Diurnal rhythms of sodium transport in the freshwater mussel

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Sodium and chloride concentrations in the blood of pondwater acclimated *Carunculina texasensis* on a 12 h light (L) : 12 h dark (D) photoperiod, undergo daily oscillations of 1 to 2 mmol/L. Changes in the active transport rate of Na are responsible for the changes in the blood sodium levels over the 24-h period. During the photophase, the blood level of Na and Cl is at a nadir and the clams are losing Na (net flux (J_n) = -0.36 μmol/g dry tissue·h^{-1}) due to a depressed Na influx (J_i = 0.30 μmol/g dry tissue·h^{-1}). The opposite response is observed during the scotophase; the blood concentration is significantly increased and the mussels are gaining sodium (J_n = 0.36 μmol/g dry tissue·h^{-1}) by increasing the influx of Na (J_i = 0.79 μmol/g dry tissue·h^{-1}).

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Les concentrations de sodium et de chlore dans le sang de moules d'eau douce *Carunculina texasensis* acclimatées à de l'eau d'étang à une photopériode 12 h lumière (L) : 12 h obscurité (O) subissent des oscillations quotidiennes de 1 à 2 mmol/L. Les changements que subit la vitesse du transport actif de Na sont responsables des variations de concentrations de Na dans le sang au cours d'une période de 24 h. Pendant la photophase, les concentrations de Na et de Cl du sang atteignent un minimum et les moules perdent du Na (J_n = -0.36 μmol/g tissu sec·h^{-1}) à cause de la chute de l'influx de Na (J_i = 0.30 μmol/g tissu sec·h^{-1}). Durant la scotophase, le phénomène inverse se produit; la concentration dans le sang augmente significativement et les moules contiennent plus de sodium (J_n = 0.36 μmol/g tissu sec·h^{-1}) par suite d'une augmentation de l'influx de Na (J_i = 0.79 μmol/g tissu sec·h^{-1}).

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Introduction

Biological rhythms are finely tuned synchronized characteristics of living organisms (Hastings and Schweiger 1975). Environmental cues such as temperature (Roberts 1962), population size (Christian 1950), and light periods (Hastings 1964; Lees 1974; Wolfson 1964) can serve as time setters (zeitgebers). To interpret and use these zeitgebers, the organism must possess a sensing tissue (receptor) and an internal timing mechansim (clock). The observed oscillating response of a particular function maintains a homeostatic condition.

Freshwater bivalve molluscs face an osmoregulatory problem associated with a hyposmotic environment. To minimize the ionic and osmotic movements, freshwater clams maintain a low concentration of blood solutes (Dietz 1979). These mussels have ion transport systems capable of accumulating sodium and chloride from dilute solutions (Dietz 1978, 1979). Nevertheless, freshwater mussels do not maintain an "absolute" constancy of their blood ion concentration.

In this study, we observed that the blood sodium and chloride concentrations vary on a diurnal basis. The changes in blood sodium concentration are due to alterations in the rate of epithelial sodium transport between the animal and the bathing medium.

Materials and methods

Animals

Carunculina texasensis were collected from freshwater ponds in Louisiana and stored in aerated, artificial pond water (0.5 m*M* NaCl, 0.4 m*M* $CaCl_2$, 0.2 m*M* $NaHCO_3$, 0.05 m*M* KCl). The clams were not fed.

Photoperiod

The animals were transferred to an environmental room with a light regimen of 12 h light (L) and 12 h dark (D) for a 10-day acclimation period. The pond water was changed at irregular times of the day every 3rd day with the last change of the bath occurring 3 days before use.

Blood ion composition

Blood samples were collected by pericardial puncture (Fyhn and Costlow 1975). The animals (n = 67) were used only one time and the experiment was repeated to confirm initial findings (see Statistics). The blood samples were centrifuged and the total solutes determined by freezing point depression. Blood samples were diluted and chloride was determined by electrometric titration and sodium concentrations by flame photometry.

Net flux of sodium and chloride

Ten *C. texasensis* were entrained to the 12 h L : 12 h D photoperiod for 10 days. They were placed in separate containers of pond water and samples of the pond water baths were

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taken from each individual at 4-h intervals for 24 h. The bath volume was kept constant at 25 mL. All samples were analyzed for sodium and chloride concentrations and the soft body tissues removed and dried for 24 h to determine dry tissue weight. The mean weight (in grams) ± 1 SEM for the whole animal equaled 11.32 ± 1.13; dry shell = 5.60 ± 0.69; and dry tissue = 0.49 ± 0.05. Concentrations of sodium were determined by flame photometry (corrected for the replacement of fresh pond water) and the net flux (J_n) was calculated for each individual (expressed as micromoles Na per gram dry tissue per 4 h).

