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Electrometric measurement of plasma and tissue cholinesterase activities of four wild birds in Iraq

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Determination of cholinesterase activity is an important tool for biomonitoring environmental exposure of wild birds to cholinesterase inhibiting pesticides such as organophosphates and carbamates. In the present study an electrometric method is described and used for measurement of cholinesterase activities in the plasma, brain, liver and pectoralis muscle of four indigenous wild birds commonly found in Northern Iraq. The birds examined were quail (*Coturnix coturnix*), large pin-tailed sand grouse (*Perocles alchata caudacutus*), starling (*Sturnus vulgaris*) and rock dove (*Columba livia gaddi*). The mean values of cholinesterase activity (ΔpH/30 min) in the plasma, brain, liver and pectoralis muscle of the birds were, respectively, as follows: quail (1.23, 0.39, 0.19 and 0.06), large pin-tailed sand grouse (1.81, 0.37, 0.06 and 0.07), starling (1.1, 0.24, 0.08 and 0.08) and rock dove (1.28, 0.59, 0.12 and 0.08). Using the technique of *in vitro* cholinesterase inhibition by quinidine sulfate, the estimated percentages of true cholinesterase activity in the plasma of the quail, large pin-tailed sand grouse, starling, and rock dove were 77, 69, 71 and 73%, respectively. These findings are the first report of plasma and tissue cholinesterase activities of the four wild birds in Iraq, using the described electrometric method. The electrometric values of cholinesterase activity of the wild birds could be used as reference points for future studies concerning biomonitoring of exposure of these birds to anticholinesterase pesticides.

Key words: Biomonitoring, cholinesterase, electrometric method, organophosphate, wild birds.

INTRODUCTION

Measurement of serum or plasma and brain cholinesterase (ChE) activities in wild birds is used to diagnose and monitor their exposure to organophosphate and carbamate insecticides (Marden *et al*., 1994; Burn & Leighton, 1996; Iko *et al*., 2002; Osten *et al*., 2005). Reduced enzyme activity is an indicator of exposure and adverse effects of anti ChE insecticides even in the absence of overt signs of toxicosis or lethality (Wilson & Henderson, 1992; Wilson, 1998, 1999). These insecticides inhibit acetylcholinesterase activity in the nervous tissue causing accumulation of acetylcholine at the nerve endings with subsequent appearance of signs of toxicosis characterized by muscarinic, nicotinic and central nervous system effects (Osweiler, 1996; Wilson, 1998). The extent of ChE inhibition and the appearance of signs of toxicosis depend on many factors such as the type of the ChE inhibiting compound, amounts exposed to, duration, frequency and route of exposure and species variation (Osweiler, 1996; Wilson, 1998, 1999; Wilson *et al*., 1998).

Various colorimetric and electrometric methods are available for measurement of blood and tissue ChE activities (Fairbrother *et al*., 1991; Wilson, 1999). Electrometric methods are widely used in veterinary practice because of their simplicity, accuracy and they do not require sophisticated equipment and laboratory supplies (Mohammad & St. Omer, 1982; Osweiler *et al*., 1985; Imerman, 1993). Recently, a modified electrometric ChE method has been validated and used in several animal species (Mohammad *et al*., 1997, 2002; Al-Jobory & Mohammad, 2004; Ahmed & Mohammad, 2005) as well as in the chicken (Faris *et al*., 1999; Abass & Mo-

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hammad, 2004; Mohammad & Al-Baggou', 2005). The method is based on measurement of the decrease in pH of the enzymatic reaction mixture as a result of hydrolysis of the substrate acetylcholine iodide or acetylthiocholine iodide and the production of acetic acid (Mohammad *et al*., 1997; Ahmed & Mohammad, 2005). The procedure is simple and efficient in detecting ChE inhibition induced by both organophosphates and carbamates (Mohammad *et al*., 1997; Ahmed & Mohammad, 2005; Mohammad & Al-Baggou', 2005). It also correlates well with the original electrometric method of Michel (Mohammad *et al.*, 1997) and with the colorimetric method of Ellman (Abass & Mohammad, 2004).

The described electrometric method, however, has not been used in measuring plasma and tissue ChE activities in wild birds. Therefore, the purpose of the present study was to apply the previously described electrometric method (Mohammad *et al*., 1997) for the measurement of plasma and tissue ChE activities in four indigenous wild birds in northern part of Iraq. This is an initial attempt for establishing normal ChE values in these birds, since diagnostic interpretation of ChE data requires knowledge of normal ChE values of the wild birds (Hooper, 1988; Marden *et al*., 1994; Wilson, 1999).

MATERIALS AND METHODS

Studied organisms and plasma and tissue preparation

The wild birds investigated in the present study were quail (*Coturnix coturnix*), large pin-tailed sand grouse (*Perocles alchata caudacutus*), starling (*Sturnus vulgaris*) and rock dove (*Columba livia gaddi*). Adult birds of both sexes from each species, captured at nearby regions of Mosul, Iraq, were examined for the determination of ChE activities. There were no histories of insecticide applications in the regions when the birds were captured during winter of 2002, and the birds were apparently healthy. Birds from each species were kept in captivity, for 3-7 days before experiments under laboratory conditions at a temperature of 20 ± 2˚C with water and poultry feed available *ad libitum*. All experiments complied with regulations addressing animal use, and proper attention has been given to ethical consideration towards the birds used in the present study.

