RAPID COMMUNICATION

ZIPGRAM

THE EFFECT OF OUABAIN ON BLOOD NaCl IN THE OSMOREGULATING CLAM RANGIA CUNEATA (1)

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ABSTRACT Exposure of R. cuneata to exogenous ouabain is not lethal for several days. In both hypo- and hypersaline media, O_2 uptake is uninhibited for periods up to 17 hr. Blood NaCl changes significantly, both before and after O_2 uptake finally ceases. Changes in blood osmolality, however, are closely related to blood $CaHCO_3$ and normal aerobic respiration.

Since their discovery several decades ago, the $Na^+ + K^+$-ATPases have been strongly implicated in the transport of cations into and out of the blood of euryhaline animals. These enzymes are located in the plasma membrane of the cell rather than the mitochondria, their activity in salt absorbing organs is often very high and this activity is enhanced when NaCl balance shifts from conformity to a gradient between blood and ambient water (Towle et al., '76; Conte, '77). The osmoregulatory role of the $Na^+ + K^+$-ATPases has been recently questioned, however. The hypothesis that they maintain a hyposaline blood is difficult to reconcile with their location on the inside of the epithelial barrier separating blood from ambient water, where they should pump Na^+ in, not out (Karnaky et al., '76). Moreover, the deviations of measured from predicted electrical potentials across transporting epithelia suggest that NaCl is regulated entirely by Cl^- pumping (e.g., Conte, '77), which may be effected by an anion requiring ATPase (De Renzis and Bornancin, '77). Thus the question arises: does the activity of the $Na^+ + K^+$-ATPases

influence the balance between blood and ambient NaCl? This question has not been answered, in part because the activity of these enzymes is critical to the function of the central nervous system, and thus indirectly, to the O_2 supply at the immediate site of NaCl absorption, at least in many aquatic animals. While injections of ouabain into animals such as crabs and eels may influence NH_4^+ or Na^+ movements, they have conspicuous systemic effects resulting in death within several hours (Silva et al., '77; Mangum et al., '78).

Exposure to exogenous ouabain, a specific inhibitor of the $Na^+ + K^+$-ATPases, is not lethal to simpler aquatic animals, and in several annelids and molluscs with either hyper- or isosaline bloods, it depresses NH_4^+ excretion (Mangum et al., '78). Ouabain exposure of the clam Rangia cuneata also stimulates NH_4^+ excretion when the blood is hyposaline to the medium (Henry and Mangum, '79). The explanation of these finds which is most consistent with current knowledge is that ouabain permeates into the blood and blocks a catalyzed exchange of NH_4^+ for Na^+. If this inference is true and if the process results in a net change in NaCl balance as well, then exogenously added ouabain should directly influence the steady state level of NaCl in the blood. Since circulation and ventilation in lamellibranchs is not completely dependent on the central nervous system, the influence of ouabain can be studied in intact animals and, initially, the effects on salt balance can be distinguished from respiratory consequences.

MATERIALS AND METHODS After acclimation to a mixture of natural estuary waters, clams were transferred to 1 l. of medium containing (1) no ouabain and aerated, (2) no ouabain and deoxygenated or (3) ouabain and aerated. O_2 uptake was measured with Yellow Springs Instr. Model 54 electrodes, and then blood was taken from the mesosomal and pedal sinuses. Free cation concentrations were measured with ion selective electrodes (Mangum

TABLE 1

Effect of ouabain on blood osmolality and ionic composition in Rangia cuneata. 22-24°C. Mean ± S.E. No variation detected in 3 replicate measurements of free ions in medium.

