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INHIBITORY ACTION OF THE DEFENSIVE DISCHARGE OF THE GRASSHOPPER, *POECILOCERUS PICTUS*, ON CERTAIN ENZYMES IN THE LIZARD, *CALOTES NEMORICOLA*

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Administration of the defensive secretion of the grasshopper, *Poecilocerus pictus* inhibited acetylcholinesterase (AChE) and adenosine triphosphatases (ATPases) in the brain and muscle tissues of the garden lizard, *Calotes nemoricola*. The inhibition was gradual, continuous and irreversible with lethal doses of the defensive secretion, whereas the inhibition observed with sublethal doses was followed by an increase towards control levels within 24 h after injection. *In vitro* application of defensive secretion also showed concentration-dependent inhibition in the activity of AChE and ATPases in the tissue homogenates. Inhibition in AChE activity might be a factor for the observed mortality in the defensive fluid-treated lizards. Since the cardenolides are known to inhibit the activity of ATPases, the inhibition in the activity of ATPases observed in the present study suggests the presence of cardenolides in the defensive fluid of *P. pictus*.

Keywords: Grasshopper; lizard; acetylcholinesterase; adenosine triphosphatase; inhibition.

INTRODUCTION

Natural toxins such as scorpion venom,[1,2] mycotoxin[3] and cytotoxin,[4] and chemical compounds such as monocrotophos,[5] phosalone[6] and propoxur[7] have been shown to inhibit the activity of the enzymes acetylcholinesterase (AChE) and adenosine triphosphatases (ATPases). Acetylcholinesterase is present in high concentration in the nervous tissues of both vertebrates and invertebrates[8] and the presence of AChE has also been reported in several non-excitable tissues, such as red blood cells.[9] Adenosine triphosphatases represent a complex enzymatic system requiring Mg^{2+}, Ca^{2+}, Na^{+} and K^{+} ions for their activity. Magnesium adenosine triphosphatase

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(Mg^{2+}-ATPase) is localised in the mitochondria and presumed to be present in all types of cells.[10] It has been shown to participate in oxidative phosphorylation and thus has a unique role in energy production.[11] Na^+, K^+-stimulated ATPase (Na^+, K^+-ATPase) on the other hand is shown to regulate the active ion transport across the cell membrane[12] and therefore plays a key role in nerve conduction.

The present investigation is an attempt to study the effect of the defensive fluid of the grasshopper, *Poecilocerus pictus* on the activity levels of the enzymes AChE and ATPases in the brain and leg muscle of the garden lizard, *Calotes nemoricola*.

MATERIALS AND METHODS

The late instar (3rd and 4th) grasshoppers, *Poecilocerus pictus* were stimulated electrically (single pulse of 100 V, 5 mV) for the release of defensive secretion. The defensive discharge was collected into a glass vial and diluted with physiological saline.

The garden lizards, *Calotes nemoricola* (Jerdon) collected locally, were acclimatised to laboratory conditions ($28 \pm 2°C$ and LD 12:12) for a week prior to the experiments. The animals were fed with cockroaches daily on a fixed time schedule. Male lizards of uniform size (20 ± 3 gms) were chosen for the present study and different doses of defensive secretion were injected into the thigh muscle. The fifty per cent lethal dose for a period of 24 h was determined by the method of Finney,[13] and 1/3rd of the lethal dose (0.0824 μl/g body weight) was considered as a sublethal dose. Control lizards were injected with equal volumes of physiological saline and were maintained separately. The animals were dissected for the isolation of the brain and muscle from the thigh region of leg. The experiments were performed at different time periods following the administration of the defensive secretion. *In vitro* studies were also made with different concentrations of the defensive secretion. AChE activity was assayed following the method of Ellman *et al.*,[14] and Mg^{2+} and Na^+, K^+-ATPase activity was estimated by the method of Tirri *et al.*[15] The data was analysed using ANOVA (Analysis of Variance) and SNK (Student-Newman-Keul) test.[16]

RESULTS

In vivo administration of lethal doses of *P. pictus* defensive secretion produced inhibition in AChE activity continuously upto 24 h in the brain and leg muscle of lizards whereas the inhibition in enzyme activity due to sublethal doses was seen upto 6 h in brain and 12 h in muscle followed by an increase towards control levels by 24 h (Table I).

