness, anxiety, restless, headache, tremor, confusion, failure to concentrate, convulsions and respiratory depression.
Muscarinic	Glands; Nasal mucosa Bronchiole mucosa Lachrymal Salivary	Rhinorrhea, bronchorrhea, sweating, lachrymation and salivation
	Smooth muscle; Iris Ciliary muscle Gut Bladder Heart	Meiosis, failure of accommodation, abdominal cramp, diarrhea, frequency, involuntary micturition, and bradycardia
Nicotinic	Autonomic ganglia Skeletal muscle	Sympathetic effects, pallor, tachycardia, hypertension Weakness and fasciculation

Effects of Carbamate and Organophosphate pesticides

Organophosphorous and carbamate pesticides are used extensively as pesticides in urban, agricultural and aquacultural situations. Despite their rapid hydrolysis in air, water and soil, bioaccumulation of organophosphorous compounds in sediments has been documented at appreciable levels in estuaries and coastal locations, representing a potential threat to biota [24]. The primary toxicity of these substances is due to a reversible inhibition of AChE, a key enzyme of the nervous system [14]. These anticholinesterase agents are widely used to control various agricultural pests, but are potentially toxic for other animals [25]. Individuals poisoned by these compounds experience partial cholinesterase inhibition, which can be lethal [16]. Insect AChE is the biological target of most of the important insecticides used in agriculture but their effects went beyond the target species [26].

Carbamate insecticides used in the crop protection, for their low persistence, wide action spectra, and ability to control pests. One of the mostly used carbamate insecticides is carbofuran (2, 3, dihydro-2, 2 dimetyl-7-benzofuranyl methyl carbamate) [27]. In Malaysia, carbofuran is one of the extensively used pesticides in west Malaysia paddy field. The carbofuran are neurotoxin and potent inhibitor of the enzyme AChE [24]. Carbamates and organophosphates are been apply to control insects by binding to the active site of acetylcholinesterase through carbamylation for carbamates or phosphorylation for organophosphate and resulted in the inhibition of neurotransmitter metabolism in the nervous system and causes lethality [28].

Organophosphates and carbamates insecticides are reported to inhibit AChE activities in fish [11]. Biochemical changes in *C. batrachus* following exposure to carbofuran were investigated by [27]. AChE inhibition in the brain produces changes in behavior, but in the muscle, it leads to hyperstimulation of the muscle fibers and can lead to tetania, paralysis and even dead. In the fish study, brain AChE has been studied more as compared to the muscle [29].

Anticholinesterase

Understanding of the catalytic properties of the protein has assisted in understanding of its inhibition by anticholinesterase. Owing to its critical role in ACh-mediated neurotransmission, AChE is a sensitive target for both natural and synthetic cholinergic toxins [30]. The anticholinesterase ability of some neurotoxic pesticides are widely been used as a good bioindicator of environmental pollution, also the tendency of any pesticide to inhibit the acetylcholinesterase of any organism in particular is vital in assessing the impact of the chemical on the target organism [31].

Among the natural anti-AChEs are plant derived carbamates and glycoalkaloid inhibitors. A natural inhibitor of AChE was also found in a mollusc and green mamba venom includes the neurotoxic peptide fasciculin, which blocks entrance to the active and the peripheral sites of AChE [32]. Synthetic anti-AChEs were first studied and manufactured as highly poisonous organophosphate and carbamate, nerve gases and insecticides. In the clinic, controlled use of AChE inhibitors has proved valuable for the treatment of diseases that involve compromised ACh-mediated neurotransmission [33].

Susceptibility to inhibition by eserine, carbamate and organophosphate compounds were one of the distinctive characteristics of cholinesterase. In addition to the carbamate and organophosphorus inhibitors which are effective in very low concentrations, many other agents also inhibit AChE activity [34]. Among these are acetone [35], zinc and various anions including fluoride [36]. Chlorinated hydrocarbon insecticides are expected to have an affinity for hydrophobic sites in enzymes including AChE, but such chlorinated compounds are not important in the inhibition of active sites [37].

Substances which inactivate AChE are less numerous but include cadmium and magnesium [38], lithium, potassium and sodium [36]. Magnesium seems to be the most potent but as pointed out; the effects of ions vary greatly with other conditions of the experiment. This is particularly true in the case of sodium and potassium because they are not only activate the enzyme but also increase the value for the optimum substrate concentration [39].

If pesticides and inorganic compounds are unlikely sources of inhibitors, detergent are the other classes of compounds that could inhibit AChE. Moreover, one of the principal classes of chemicals in domestic wastewater is detergents. This is of interest since many detergents contain tertiary and quaternary ammonium compounds which have been shown to inhibit AChE in vitro [40].

