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Effect of Bicarbonate on Chloride-Dependent Transmural Potential and ATPase Activity in the Rectal Wall of *Schistocerca gregaria* *

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An activating effect of bicarbonate on the active transport of Cl^- across the rectal wall of *Schistocerca gregaria* has been found, as revealed by an increase of the transmural potential and short-circuit current. Acetazolamide, an inhibitor of both parameters, does not show this inhibitory effect, even at much higher concentrations, when added in the presence of bicarbonate. A Cl^- or SO_4^- anion dependent ATPase, different from the Na^+, K^+ ATPase, has also been found, which can be further stimulated by bicarbonate.

The existence of an active mechanism for transporting Cl^- from the lumen to the haemolymph across the rectal wall in the desert locust, *Schistocerca gregaria* has been already established (2, 3).

This transport can be measured by the variations of Potential Difference (PD) and short-circuit current (Isc) between both compartments; it is also already known how these parameters are affected by metabolic inhibitors as well as by certain ions. Thus it has been demonstrated that both PD and Isc are directly dependent on the active transport of Cl^- from the luminal side to the haemolymph and also that the aerobic metabolism is the main energy source supporting this active transport. This conclusion has been reached after observing the inhibition of PD and Isc in the absence of oxygen, or in the presence of metabolic inhibitors such as KCN or dinitrophenol.

Bicarbonate seems to be implied in this transport mechanism, since a marked inhibitory effect has been observed on PD and Isc when acetazolamide is present in the medium (3). This effect could be explained in two different ways: 1) That bicarbonate could be exchanged on the lumi-

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Table I. *Composition of bathing solutions (mM)*

Compounds	Solution O'Riordan	Krebs-Henseleit
NaCl	154.8	153.8
KCl	12.8	8.0
$CaCl_2 6H_2O$	4.5	1.6
$MgCl_2 6H_2O$	4.0	—
KPO_4H_2	—	1.5
$MgSO_4 7H_2O$	—	1.5
Tris-HCl	5.5-5.0	—
$NaHCO_3$	—	32.5

minal side by Cl^-, and therefore in its absence the entry of Cl^- would be hindered, or 2) That the transport mechanism of Cl^- itself were sensitive to bicarbonate.

In order to study which of these two hypotheses could be correct the effect of bicarbonate on PD and Isc has been followed when this anion was present in the luminal or in the haemocoelic compartment or simultaneously in both compartments. The ATPase activity of the tissue was also studied, as well as its relationship with the ions participating in this transport.

Materials and Methods

Adult male and female specimens of the desert locust *(Schistocerca gregaria)* were used. The way to obtain the everted rectal preparations and the procedure for measuring PD and Isc were described in a former paper (2). The solutions have been those of O'RIORDAN (6) for insects and that of KREBS-HENSELEIT (4) slightly modified according to the experiments to be performed (table I).

After a control incubation period of the preparations in the standard O'Riordan medium during approximately 13 min the PD and the Isc were measured. The medium was then rapidly replaced in the lumen or in the haemocoele or in both compartments by the Krebs-Henseleit solution (medium with HCO_3^-) and bubbling of 95 % O_2 and 5 % CO_2 instead of O_2 with the purpose of maintaining the pH. After 15 minutes of incubation the preparations were then taken back to the initial conditions (O'Riordan medium and O_2 bubbling). Acetazolamide when used was always dissolved in Krebs-Henseleit medium.

The ATPase activity of the tissue has

Table II. *Effect of HCO_3^- and acetazolamide on electric potential differences*
The change per cent values represent the mean of per cent changes for each independent [illegible], $P < 0.05$. Mean values ± S.E. PD, in mv (Lumen always positive).