The wild birds (N = 10 individuals per species) were euthanized by decapitation and blood samples were collected using heparinized test tubes (Coles, 1986). Plasma was separated from erythrocytes by centrifugation at 3000 rpm (Centurion, U.K.) for 15 min. The whole brain and samples of the liver and pectoralis muscle were also obtained from the birds. All samples were kept frozen at -20˚C pending analysis within two weeks.

Electrometric procedure for measurement of ChE activity

A modified electrometric method (Mohammad *et al*., 1997) as further described for chicken ChE (Faris *et al*., 1999; Abass & Mohammad, 2004) was used. For plasma ChE determination, the enzymatic reaction mixture in a 10-ml beaker contained 3 ml distilled water, 0.2 ml plasma and 3 ml barbital-phosphate buffer. The buffer solution (pH = 8.1) consisted of 1.237 g sodium barbital (BDH, U.K.), 0.63 g potassium dihydrogen phosphate (E-Merck, Darmstadt, Germany) and 35.07 g sodium chloride (BDH, U.K.) in 1 L of distilled water (Mohammad *et al*., 1997). The mixture was incubated at 37˚C for 30 min and the pH of the mixture (preincubation-pH1) was measured with a glass electrode of a pH meter (Phillips, U.K.). The reaction was started by adding 0.12 ml of 7.5% aqueous solution of acetylthiocholine iodide (BDH, U.K.) to the mixture. At the end of the 30 min-incubation period, the pH of the reaction mixture (pH2) was measured. As control, a mixture without containing plasma was used. The pH of control was measured at 0 min and after 30 min of incubation at 37˚C. Plasma ChE activity was calculated as follows:

$$\text{ChE activity } (\Delta pH/30 \text{ min}) = (pH1 - pH2) - \Delta pH \text{ of blank}$$

For brain, liver and pectoralis muscle ChE activities, the tissues were separately homogenized in the barbital-phosphate buffer solution (pH = 8.1) at 3 ml per 100 mg wet weight with a teflon homogenizer (Karl Kolb, Germany) using 25% of the maximum velocity of the homogenizer. Homogenization was performed on an ice bath, and all tissue homogenates were kept on ice before ChE determination. For determination of brain, liver and muscle ChE activities, 0.2 ml of the tissue homogenate was used instead of the plasma aliquot in the enzymatic reaction mixture described above.

Determination of true ChE activity in the plasma

Aliquots (0.2 ml) of the same plasma samples of the above mentioned wild birds were incubated in separate reaction mixtures with 40 µl of 0.1% quinidine

sulfate (Sigma Chemical Co., St. Louis, USA) for 10 min at 37°C to inhibit pseudo ChE activity (Mohammad *et al*., 1997). Thereafter, the remaining (true ChE) activity in the plasma was measured as described before; pseudo ChE activity = ChE activity (without quinidine) - true ChE activity (with quinidine). Quinidine specifically inhibits pseudo ChE activity in the plasma (Farage-Elawar & Francis, 1988; Wilson, 1999).

The mean, standard deviation, standard error, range and 95% confidence interval of plasma, brain, liver and muscle ChE activities were determined (Petrie & Watson, 1999).

RESULTS

Table 1 shows the normal reference range values, 95% confidence interval and related statistics for plasma, brain, liver and pectoralis muscle ChE activities of the four bird species studied. Using the technique of *in vitro* ChE inhibition for 10 min by 0.1% quinidine sulfate, the estimated percentages of true ChE activity in the plasma of the quail, large pin-tailed sand grouse, starling, and rock dove were 77, 69, 71 and 73%, respectively (Table 2).

TABLE 1. Cholinesterase activity values for plasma, brain, liver and pectoralis muscle as determined by the described electrometric method in the quail (*Coturnix coturnix*), large pin-tailed sand grouse (*Perocles alchata caudacutus*), starling (*Sturnus vulgaris*) and rock dove (*Columba livia gaddi*). N=10 individuals per species

	Cholinesterase activity (ΔpH/30 min)			
	Plasma	Brain	Liver	Muscle
Quail				
Mean	1.23	0.39	0.19	0.06
Standard error	0.098	0.013	0.021	0.004
Standard deviation	0.310	0.040	0.066	0.013
95% confidence interval	1.008, 1.452	0.360, 0.418	0.124, 0.146	0.050, 0.068
Range	1.71-0.72=0.99	0.45-0.33=0.12	0.34-0.09=0.25	0.08-0.04=0.04
Large pin-tailed sand grouse				
Mean	1.81	0.37	0.06	0.07
Standard error	0.109	0.025	0.009	0.005
Standard deviation	0.345	0.081	0.029	0.016
95% confidence interval	1.512, 2.068	0.304, 0.434	0.037, 0.068	0.054, 0.077
Range	2.58-1.44=1.14	0.51-0.25=0.26	0.12-0.01=0.11	0.09-0.04=0.05
Starling				
Mean	1.10	0.24	0.08	0.08
Standard error	0.074	0.029	0.006	0.008
Standard deviation	0.235	0.093	0.019	0.025
95% confidence interval	0.936, 1.272	0.174, 0.308	0.066, 0.081	0.057, 0.093
Range	1.56-0.84=0.72	0.38-0.12=0.26	0.11-0.04=0.07	0.13-0.04=0.09
Rock dove				
Mean	1.28	0.59	0.12	0.08
Standard error	0.08	0.026	0.010	0.008
Standard deviation	0.256	0.084	0.030	0.025
95% confidence interval	1.100, 1.466	0.525, 0.585	0.094, 0.138	0.062, 0.099
Range	1.70-0.85=0.85	0.75-0.45=0.28	0.17-0.07=0.10	0.14-0.05=0.09