Unidirectional sodium flux

Two groups of *C. texasensis* were acclimated for 10 days to the 12 h L : 12 h D photoperiod. Each group was rinsed in distilled water for 1 h prior to placing them into individual containers of 0.5 m*M* Na_2SO_4 (labeled with 2×10^3 cpm ^{22}Na/mL). Ion transport rates were determined on one group during the light phase (0900–1200 hours) and the other group during the dark phase (1900–2200 hours). Samples of the bath were taken from each group at time 0 and 3 h later. Aliquots of the bath were counted with a Beckman LS-8000 liquid scintillation counter to give the change in radioactivity and bath sodium concentrations were measured by flame photometry. The net flux (J_n) of sodium was determined by the changes in the sodium concentration of the bath. The unidirectional influx (J_i) was determined by the disappearance of ^{22}Na from the bathing media (Dietz and Branton 1975), and the efflux (J_o) was estimated by the difference: $J_o = J_i - J_n$. The volume of the bath was kept as small as possible (22.5 mL) to enhance the changes in the radioactivity of the bathing compartment with the small changes in the animal compartment. The mean weights (in grams) ± 1 SEM were the following: whole animal = 15.73 ± 1.07; dry shell = 8.57 ± 0.69; dry weight = 0.87 ± 0.07.

Statistics

Analysis of variance (block design) was carried out on the data for the concentrations of sodium and chloride in the blood over a 24-h period. It was blocked on the basis that the experiment was repeated and identical to the first investigation. Orthogonal (independent) comparisons were made between light and dark phases and within each phase. Completely randomized analysis of variance was used to test the effect of light on the net flux of sodium. All other data were analyzed using a two-tailed Student's *t*-test.

Results

Analysis of the blood samples from the dual experiments of *Carunculina texasensis* on a 12 h L : 12 h D light regimen shows a distinct rhythm in the sodium and chloride levels (Fig. 1). However, measurements of the total solutes in these blood samples did not vary significantly from the mean of 49.9 ± 1.5 mosmol/L. An analysis of variance of the concentrations of the blood ions throughout the 24 h is significant ($P < 0.05$). There is a steady decline in the sodium and chloride concentrations during the light (photophase) period and a progressive increase of these ions during the dark (scotophase) period. Orthogonal comparisons between the sampling times reveals a significant peak concentration of sodium (14.6 mmol/L) and chloride (12.1 mmol/L) at approximately 0700 hours (lights on) with the nadir concentration of these ions (12.3 mmol Na/L and 10.4 mmol Cl/L) occurring between 1100 and 1500 hours (Fig. 1, Table 1).

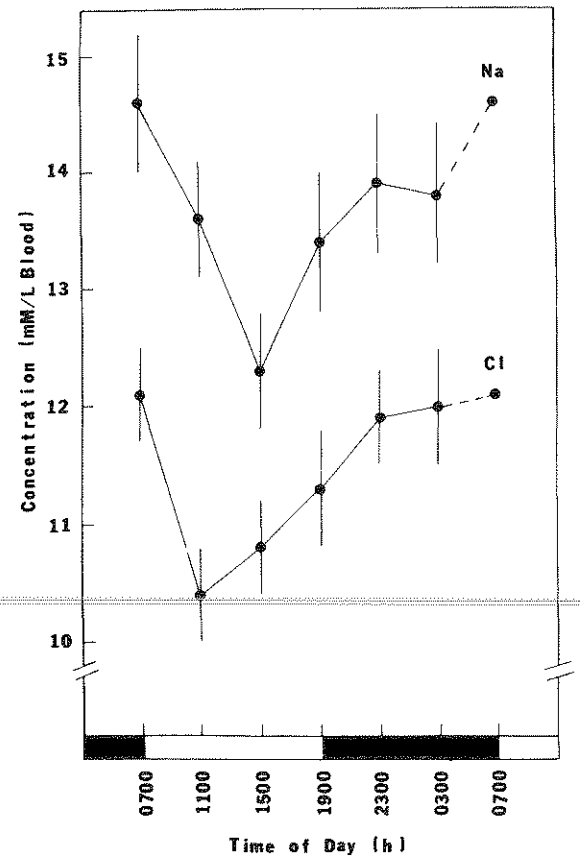

FIG. 1. Changes in blood Na and Cl concentration in pondwater acclimated *C. texasensis* on a 12 h L : 12 h D photoperiod. Each point represents 10–12 animals and the vertical line is ± 1 SEM. The dark bar at the base of the figure indicates "lights-off."

