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Protocerebral Deafferentation Effects on Crayfish Glycemic Response: a Protocerebral Circadian Pacemaker Regulates the Hemolymph Sugar Concentration

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Similar to intact crayfish animals with an isolated protocerebrum exhibit a competent control for circadian variations of glucose concentration in the hemolymph. However, the sudden increase in glucose concentration induced by stressing influences in intact or partially deafferented animals dropped or became totally suppressed in the preparations with isolated protocerebrum.

Key words: Protocerebrum, Crayfish, Glycemia, Circadian pacemaker, Circadian rhythm.

The role of eyestalk neurohemal system in the regulation of glucose blood level in crustaceans has been under study since the pioneer work by ABRAMOWITZ *et al.* (1). The hyperglycemic effect of physiological, pharmacological, and toxicological agents upon a variety of decapod crustaceans has been tested (9, 14, 15, 18). In recent years, a crustacean hyperglycemic hormone (CHH) has been isolated from sinus gland. Its chemical composition (11, 14), has been sequenced and the site of its synthesis in the neuroendocrine system of *medulla terminalis* (6-8, 10) has been established. But the physiological control of hyperglycemia related to the integrity of the central nervous system of crayfish has not yet been determined. The goal of this study was to analyze the effects of neural disruption of brain sensory systems upon the protocerebral pacemaking structure which governs the glycemia of crayfish.

Materials and Methods

The experiments were carried out in 83 specimens of the crayfish *Procambarus*

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bouvieri of both sexes and with a 14 to 35 g body weight. The hemolymph glucose content was determined by means of an enzymatic method by BERGMEYER and BERNT (5). Except for 6 animals in which glucose was continuously determined, the observations were performed within 8 to 12 hours to minimize the effect of diurnal variations.

To study the CO_2 effect on hyperglycemic response, one group of crayfish was placed into a container (3L) with a 10 g piece of dry ice. In the second group autotomy of one of the claws promptly took place, after a puncture had been applied to the claw.

The third group was stimulated by asphyxia placing the animals in dry containers for 60 minutes after completely removing the water from their gills. This group was divided into three and a specific type of surgery was performed on each group: 1) the bilateral section of optic tracts; 2) the unilateral or bilateral section of circumesophageal connectives. 3) the complete removal of supraesophageal or cerebral ganglion. A fourth group in which the neural deafferentation of protocerebrum was performed (n = 12), served to explore the site of glucose pacemaker regulation. These surgical maneuvers were performed following a previously reported technique (2, 4).

Results

The mean blood glucose concentration of the crayfish (n = 30) in its natural environment measured 10.0 to 17.73 mg %. In our laboratory their glycemic level decreases from 10.6 to 8.15 mg % after the 5 and 16 days of captivity.

The effects of anaesthesia induced by carbon dioxide and the effects of asphyxia on the glucemia of intact crayfish are illustrated in figure 1. It shows that asphyxia (n = 5) led to a greater hyperglycemia than did the direct effects of anaesthesia (n = 4) which had been induced by carbon dioxide. The hyperglycemic response to carbon dioxide was 0.30 to 1.25 times higher than the control (fig. 1A) and from 2.75 to 5.0 times the control values in the animals subjected to asphyxia.

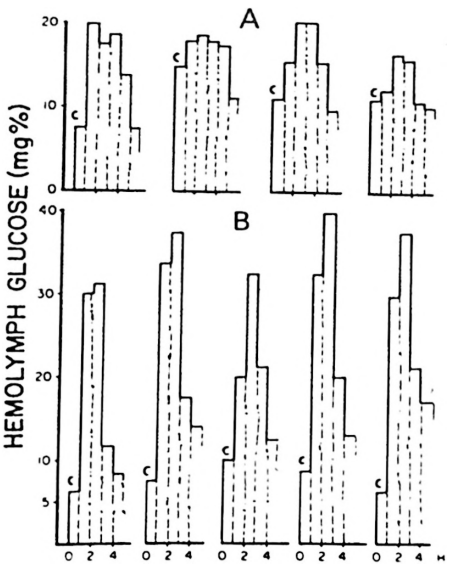

Fig. 1. *The stressing hyperglycemic response in anesthetized crayfishes under a satured carbon dioxide atmosphere (A) is compared with their hyperglycemic response induced by asphyxia (B), two days later.*
«C» represents control values.

In order to test the effects of the other sensory afferences upon the glycemia, autotomy was done (fig. 2) in four intact animals in which a clear increase (3 to 5 times) in sugar was obtained.

