On the possible mode of action of neurohormones on cholinesterase activity in the ventral nerve cord of scorpion, Heterometrus fulvipes

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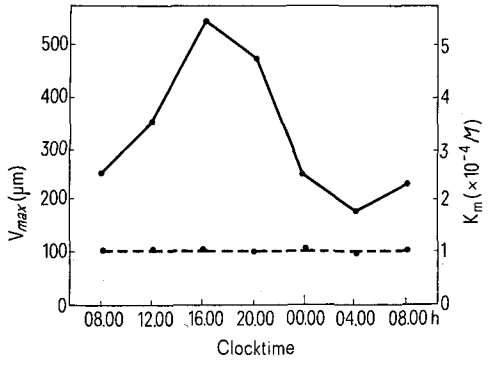
Summary. It is shown that K_m of ChE is not affected by the neurohormones but V_{max} is increased and decreased in presence of acceleratory and inhibitory neurohormones respectively. Hence it is suggested that the neurohormones might modulate the enzyme activity by altering the maximal velocities (V_{max}) rather than affecting the enzyme affinity (K_m) towards the substrate.

The enzyme, Cholinesterase (ChE), is found in different parts of the nervous system and innervated organs of the scorpion and the level of activity is higher in the nervous tissue[3]. The enzyme activity in the ventral nerve cord shows a diurnal rhythmicity, being maximum at 16.00 h and minimum at 4.00 h[4]. In view of the occurrence of a similar diurnal rhythmicity in the spontaneous electrical activity of the central nervous system[5-7] and its modifiability by the 2 types of neurohormones produced in the cephalothoracic nerve mass (CTNM) out of phase with each other[8], it is suggested that the rhythmic activities of the animal are regulated by the neurohormones through their influence on electrical activity, thereby determining the central excitability of the animal[8]. A recent study showed that the neurohormones also modulate the ChE activity of the ventral nerve cord, and hence it is suggested that the neurohormones influence the level of electrical activity through their effects on the enzyme activity[9]. However, it is not known how the neurohormones influence the enzyme activity. The present paper suggests the possible mechanism.

Effect of neurohormones on kinetic parameters of ChE activity in the ventral nerve cord of scorpion (the values are the averages of 6 determinations)

Neurohormone added	Control K_m	Control V_{max}	Experimental K_m	Experimental V_{max}
Acceleratory neurohormone (12.00 noon CTNM extract)	1.1×10^{-4} M	328	1.1×10^{-4} M	525
Inhibitory neurohormone (12.00 night CTNM extract)	1.2×10^{-4} M	348	1.2×10^{-4} M	158



K_m (●---●) and V_{max} (●——●) values for the ChE activity in the ventral nerve cord of scorpion measured at different times of the day.

Material and methods. The scorpion, Heterometrus fulvipes, was used. The nerve cords were isolated at different times of the day into cold scorpion ringer[10]. 3–4 cords were pooled and 1% (w/v) homogenates were prepared in 0.25 M sucrose solution. The enzyme activity was determined using the crude homogenate, acting as cell free systems, following the method of Metcalf, as suggested earlier[3]. The enzyme activity was studied at various substrate concentrations ranging from 1 to 10 µmoles. The protein content was determined by the method of Lowry et al.[11], and the enzyme activity was expressed as µM Ach hydrolyzed/mg protein/h. 2 samples were taken each time and the experiment was repeated for 3 days. The average values of 6 observations made each time for each concentration were used to construct the Lineweaver-Burke plots, and straight lines were fitted using the principle of least squares. K_m and V_{max} values were determined from the points of intercepts on X and Y axes respectively.

Results and discussion. The K_m and V_{max} values measured at different times of day are shown in the accompanying figure. It is evident that K_m value had been 1.1×10^{-4} M and it did not change with time of the day. V_{max} values, however, varied from 176 to 552 µM Ach hydrolyzed/mg protein/h. The value increased initially and reached maximum at 16.00 h. Thereafter, it declined gradually and came to minimum by 4.00 h. V_{max} values, thus followed a typical circadian rhythm.

K_m values for ChEs generally lie in the range of 1.3 to 9.0×10^{-4} M[12] and the present value is in the vicinity of this range, though determined with cell free systems.