TABLE 2. Estimation of true cholinesterase activity as determined by the described electrometric method in the plasma of quail (*Coturnix coturnix*), large pin-tailed sand grouse (*Perocles alchata caudacutus*), starling (*Sturnus vulgaris*) and rock dove (*Columba livia gaddi*)

Bird	Variable	(ΔpH/30 minutes)	% activity
Quail	Total cholinesterase	1.230±0.093	100
	True cholinesterase*	0.950±0.069	77
	Pseudo cholinesterase	0.280±0.054	23
Large pin-tailed sand grouse	Total cholinesterase	1.810±0.109	100
	True cholinesterase*	1.240±0.076	69
	Pseudo cholinesterase	0.570±0.126	31
Starling	Total cholinesterase	1.100±0.074	100
	True cholinesterase*	0.780±0.025	71
	Pseudo cholinesterase	0.320±0.042	29
Rock dove	Total cholinesterase	1.280±0.080	100
	True cholinesterase*	0.930±0.063	73
	Pseudo cholinesterase	0.350±0.028	27

Cholinesterase values are the mean±SE of 10 plasma samples for each of bird species
*Quinidine sulfate was used to inhibit pseudo cholinesterase activity in the plasma

DISCUSSION

The present report introduces for the first time normal ChE activities of the plasma, brain, liver and pectoralis muscle of four indigenous wild birds (quail, large pin-tailed sand grouse, starling and rock dove) as determined by a simple modified electrometric method (Mohammad *et al*., 1997). These values could be preliminary reference values for future studies using the described electrometric method for rapid measurement of plasma and tissue ChE activities in wild birds described in the present study. Determination of plasma or serum and tissue (especially brain) ChE activities in wild birds is used as a biomarker of exposure to organophosphate and carbamate insecticides which are widely used in agriculture (Marden *et al*., 1994; Burn & Leighton, 1996; Iko *et al*., 2002; Osten *et al*., 2005). The described method has been applied successfully to evaluate poisoning induced by organophosphate and carbamate insecticides in chickens (Abass & Mohammad, 2004; Mohammad & Al-Baggou', 2005) as well as in rodents (Al-Baggou' & Mohammad, 1999; Mohammad *et al*., 2002; Ahmed & Mohammad, 2005).

The described method was also used to estimate the level of true ChE activity in the plasma of the wild birds. The reported values in the present study correlate with those reported in wild birds by other researchers (Wilson *et al*., 1998; Wilson, 1999). Comparatively, true ChE activity in the plasma of mammals (about 10-30%) is much lower than those of the wild birds (Wilson *et al*., 1998; Wilson, 1999; Ahmed & Mohammad, 2005). It might be differentially inhibited by organophosphate or carbamate pesticides (Osweiler *et al*., 1985; Osweiler, 1996; Wilson, 1998, 1999; Wilson *et al*., 1998).

There were physiological differences in the values of plasma and tissue ChE activities across (as well as within) the species of the birds of the present study. Such differences were reported earlier in wild birds and mammals, and they might form the basis of the differential responses of the ChEs to organophosphates and carbamates (Fairbrother & Bennet, 1988; Hooper, 1988; Wilson *et al*., 1998; Wilson, 1999).

The 30-min one step incubation time and the 0.2 ml sample volume in the described method of the present study appeared to be suitable for the enzymatic assay condition in the wild birds. This is in agreement with earlier findings using the described assay conditions in chickens (Faris *et al*., 1999; Abass & Mohammad, 2004; Mohammad & Al-Baggou', 2005) and mammals (Al-Baggou' & Mohammad, 1999; Mohammad *et al*., 2002; Ahmed & Mohammad, 2005). The one step of short incubation time of the described method would be useful in increasing the efficiency of the procedure for multiple samples when compared to more than 60 min of the original electrometric method of Michel (1949). Our tech-

nique also decreases substantially handling of the reaction mixture compared with other electrometric methods (Mohammad & St. Omer, 1982; Mohammad *et al*., 1997).

In conclusion, the present study reports normal values of plasma, brain, liver and pectoralis muscle ChE activities in four indigenous wild birds (quail, large pin-tailed sand grouse, starling and rock dove) in Northern Iraq as determined by a simple electrometric method. These values could be reference points for future studies concerning biomonitoring of exposure of these birds to anti ChE pesticides.

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