Experimental condition	Parameter	Time (min) Standard 0	Standard 13	Experimental 15
HCO_3^- in Lumen (6)	PD	44.2± 4.2	42.3± 3.9	48.2± 3.4
	I_{sc}	331.8±46.3	330.9±36.2	383.9±39.6
HCO_3^- in Lumen and Haemocoele (7)	PD	31.9± 4.6	16.4± 2.6	31.1± 1.8
	I_{sc}	423.0±26.1	218.3±15.1	310.4±19.1
HCO_3^- in Haemocoele (7)	PD	36.8± 2.5	26.0± 5.4	38.7± 5.7
	I_{sc}	391.7±30.9	299.9±61.7	526.6±26.1
HCO_3^- and acetazolamide 10^{-3} M in Lumen (6)	PD	41.3± 3.3	32.8± 4.3	40.3± 4.2
	I_{sc}	396.3±23.8	342.2±21.7	458.3±36.5

been measured according to the following procedure. The rectal walls of three specimens of *Schistocerca gregaria* were homogenized in 5 ml of 0.25 M sucrose, and centrifuged at 14,000 × *g* for 10 min, and sediments resuspended again in 5 ml of 0.25 M sucrose; supernatants were discarded since no activity was found in them. ATPase activity was measured determining the orthophosphate (1) released from ATP. The incubation medium contained in 0.8 ml 5 μmoles of Mg $(NO_3)_2$. Tris-anions (Cl^-, $SO_4^=$, HCO_3^-, NO_3^-), ph 7.4, at the concentrations indicated in each experiment, an aliquot of the enzyme suspension (50-100 μg of protein); after an incubation of 5 min at 30° C the enzymatic reaction was initiated by the addition of 6 μmoles of ATP dissolved in 0.2 ml of distilled water. After 10 min the reaction was stopped by the addition of 0.1 ml of 50 % trichloroacetic acid. Reagent and enzyme blanks were determined in each experiment. Protein was determined following the technique of LOWRY *et al.* (5).

Results

Effect of bicarbonate. The effect of the addition of bicarbonate in the luminal, haemocoelic compartment or in both compartments, has been studied. After a control incubation period the saline medium of O'Riordan was replaced by that of Krebs-Hanseleit (with bicarbonate), in the luminal or in the haemocoelic side, or in both compartments simultaneously. An increase of PD and Isc was observed and their values decreased after returning the preparations to the control medium without bicarbonate (table II and fig. 1).

The effect of the presence or absence of bicarbonate on PD and Isc was more marked when added in, or when removed from the haemocoelic than from the luminal side. This could be explained by a much easier access of the bicarbonate to the cell from the haemocoelic side.

Effect of acetazolamide. It had already been observed in previous work (3) that acetazolamide inhibited PD and Isc when present in the luminal side at 10^{-3} and 5×10^{-3} M concentrations. Since acetazolamide is a specific inhibitor of carbonic anhydrase its effect was explained as due to a decrease in intracellular bicarbonate concentration. This seems to

and short-circuit current strength across the rectal wall of locust.
experiment. Student's t test for no independent values: [1], $P < 0.001$; [2], $P < 0.01$; [3], $P < 0.02$; I_{sc}, in μA cm^{-2}. Number of experiments in parentheses.

and condition				
condition	Standard		Change %	
30	32	42	Addition of HCO_3	Omission of HCO_3
42.0± 3.7	36.7± 3.9	32.8± 3.9	15.4± 4.0[3]	—11.6 ±3.5[3]
337.2±37.4	274.3±45.4	217.7±22.4	17.7± 5.8[4]	—26.3 ±6.4[2]
24.9± 2.0	10.3± 2.2	8.6± 2.0	123.8±39.0[3]	—58.9 ±7.3[1]
258.1±11.0	113.0±15.0	111.4±18.2	44.1± 6.7[1]	—56.0 ±6.0[1]
38.5± 6.4	22.0± 3.8	21.5± 3.5	73.9±20.5[3]	—42.74±1.9[1]
471.8±48.4	275.6±51.4	270.9±24.2	139.7±54.3[4]	—42.30±7.0[2]
38.7± 4.7	29.8± 4.7	28.8± 4.5	25.6± 5.2[2]	—23.8 ±3.7[2]
431.0±43.4	431.0±41.8	269.7±24.1	35.9±12.6[4]	—27.1 ±5.9[2]