TABLE I AChE (Acetylcholineterase) activity levels (expressed as μ moles of ACh hydrolysed/mg protein/h) in brain and muscle tissues of the garden lizard, *C. nemoricola* at different time periods after injecting lethal and sublethal doses of the defensive fluid of the grasshopper, *P. pictus*.

Tissue	*Control*	*1 h*	*3 h*	*6 h*	*12 h*	*24 h*
Lethal						
Brain	13.330	12.610*	10.042	7.740	6.343	5.900
SD ●	0.887	0.520	0.612	0.867	0.515	0.672
		(−5.40)	(−24.79)	(−41.93)	(−52.42)	(−55.74)
Muscle	1.140	0.931*	0.808*	0.668	0.601	0.606
SD ±	0.191	0.010	0.004	0.006	0.018	0.024
		(−18.33)	(−29.12)	(−41.40)	(47.28)	(−46.84)
Sublethal						
Brain	13.330	12.813*	11.490*	9.890	11.560*	12.200*
SD ±	0.887	0.641	0.540	0.986	0.913	0.615
		(−3.88)	(13.80)	(−25.81)	(−13.28)	(−8.48)
Muscle	1.140*	0.976*	0.915*	0.898*	0.868*	0.964*
SD ±	0.191	0.008	0.009	0.005	0.008	0.009
		(−14.39)	(−19.74)	(−21.23)	(−24.30)	(−15.44)

The changes from the control are significant ($P < 0.05 - 0.001$) except the values marked with asterisk (*). All values are mean ± SD where $n = 6$. Values in parentheses represent percentage change over control.

TABLE II Mg^{2+}-ATPase activity levels (expressed as μ moles of Pi formed/mg protein/h) in brain and muscle tissues of the garden lizard, *C. nemoricola* at different time periods after injecting lethal and sublethal doses of the defensive fluid of the grasshopper, *P. pictus*.

Tissue	*Control*	*1 h*	*3 h*	*6 h*	*12 h*	*24 h*
Lethal						
Brain	15.490	13.120	12.820	10.680	9.190	9.320
SD ±	0.559	0.329	0.300	0.632	0.323	0.547
		(−15.30)	(−17.24)	(−31.05)	(−40.67)	(−39.83)
Muscle	18.960	16.168	13.250	10.120	9.930	8.060
SD ±	0.672	0.470	0.477	0.584	0.283	0.272
		(−14.73)	(−30.12)	(−46.62)	(−47.63)	(−57.49)
Sublethal						
Brain	15.490	17.540	14.650*	13.560	13.780	14.360*
SD ±	0.559	0.439	0.367	0.402	0.395	0.347
		(−13.23)	(−6.01)	(−12.46)	(−11.04)	(−7.30)
Muscle	18.960	21.960	16.203	14.300	15.670	18.670*
SD ±	0.672	0.881	0.410	0.505	0.771	0.634
		(15.82)	(−14.54)	(−24.58)	(−17.35)	(−1.53)

The changes from the control are significant ($P < 0.05 - 0.001$) except the values marked with asterisk (*). All values are mean ± SD where $n = 6$. Values in parentheses represent percentage change over control.

TABLE III Na^+, K^+-ATPase activity levels (expressed as μ moles of Pi formed/mg protein/h) in brain and muscle tissues of the garden lizard *C. nemoricola* at different time periods after injecting lethal and sublethal doses of the defensive fluid of the grasshopper *P. pictus*.