Salinity (o/oo)	PO_2 (mm Hg)	Ouabain conc. (M)	Exposure period	Fluid	Ion Conc. (mM) Na^+	K^+	NH_4^+	Ca^{+2}	Cl^-	HCO_3^-	Osmolality (mOSM)	N
1-2	150-159	0	7-14 da.	medium	8.5	0.44	0.01	0.46	14.7		25	12
				blood	23.2±0.8	1.09±0.66	0.19±0.22	2.51±0.19	19.2±0.5		112±2	
	0-5	0	48-60 hr.	medium	8.0	0.28	0.01	0.21	14.5			8
				blood	12.4±0.7	0.96±0.10	3.3±1.3	9.83±1.64	10.0±0.5			
	150-159	10^{-4}	60 hr.	medium	8.5	0.77		0.61	14.9		27	
				blood	12.4±1.3	0.92±0.14		15.26±0.84	13.3±2.0		119±2	8
			24 hr.	medium	9.6		0.01	0.29	16.2		37	
				blood	14.8±0.4		2.04±0.30	6.98±0.8	16.8±0.8		88±4	4
			12-16 hr.	medium	9.7			0.28	15.5		27	
				blood	18.5±0.7			6.96±2.13	18.8±0.6		67±1	4
19-20	150-159	0	7-14 da.	medium	258	4.75	0.01	5.48	312	1.07	536	
			(undisturbed)	blood	236±2	5.69±0.25	0.15±0.01	6.99±0.40	251±4		473±1	4
			(valves closed)	blood	249±4			6.96±0.73	261±4		556±2	6
				mantle fluid				8.50±0.20		1.01±0.08		4
	0-5	0	24 hr.	medium				6.00				
				blood				14.50±0.5				
				mantle fluid				15.80±0.6		11.8±2.8		6
	150-159	10^{-4}	12-16 hr.	medium	253		0.01	6.32	307		565	
				blood	245±4		0.35±0.01	8.37±0.71	280±2		596±1	4
			3-6 hr.	medium	248		0.01	7.00	296		542	
				blood	240±3		0.16±0.05	8.01±0.31	280±1		591±9	6
23-24	150-159	0	6 da.	medium	305			5.60	354		675	
				blood	292±1			7.64±0.25	320±7		710±0	6
	0-5	0	20 hr.	medium	303			5.92	353		682	
				blood	299±2			23.4±2.2	342±8		723±9	4
	150-159	10^{-4}	3-17 hr.	medium	303			6.01	353		700	
				blood	304±4			8.07±0.45	351±7		733±7	8

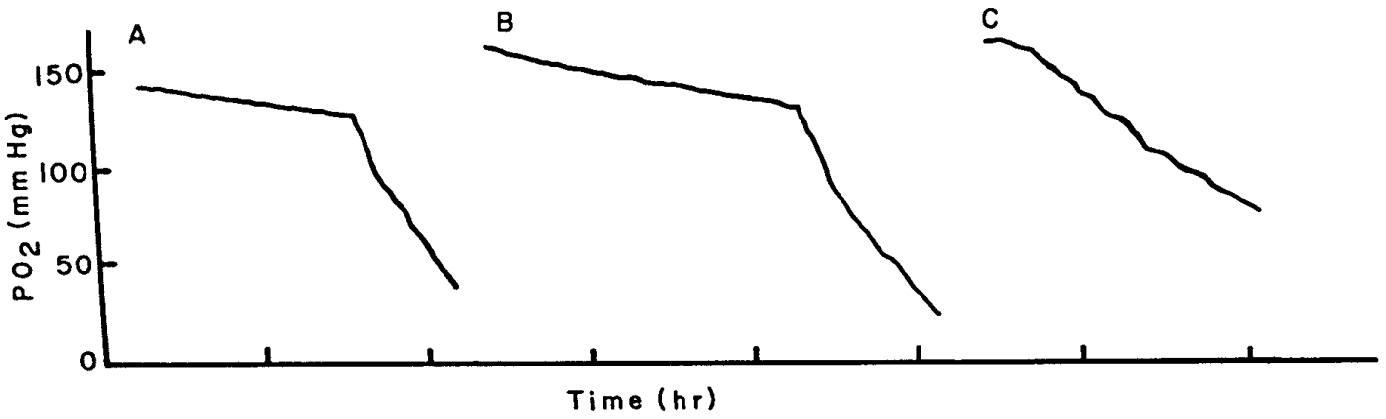
et al., '78). Appreciable interference with the Cl^- electrode, presumably from sulfur compounds, was encountered in preliminary attempts to analyze samples from hypoxic animals. Therefore Ag titration (Buchler-Cotlove) was used instead. Osmolality was determined with a Wescor 5130A osmometer, and NH_4^+ by the phenol hypochlorite method (Solòrzano, '69). In separate animals, heartrate was measured with an impedance pneumograph (deFur and Mangum, '79), and pH and PCO_2 with a Radiometer BMS1 Blood Gas Apparatus.