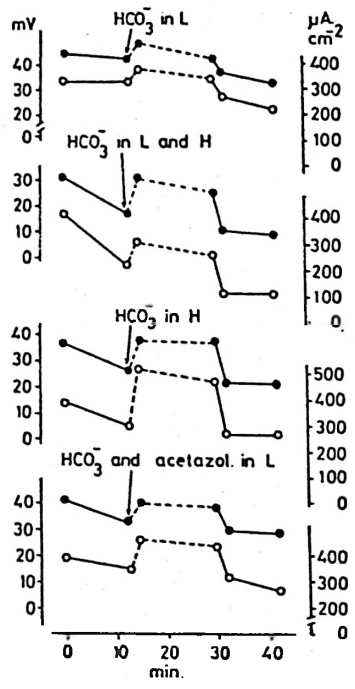

Fig. 1. *Electropotential difference (PD) and short-circuit current (Isc) across the rectal wall of locust.*
Effect of bicarbonate (32.5 mM), or bicarbonate and acetazolamide (10 mM) on the lumen (L), haemocoele (H) or both compartments. PD (●); Isc (○); Control, ——; HCO_3^-, -----.

be confirmed with the results here presented which show that at concentrations 10 times higher than those used in previous work, and which caused inhibition of PD and Isc in the absence of bicarbonate, no effect was observed when bicarbonate was present in the medium either in the lumen or in the haemocoele (table II and fig. 1).

ATPase activity. Table III shows the effect of anions on ATPase activity of the homogenate of the rectum of *Schistocerca gregaria.* The basal activity observed was 50 mμmoles Pi mg^{-1} protein min^{-1} measured in the presence of Mg^{++} added as $Mg(NO_3)_2$.

It may be seen that 60 mM Cl^- or 60 mM $SO_4^{=}$ had a similar effect; their effects were also additive in such a way that varying the concentrations of these two anions but maintaining their sum equal to 60 mM the ATPase activity did not change.

NO_3^- anion was found to be much less stimulatory.

The ATPase activity found in the homogenates was different from that of Na^+ and K^+ ATPase since these cations were

Table III. *Effect of anions on ATPase activity in homogenates from the rectal wall of* Schistocerca gregaria.
Number of experiments in parentheses.

	ATPase activity * [mμmol ATP hydrolyzed/mg prot × min]	
	Mg^{++} [5 mM]	Mg^{++} [5 mM] and HCO_3^- [10 mM]
None (15)	50± 4	53± 4
Cl^- [60 mM] (10)	205±10	415±15
$SO_4^{=}$ [60 mM] (10)	200±10	400±18
NO_3^- [60 mM] (10)	108± 5	137± 7
Cl^- [30 mM] + $SO_4^{=}$ [30 mM] (6)	204±11	410±15
Cl [30 mM] + $SO_4^{=}$ [10 mM] (6)	138± 5	270± 7
Cl [60 mM] + NO_3^- [10 mM] (6)	219±11	420±15

* Mean values ± S.E.

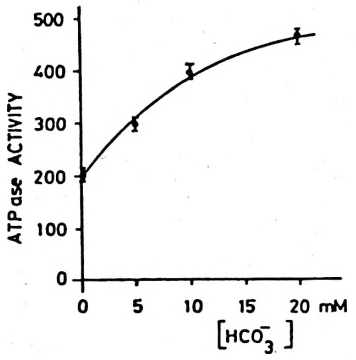

Fig. 2. *Effect of bicarbonate on ATPase activity measured in the presence of Cl⁻.*
ATPase activity has been expressed as mμmoles ATP hydrolyzed $min^{-1} \cdot mg^{-1}$ protein. The concentration of Cl^- was 60 mM. Bars represent mean values ± S.E.