Asphyxia and autotomy in commissurectomized preparations. — In commissurectomized preparations of the crayfish there was a minimal glycemic response. But a clear increase in the glycemic response, from 2.6 to 6.0 times the control valves, was immediately obtained by the effects of both asphyxia and autotomy. This response demonstrates the additive

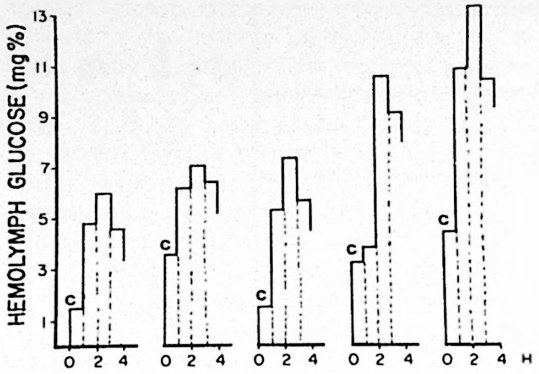

Fig. 2. *Hyperglycemic response in five animals under the effects of autotomy.*
«C» represents control values.

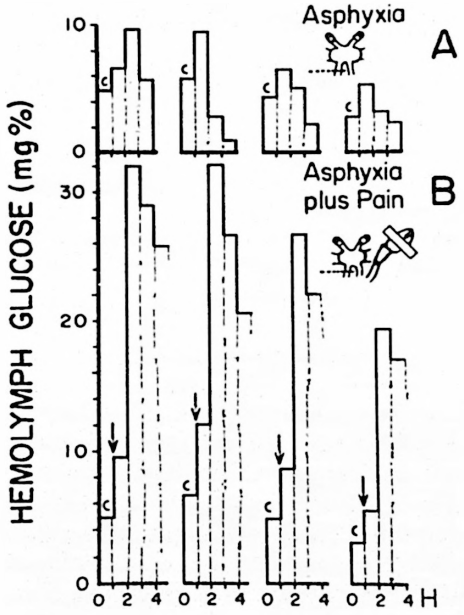

Fig. 3. *The simple asphyxia effects on crayfish glycemia (A) is compared with the same response after either claw was completely damaged (B) in animals with one circumesophageal commissure severed.*
«C» represents control values.

action of two stressful stimuli successively applied to the same animals (fig. 3B).

Partial dennervation of the eyestalks on crayfish glycemia. — Compared with the strong hyperglycemic response on intact (n = 4) animals (fig. 4A), those with restricted neural afference (n = 8) towards the neurohemal complex present a discrete hyperglycemia from 0.4 to 2.4 times the control values (fig. 4B, 4C). The animals with bilateral transection of circumesophageal connectives, kept their control on glycemic values almost unchanged after asphyxia (fig. 4D). The lack of any afferent neural connection to the main eyes talk neurohemal structures (fig. 4E) did not produce any hemolymphatic sugar change.

Protocerebral circadian regulation of crayfish glycemia (fig. 5). — In order to determine if those preparations with isolated protocerebrum still retained any

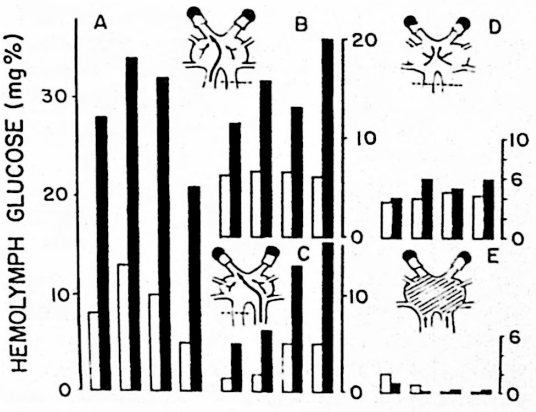

Fig. 4. *Compared with a partial (B, C, D) and complete suppression of neural afferences (E) upon hyperglycemic response to asphyxia, intact (A) asphyxiated crayfish depend on ipsilateral (B) and contralateral (C) thoracoabdominal and supraesophageal ganglion afferences alone (D).*
White and black bars represent the control and the hyperglycemic values respectively.