RESULTS AND DISCUSSION The initial experiments showed that 48-60 hr exposure to ouabain or hypoxia in low salinity water virtually halves blood NaCl ($P < .001$) but does not change K^+ (table 1). However, Ca^{+2} increased to very high levels, and osmolality increased. Even though no mortality occurred, the ouabain exposed animals failed to retract their siphons when mechanically stimulated, and the siphons had become quite flaccid. In both cases the low blood NaCl could be attributed to O_2 deprivation at the immediate site of salt absorption. Subsequently, O_2 uptake was measured during the last few hours of ouabain exposure, immediately prior to blood sampling. After 24 hr in ouabain at low salinity (table 1), blood NaCl was still significantly low ($P < .001$). O_2 uptake was also low, however, and Ca^{+2} highly variable. After only 12-16 hr in ouabain, there was no evidence of an appreciable depression of $\dot{V}O_2$ ($P = .80$, fig. 1) and no apparent change in ventilation behavior, yet blood NaCl was significantly low (table 1, $P < .01$).

In animals assuming the normal ventilation posture, heartrate decreases from 17.5 ($\pm$1.3 S.E.) to 7-9 bts/min within 1 hr of exposure to ouabain,

FIGURE LEGEND

1 O_2 uptake by R. cuneata in a closed container. 23 C. A, Control, 1-2 o/oo. B, same animal, after 13 hr exposure to ouabain. High velocity ventilation resumed at about 120 mm Hg. C, different animals, after 16 hr in 20 o/oo water plus ouabain.


A
B
C
150
100
50
0
PO_2 (mm Hg)
Time (hr)

remains constant for the next 21 hr and then drops to 3-6 bts/min at 21-29 hr (N=4). Cardiac arrest never occurred during this period. The respiratory role of the blood in lamellibranchs is small (Booth and Mangum, '78), and the organs with the highest $Na^+ + K^+$-ATPase activity comprise little of the total mass of respiring tissue (Saintsing and Towle, '79). Even though O_2 uptake in these organs is sensitive to ouabain (Henry and Mangum, '79), it is not surprising that the decrease in a whole animal is not significant.

Above 12 o/oo the blood is invariably hyposaline and the deficit increases at 20 o/oo, which approaches the upper limit of prolonged (> 2 wk) salinity tolerance (Henry and Mangum, '79). At 19-20 o/oo, exposure to ouabain results in a clear increase in blood Cl^-, and the blood medium difference in Na^+ becomes insignificant, prior to a pronounced change in O_2 uptake (fig. 1). At 23-24 o/oo, both hypoxia and ouabain result in even larger increases in blood NaCl.

Regardless of the relationship between blood and ambient NaCl, osmolality is surprisingly labile and strongly dependent on uninterrupted gas exchange. At 19-20 o/oo, blood taken from animals kept in the dark, undisturbed for several days, was both hyposaline and hypoosmotic (table 1). After only intermittent disturbance during the next 24 hr, animals taken from the same aquaria but with valves closed had less hyposaline blood which was actually hyperosmotic to the medium (table 1). While we were unable to obtain anaerobic samples of blood in sufficient quantity for CO_2 measurements, mantle fluid from hypoxic animals had increases in Ca^{+2} and HCO_3^- in a ratio approaching 1:2, suggesting dissolution of the shell as body fluid pH falls (table 1). Assuming the same ratio whenever blood Ca^{+2} rose, elevated $CaHCO_3$ explains some but not all of the observed increases in osmolality (table 1).

Even though the osmotic balance between blood and ambient water may be

influenced by other salts and, in all likelihood, by metabolites, it is clear that NaCl balance requires an unimpaired rate of O_2 uptake and uninhibited $Na^+ + K^+$-ATPases, whose mode of action may prove to be extremely complicated (Silva et al., '77). Although the present data do not exclude all conceivable indirect effects of these inhibitions, their simplest and most probable explanation is that blood NaCl is regulated by the enzymes.

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