The mechanism involved in the cyclic changes of the concentration of blood sodium ions is by exchange with the bath ions rather than sequestering the blood ions in some tissue compartment. This is demonstrated by measurements of the net flux (J_n), or the exchange of blood sodium with the bath

TABLE 1. Specific orthogonal comparisons of blood sodium and chloride concentrations in *C. texasensis* over 24 h

Comparison, time	*F*(Na)	*F*(Cl)
Light vs. dark	0.18	2.49
0700 + 1100 vs. 1500 hours	7.23**	0.62
0700 vs. 1100 hours	1.60	8.66**
1900 + 2300 vs. 0300 hours	0.08	0.56
1900 vs. 2300 hours	0.47	0.63

**$P < 0.01$.

TABLE 2. Sodium fluxes (in micromoles per gram dry tissue per hour) in pondwater-acclimated *C. texasensis* on a 12-h photoperiod

Time of day	N	J_n	J_i	J_o
0900–1200 hours	7	-0.36 ± 0.10	0.30 ± 0.10	0.66 ± 0.12
1900–2200 hours	8	0.36 ± 0.19	0.79 ± 0.15	0.43 ± 0.16
P		<0.01	<0.05	<0.20

sodium (Fig. 2). During the photophase, when blood levels of sodium are decreasing, there is a highly significant ($P < 0.01$) increase in the sodium concentration of the milieu concentration (J_n negative). Conversely, during the scotophase, the increasing blood levels are reflected by a decrease in the milieu concentration (J_n positive). During the photophase, 6–10 μmol of sodium is lost from the animals. On a whole-animal basis, this loss of sodium accounts for the 1–2 mmol/L depression of blood sodium concentration. Although the sodium flux varies diurnally, the net flux of chloride did not change significantly and the animals essentially remained in a chloride steady state. Therefore, further investigations were confined to sodium transport.

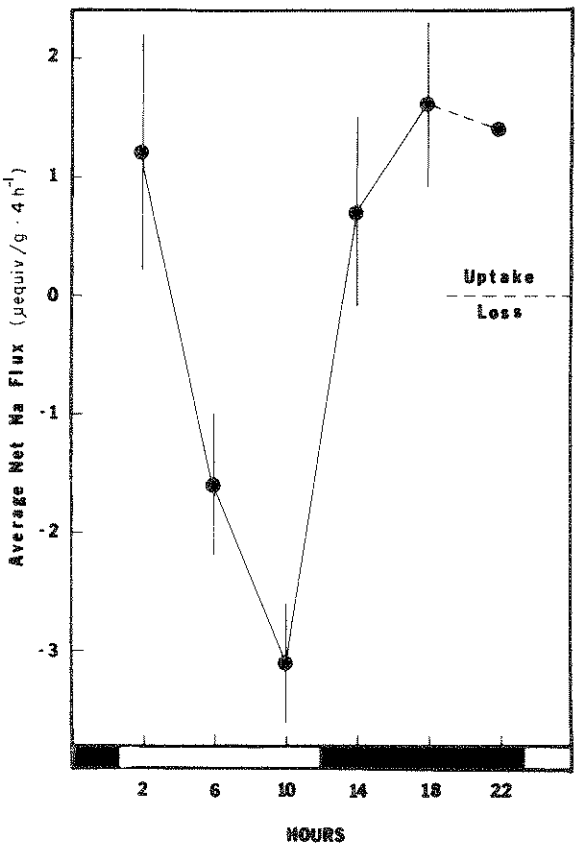

FIG. 2. Changes in net Na flux in pondwater-acclimated *C. texasensis* on a 12 h L : 12 h D photoperiod. The dark bar at the base of the figure represents "lights-off" and the hour refers to the midpoint of the sample interval (t_0–t_4).