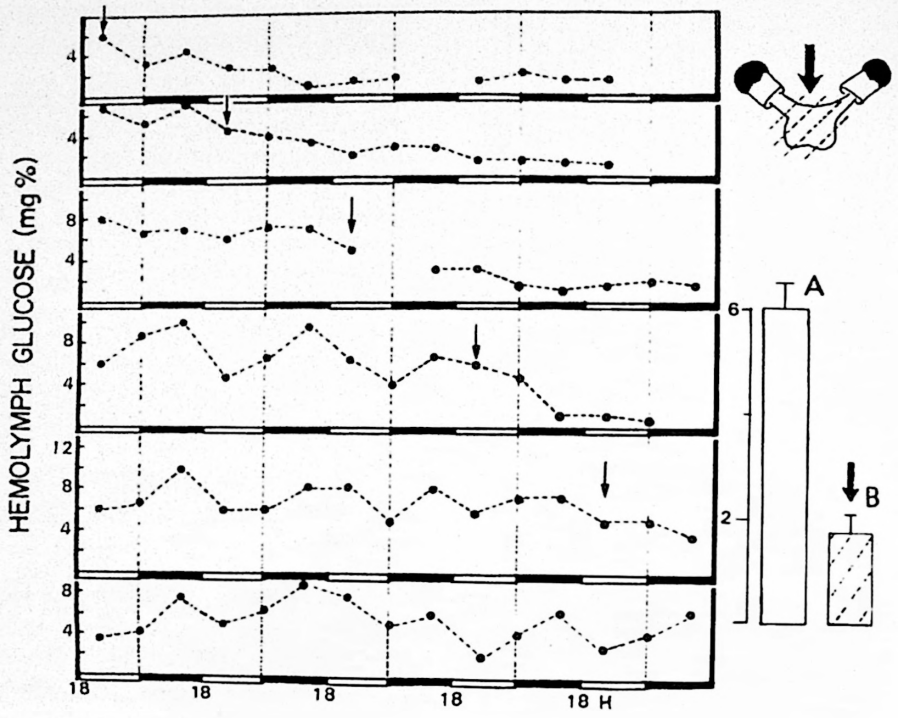

Fig. 5. *Cyclical variations of glycemic values (2-10 mg %) measured in six animals with neurally disconnected protocerebral structures, which finally were removed by surgery (↓).*
This maneuver abolished the apparent rhythmic variations of glycemia whose mean values (A) became also diminished from 6 (A) to 1.8 mg % (B). Standard deviations are signaled in A and in B.

kind of long term control for glucose concentration in hemolymph, the glycemic concentration was measured in a serial of regular samples (fig. 5A), during several days. Six out of twelve animals remained alive at the end of these experiments, while the others died, probably either by the stress of the seventh to ninth successive extraction of hemolymph, or by the aversive effects of surgery while the ablation of the protocerebrum was being practiced at different times of day. An apparent circadian rhythm of glycemia decreased or was totally absent as a consequence of the protocerebrum removal. In all these cases, the glucose concentration values were drastically reduced.

Discussion

From experiments carried out by Sttot (17) and Roche and Dumarzet (16), it is known that asphyxia results in a strong stimulus for the induction of hyperglycemia in crayfish. It is evident from figure 1B that 60 minutes of asphyxia in intact crayfish always induce an increase of 300 to 600 percent in glycemia which was then reversed three hours after the animals were replaced into their water containers. The weak glycemic response induced by carbon dioxide can be easily explained by the narcotic action of this substance at sensory, motor, and neuroendocrine system which normally can promote the

CHH release from the sinus gland. In contrast, the nocive stimulus effects as well as the important role of circumesophageal commissures on glycemic responses (figs. 2, 3, 4) are clearly demonstrated, as shown by the minimal or absent glycemic responses in animals with both circumesophageal commissures severed or in brainless crayfish. These data support the existence of a thoracic neural control for the glycemic response which by the redundant afferent sensory innervation of sinus gland assure an effective and intensive glycemic response in these invertebrates.

The slow variations of glycemia in circadian time during the day, clearly demonstrate that, as in the case of vision (2-4), the protocerebrum contains a similar pacemaker for the control of the metabolic rate through CHH. The apparent loss of eyestalk control of glucose concentration in hemolymph can be explained, therefore, by the potential damage that resulted from a second surgical procedure while the disconnected protocerebrum was totally removed.

Resumen

Como el camarón de río intacto, el animal con protocerebro aislado, muestra un notable control para sus variaciones nictamerales de glucosa en la hemolinfa. Sin embargo, el incremento súbito de la glicemia, inducido en los animales intactos por el estrés, es disminuido en animales con desaferentación parcial o anulado totalmente en animales con protocerebro aislado.

Palabras claves: Camarón de río, Protocerebro, Regulación de la glicemia, Marcapaso circádico, Ritmos biológicos.

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