Organophosphate as an inhibitor to AChE

Almost all types of organophosphate compound target and depress AChE activity in a dose-dependent manner [41]. Organophosphate and their metabolites are potent inhibitors of serine esterase through phosphorylation of the serine hydroxyl moiety within the active site of the esterase. Organophosphate is also highly labile and with their generally extreme lack of lipophilicity, the sensitivity of organophosphate towards AChE is reducing as a result of low bioaccumulation by target organism [42]. Organophosphates are poorly water soluble, a high oil-water partitioning coefficient and low vapour pressure. All organophosphate can be degraded by water hydrolysis especially in alkaline condition, yielding water soluble product that are generally non-toxic. Organophosphorus compounds tend to differ markedly in their inhibition ability against cholinesterases from different sources [43]. The reaction of organophosphate inhibitors with the enzymes involves the catalytic machinery of the enzymes. During inhibition, the enzyme acts as nucleophile and the hydroxyl group of serine is phosphorylated (**Fig. 1**). The behaviour of organophosphate action mimics those of carbamate but with much smaller k_3 value [44,45].

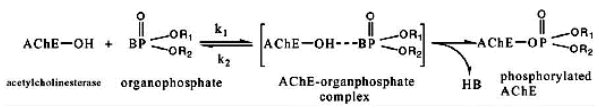

Fig. 1. Inhibition scheme of AChE by organophosphate pesticides [46].

Carbamate as an inhibitor to AChE

Carbamates are important class of anti-AChE insecticide. The functional group of carbamate is urethane. Most carbamates are slightly soluble in water but dissolve readily in organic solvents, thus conferring varying degrees of lipid solubility [47]. Carbamates promote a reversible inhibition [45]. Thus dilution or dialysis will restore enzyme activity and it was at once time thought that carbamate were irreversible inhibitors in contrast to organophoshate that were thought to be reversible inhibitors [47]. Actually, the mechanism of inhibition for both groups of compounds is the same but the pharmacologically interesting carbamates have a value of k_3 that is kinetically significant, whereas the organophosphates have a value of k_3 that is much smaller (**Fig. 2**). The biological effect of the accumulating ACh tend to be of short duration of hours rather than days or weeks as observed in organophoshate [48].

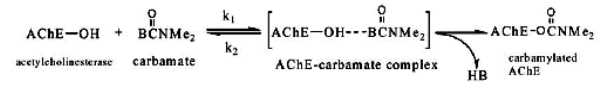

Fig. 2. Inhibition scheme of AChE by carbamate pesticides [46].

Eserine as a potent inhibitor to AChE

Eserine is probably the best known of all anticholinesterase. It is a alkaloid containing a metyl carbamino group and is obtained from Calabar beans, the seeds of the West African vine *Physostigmina venosum* Balfouri. These seeds are also known as Ordeal beans and as Esere nuts, hence the use of eserine as an alternative term for physostigmine [11]. Eserine is an extremely potent anticholinesterase and has two main uses as an experimental tool. In bioassay it will prevent the cholinesterase of the test-organ from hydrolyzing any ACh or ATC in the solution under test. Similarly, in physiological investigations of cholinergic mechanism it will preserve endogenous ACh and other cholinomimetics which are injected into the animal locally or systemically [49].

Therefore without such an inhibitor, cholinesterase susceptible drugs would be hydrolyzed before they could exert their effects [47]. The second important laboratory use of eserine is in biochemical or histochemical studies where it is necessary to distinguish reactions which may be due to cholinesterase from those due to other, eserine resistant esterases (BuChE) [50]. For most vertebrate species a concentration of 10^{-5} M is more than adequate to inhibit all cholinesterase activity in tissues in vitro.

AChE as biosensor and biomarker

Men's interest in pollution being centred on its effects on living organisms, there has been a rapid international growth of environmental monitoring over the past two decades [51]. The understanding and predictions of the reactions and influences of chemicals (toxicants) in and on the environment have become important issues in environmental quality assessment [52]. Until recently, monitoring and assessing of the "health" of the aquatic environment was mainly based on chemical and physical measures of water quality. A limitation to this way of monitoring the environmental health is that physical and chemical information is based towards the momentary conditions that exist at the time of sample collection. This often means missing the short-term events that may be critical to ecosystem health [51].