The mechanism responsible for the changes in the net flux could be either alterations in the influx or efflux of the ion. The unidirectional fluxes of sodium in *Carunculina texasensis* are given in Table 2. The net flux confirms the previous pattern of loss of sodium ($J_n = -0.36$ μmol/g dry tissue · h^{-1}) during the photophase and gain of sodium ($J_n = 0.36$ μmol/g dry tissue · h^{-1}) during the scotophase. The changes in the ionic balance are due to significant changes in the influx ($P < 0.05$). During the early daylight, the influx (0.30 μmol/g dry tissue · h^{-1}) is less than the influx (0.79 μmol/g dry tissue · h^{-1}) measured during the night period. Since there is no significant difference between the efflux measured during the light versus dark the animals are not simply closing their valves.

Discussion

Carunculina texasensis has a diurnal rhythm of sodium and chloride concentrations in the blood. Photostimulation is apparently the entraining element. The highest concentrations of sodium and chloride in the blood are reached during the scotophase. The nadir points for the two ions may be slightly out of phase with each other. However, this apparent phase shift could be an artifact of the selected measurement times. The true nadir may be the same for both ions and occurring somewhere between 1100 and 1500 hours.

The blood total solute concentration is relatively stable during the fluctuating blood ion concentrations. Murphy and Dietz (1976) found a similiar situation in salt-depleted freshwater mussels. They attributed the stability of the blood total solutes in the face of losses of blood NaCl to an increase in blood HCO_3 and possibly an increase in blood Ca concentrations.

It is interesting that chloride transport rhythms are not evident in these animals. Possibly the handling associated with transferring the animals to individual containers for the flux studies dampens the oscillations in chloride transport. Diurnal rhythms in blood chloride have been reported in the

gulf killifish (Spence et al. 1977). Although chloride transport rates were not measured in the killifish, changes in total animal chloride content paralleled blood chloride concentration. These data suggest that chloride is exchanged between the fish and the bathing medium.

The mechanism responsible for the oscillations of sodium in the blood is due to variations in active sodium transport. The mussels gain sodium from the milieu during the scotophase ($J_i > J_o$) and lose sodium ($J_o > J_i$) during the photophase. The amount of sodium exchanged with the milieu accounts for the 1–2 m*M* oscillations of sodium in the blood.

Recently, a transient stimulation of sodium transport has been reported for the freshwater mussel, *Margaritifera hembeli*, in response to handling (Dietz 1979). These studies suggest that sodium transport is being regulated by a rapid acting hormonal or neurohormonal control system. However, there is virtually no information on the endocrine system in freshwater bivalves. We recently reported that prostaglandin E_2 is capable of inhibiting sodium influx (Graves and Dietz 1979) and there are other reports of the presence of prostaglandins in marine invertebrates (Nomura and Ogata 1976) and marine bivalves (Freas 1978).

Circadian rhythms are ubiquitous among both unicellular and multicellular organisms and a large number of oscillatory variables is involved (Hastings and Schweiger 1975; Hastings and Sweeney 1975). For these reasons, it is usually assumed that there may be a common biochemical basis for circadian rhythms (Burgoyne 1978). Several models postulating a biochemical role of membranes as the controlling mechanism of circadian rhythms have been proposed recently (Burgoyne 1978; Cumming 1975; Edmunds 1976; Hastings and Schweiger 1975; Njus et al. 1974; Schweiger and Schweiger 1977). The models assume that the biological clock is a feedback system of two or more continuously varying systems that interact to generate the oscillations.

One model is base on oscillations in the cyclic AMP levels generated by the membrane-bound enzyme adenyl cyclase. The accumulation of cyclic AMP activates the enzyme phosphodiesterase and this enzyme enhances the conversion of cyclic AMP to AMP (Cumming 1975). Alternatively, a feedback oscillator may involve circadian changes in the transmembrane ion fluxes which are mediated primarily by membrane proteins. Ion transport directly controls transmembrane ion concentrations gradients and the ion gradients may affect ion transport through rearrangement of membrane proteins. These protein molecules may be hormone receptors or may form inophores (see Njus et al. 1974). Recently, Burgoyne (1978) has proposed that membrane proteins are involved with ion transport and may be part of the Na–K ATPase or a modulator of its activity.

The manner in which sodium transport is stimulated or inhibited in freshwater mussels is unclear. The stimulation of the photoreceptor could cause increases in the endogenous levels of prostaglandins which appear to inhibit active sodium transport (Graves and Dietz 1979). The prostaglandins may be inhibiting a protein carrier molecule involved with the Na–K pump or it may alter an "ionophore" resulting a decreased transepithelial sodium influx.

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