Tissue	*Control*	*1 h*	*3 h*	*6 h*	*12 h*	*24 h*
Lethal						
Brain	11.360	9.730*	8.120	6.370	5.210	5.010
SD ±	0.865	0.654	0.588	0.804	0.658	0.487
		(−14.35)	(−28.52)	(−43.96)	(−54.14)	(−55.89)
Muscle	8.490	8.060*	7.580	7.130	6.980	6.440
SD ±	0.314	0.472	0.633	0.432	0.382	0.476
		(−5.06)	(−10.72)	(−16.02)	(−17.78)	(−24.15)
Sublethal						
Brain	11.360	10.800*	8.830	7.570	7.840	9.130
SD ±	0.865	0.277	0.228	0.189	0.198	0.233
		(−4.93)	(−22.27)	(−33.36)	(−30.99)	(−19.63)
Muscle	8.490	8.230*	7.650	6.863	7.010	7.930*
SD ±	0.314	0.165	0.127	0.232	0.094	0.205
		(−3.06)	(−9.89)	(−19.20)	(−17.43)	(−6.60)

The changes from the control are significant ($P < 0.05 - 0.001$) except the values marked with asterisk (*). All values are mean ± SD where $n = 6$. Values in parentheses represent percentage change over control.

Application of lethal doses of defensive fluid, exhibited a gradual, continuous and irreversible decrease in Mg^{2+}-ATPase activity (Table II). The enzyme activity dropped significantly in both tissues within 1 h after exposure. Unlike lethal doses, the sublethal doses of fluid showed an initial increase in Mg^{2+} ATPase activity followed by a reversible decrease (Table II).

Similar to Mg^{2+} -ATPase activity, Na^+, K^+ -ATPase activity also showed a continuous inhibition in the brain and muscle tissues of lizards injected with lethal doses of defensive secretion. The inhibition in the enzyme activity observed with sublethal doses however showed recovery toward control levels by 24 h (Table III).

In vitro application of defensive secretion of *P. pictus* to the brain and leg muscle homogenates (0.5 ml of 2%) of *C. nemoricola* showed concentration-dependent inhibition in both AChE and ATPase activities. The inhibition in enzyme activities was more pronounced at concentrations > 0.001 μl (26% and 21% in AChE activity, 46% and 51% in Mg^{2+} -ATPase activity and 42% and 39% in Na^+, K^+ ATPase activity of brain and muscle respectively). The inhibition of ATPase activity was found to be greater than that of AChE activity.

DISCUSSION

The diminished AChE activity observed in the present study correlates well with the findings of the earlier studies using animal toxins[1,3] and pesticides.[5,6] Excessive accumulation of ACh at synaptic clefts causes restlessness, tremors and convulsions[17] and some of these symptoms were observed in lizards which received the defensive fluid of *P. pictus*. Furthermore AChE, a membrane-bound enzyme, may decrease due to the disintegration of end plates.[18] The inhihition of AChE activity further leads to excessive accumulation of the neurotransmitter ACh at the synaptic junctions thereby disrupting cholinergic synaptic transmission.[19]

The decrease in Mg^{2+}-ATPase in the lizards due to the defensive fluid of *P. pictus* can be ascribed to mitochondrial damage by the toxic principles of the defensive fluid. The activities of phosphorylation enzymes have been shown to be disturbed by toxic chemicals such as propoxur[7] and patulin.[20] The defensive fluid of grasshopper species contains cardenolides as the chief toxic principles. The decreased Na^+, K^+ -ATPases activity could be attributed to the direct inhibitory action of cardenolides since cardenolides have been reported to be inhibitors of Na^+, K^+-ATPases[21] and furthermore the presence of cardenolides (calactin and calotropin) has already been reported in the defensive secretion of the grasshopper, *Poekilocerus bufonius*.[22] The inhibition of Na^+, K^+-ATPase alters the active ion transport across the cell membrane. The observed *in vitro* inhibition in AChE and ATPases suggests that the inhibitory effect of defensive secretion is greater on ATPases. The possible direct inhibitory action of the defensive secretion on the ATPases could also influence indirectly the activity of AChE and thereby impair the normal neuronal functions of predator animals exposed to the defensive discharge.

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