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The K_m of the nerve cord ChE is lower than that of heart ChE[13] showing its greater affinity towards the acetyl ester. In fact, it is shown earlier that the ChE of nervous tissue is more active than that of the innervated organs like heart, pedipalpal muscle and blood[3].

Absence of variation in K_m values at different times of the day suggests that the enzyme affinity does not change with time. However, the fact that V_{max} changes at different times shows that the enzyme is capable of hydrolyzing the substrate at different rates, depending upon the time of the day. From the V_{max} values it might be stated that the catalytic activity of the enzyme to hydrolyze the substrate is maximum at 16.00 h and minimum at 4.00 h. It is also of interest to note that the general level of ChE activity also follows a similar trend[4]. Thus the higher level of activity at 16.00 h seems to be due to the high catalytic activity of the enzyme, and the lower level of activity at 4.00 h seems to be due to the lower catalytic activity of the same.

It is known that the excitatory neurohormone produced during daytime increases the enzyme activity, while the one produced during night-time decreases the same[9]. The ventral nerve cords isolated at different times of the day are possibly under the influence of these 2 neurohormones and the observed changes in the kinetic parameters might be due to the effect of these hormones. In vitro experiments were conducted to study the effect of neurohormones on K_m and V_{max} values. The CTNMs containing the acceleratory and inhibitory principles were collected at 12.00 noon and 12.00 midnight respectively and 1% (w/v) homogenates were prepared in cold scorpion ringer[10]; 0.1 ml of the extract was added to the incubation mixture, and the enzyme activity was determined. The K_m and V_{max} values determined in presence of the neurohormones are shown in the accompanying table. It is evident that the acceleratory principle present in 12.00 noon extract increased V_{max} while the inhibitory principle contained in 12.00 night extract decreased the same. In both the cases, however, K_m values did not change. These experiments clearly showed that the observed changes in the kinetic parameters of ChE are due to the influence of the neurohormones. Thus it might be stated that the neurohormones influence the enzyme activity by altering the maximal velocities rather than affecting the enzyme affinity towards the substrate.

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Auditory and visual perception of simultaneous verbal and nonverbal stimuli[1]

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Summary. In an auditory experiment, digits and tonal sequences were presented simultaneously to both ears. In a visual experiment, words and nonsense figures had to be compared in both visual half-fields. The verbal stimuli were better reported from the right ear and right visual half-field. The nonverbal stimuli were reported equally well from both ears and visual half-fields. It appears that the processing of stimuli presented to both input channels depends on the type of the stimuli. These results point to a cerebral mechanism classifying incoming information to the brain and yielding an optimal processing of verbal and nonverbal stimuli by the cerebral hemispheres.

In experimental paradigms such as dichotic listening[2,3] and tachistoscopic vision[4], verbal stimuli (e.g. digits, words) are better reported from the right ear and right visual half-field, whereas nonverbal stimuli (e.g. tonal sequences, nonsense figures) tend to be better reported from the left ear and left visual half-field[5-7]. These findings are mostly interpreted in terms of the functional specialization of the left cerebral hemisphere for verbal information, and of the right hemisphere for nonverbal information, taking into account the connections of ears and visual half-fields with the opposite hemispheres[8,9]. On anatomical grounds, there are good reasons to assume an interaction between both sides of the brain, at either the cortical or subcortical level[10]. The function of this interaction could be the optimal distribution of information to different cerebral structures according to the functional specialization of the cerebral hemispheres[11]. Verbal information would thus be directed to the left, nonverbal information to the right hemisphere.

The present study aimed at a better definition of the interhemispheric interaction, by presenting verbal and nonverbal stimuli simultaneously to both ears and to both visual half-fields.

Table 1. Auditory experiment: average number of correct responses (N = 38)

Stimuli	Ear right	left	t*	p
Part 1: sets of 3 digits	9.6	8.3	3.15	0.002
sequences of 5 tones (max. 12 each)	7.3	7.2	0.11	0.9
Part 2: sets of 3 digits (max. 24)	19.1	16.5	2.34	0.002
Part 3: sequences of tones (max. 24)	11.2	13.2	2.58	0.01

* Student's-test for paired data.

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