The sub-lethal levels of pollutants usually cause biochemical or physiological effects at the basic level of organisation, the sub-cellular level in an organism. Data on these sub-lethal effects would help in identifying the toxicant cause the effects before dramatic changes (e.g. mass mortality) occur in the natural population in the aquatic ecosystem. Before death of the organism can occur normal physiological processes are affected and death being a too extreme criteria for determining whether a substance is harmful or not, it is important to find bio-sensor/biomarkers of health and sub-lethal toxicant effects [46,47].

A biosensor is defined as a change induced by a contaminant in the biochemical or cellular components of a process, structure or function, which can be a sensor in a biological system. A biomarker is an indicator in a living organism that reflects sub-lethal molecular and/or cellular changes (biochemical, physiological and histological) occurring along a metabolic pathway as a result of exposure to toxicant or physiological changes [53,54]. Cholinesterases have been considered as interesting biomarkers and biosensor for many years in the monitoring of environmental contamination [42,55]. This is sensitive to selected organophosphate and carbamate pesticides and may be responding to low levels of contaminants in the environment, putatively by compounds other than or in addition to pesticides [20] .

Traditional methods that used for the detection of insecticides are based on gas chromatography (GC) or high liquid chromatography (HPLC) coupled with mass selective detectors (MSD) [56]. Consequently, there is a growing interest in faster and more sensitive detection systems. As an alternative, AChE inhibition tests, and the AChE-biosensors in particular, have been shown to be suitable for the detection of insecticide [57] . Furthermore biological markers are sensitive, cost effective tools for identifying risks of environmental contamination [58].

The pioneering work of [59] and [60] focused on cholinesterase purification and inhibitor analysis, immobilization, assay development, has paved the way for many other groups involved in the development of different biosensor formats for neurotoxin detection. Generally the development of biosensor is not without its problem, the complexity of the nervous system and the multiplicity of the expression of the neurotoxic effects, along with the limited information on the mechanism of action at the target sites lead to the limited progress [46]. AChE-based biosensors give a sum parameter of AChE-inhibition without any qualitative or quantitative information about the individual analytes, i.e. different AChE-inhibiting insecticides cannot be measured selectively [54]. One approach to solve this problem involves the application of multi- sensor arrays that are combined with the data processing of artificial neural networks. Genetically engineered cholinesterase variants with specific and high inhibition constants for the desired analytes are required for the compound-specific multianalyte detection[61].

In the 1970s a relatively new concept in aquatic environmental studies arose, that analysis of changes in various physiological and biochemical parameters in resident biota could be valuable in assessing the effects of chemical contamination. Biomarkers raise the possibility of determining where an organism is located on the health-disease continuum and so enhance the ability to assess any risk on the health and survival of contaminant-exposed population [62]. There is also a need for biomarkers for assessing effects on immune and reproductive systems and neural functions. With respect to neural functions, one of the biomarkers of interest is the enzyme AChE [63]. Many organophosphate and carbamate pesticides are effective AChE inhibitors and the inhibition of this enzyme has been used to assess the nature and extent of the exposure of wildlife and humans to agricultural and forestry sprays [64].

AChE is recognized to be one of the oldest environmental biomarkers [65]. There are various advantages of biomarkers in pollution monitoring and have been identified as follows: A temporally and spatially integrated measure of bioavailable pollutants are provided by biomarkers; Some biomarkers show very specific responses and through this they attribute exposure and risk to environmental pollutants; By applying different biomarkers to species from different habitats and different trophic habitats, they help establishing the importance of different routes of exposure; They can provide information on the relative toxicities of specific chemicals and effluents; Biomarkers are applicable in the laboratory as well as in the field [66].

Assessments have commonly dealt with point sources of pesticide exposure, where the probability for contamination and enzyme inhibition are likely. However, a recent study in Europe has produced evidence for variation of AChE in the tissues of flounder over a large geographical area, namely along a pollution gradient in the North Sea [67]. Whether the inhibition observed is due to pesticides, a combination of pesticides and other factors, or other factors alone is not known. Gender, reproductive status, age and diet are among the factors that can influence the biomarker response of many invertebrates. Accordingly, the study has attracted considerable attention indicating that AChE could be greatly expanded for use as an environmental biomarker [9].

CONCLUSION

The use of acetylcholinesterase (AChE) as a biosensor and biomarker has been reviewed. There is too much pesticides load in to the environment mainly from agricultural practices which is disastrous to non-target animals especially in the aquatic environment. Pesticides inhibit the acetylcholinesterase enzyme by biding with its active site and prevent the breakdown of acetylcholine leading to the blockage of synaptic transmission of cholinergic nerves. In respect to that, cholinesterase enzyme is been considered as good biosensor and biomarker of aquatic pollutants particularly pesticides.

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