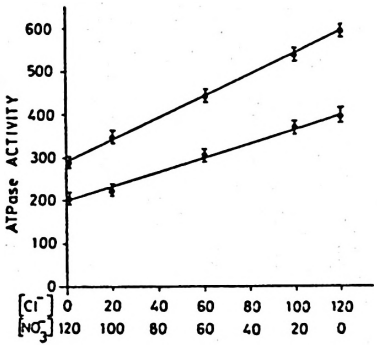

Fig. 3. *Effect of Cl^- ion concentration on ATPase activity in the presence or absence of bicarbonate.*
ATPase activity has been expressed as mμmoles ATP hydrolized $min^{-1} \cdot mg^{-1}$ protein. Bicarbonate concentration was always 10 mM (●). In abscissae concentrations of Cl^- and NO_3^- are given in mM. It should be noticed that the sum of the concentrations of both ions was kept constant and equal to 120 mM. Bars represent mean values ± S.E.

not present in the medium; however, its activity was sensitive to anions, mainly Cl^- and $SO_4^=$.

In the second column of table III the activating effect of bicarbonate on ATPase is shown. ATPase activity measured in the presence of either Cl^- or $SO_4^=$ or of both doubled when 10 mM HCO_3^- was added besides those other anions. On the contrary, only a very weak activation took place with 10 mM bicarbonate in the absence of these two latter anions and replaced by NO_3^-.

Since the main intracellular anion is Cl^- the effects of different bicarbonate concentrations on ATPase activity measured in the presence of 60 mM Cl^- has been studied. Under these conditions the activity increased with increasing bicarbonate concentrations (0 to 20 mM) (figure 2).

Figure 3 shows the effect of different concentrations of Cl^-, maintaining unchanged the ionic strength of the medium with NO_3^-. It may be seen that a linear relationship was found between ATPase activity and Cl^- concentration within the range of concentrations studied. If 10 mM HCO_3^- was also present a new enhancement of ATPase activity took place.

Discussion

Role of HCO_3^- on PD and Isc. It had already been pointed out in a previous report (3) that HCO_3^- could play an important role in the flux of ions from the lumen to the haemocoele in the rectal wall of *Sch. gregaria*, after the observation that acetazolamide produces an inhibition of PD and Isc when present in the incubation medium. The data here presented give further support to the view that bicarbonate may play a key role in the transport of Cl^-. The presence of bicarbonate in the medium bathing the luminal side enhanced the PD and the Isc; it might be thought that this effect could be due to a flux of HCO_3^- from the lumen to the haemocoele and causing therefore an increase of PD and Isc. However, this explanation is not valid since the same effect was observed when the bicarbonate was simultaneously present in the luminal and in the haemocoelic side, or only in the haemocoele. It might also be thought

that the bicarbonate effect could be explained by an exchange of HCO_3^- by Cl^- in the luminal side, taking place in this way the entry of Cl^- into the cell. If this were the case the effect due to bicarbonate should be much lower whent bicarbonate were present both in the lumen and in the haemocoele. Since the effect was the same regardless the compartment where the bicarbonate was added, and even when added to both compartments, this hypothesis can also be discarded. Besides, when acetazolamide, which inhibited PD and Isc in the absence of bicarbonate, is added in the medium at concentrations 10 times higher than those necessary to cause inhibition, no inhibitory effect was observed if bicarbonate was present in the bathing solutions.

Thus, it seems reasonable to think that the observed effect of bicarbonate on the PD and Isc, and consequently on the active transport of Cl^-, is a direct effect on the mechanism of this transport. It is possible to think that some potential changes may have arisen from junctions potentials, because the solutions which are used in both sides (lumen and haemocoele) are different but we have carried out experiments which prove that using identical solutions on both sides (O'Riordan solution) the addition of bicarbonate in the presence of acetazolamide in the haemocoele, in the lumen or in both sides, always increased the transmural potential, and in that case there was no possibility of the existence of junction potentials.

ATPase activity. The existence of an ATPase in the tissue of the rectal wall dependent on the presence of Cl^-, and activated by bicarbonate, could very well be related to the transport mechanism of Cl^- ions into the cells, since, as discussed above, Cl^- and HCO_3^- affect the PD and Isc in the rectal sac of *Schistocerca gregaria*.

Resumen

El ion bicarbonato produce un incremento del potencial transmural y de la corriente de cortocircuito en la pared rectal de *Schistocerca gregaria*. La acetazolamida que inhibe ambos parámetros no produce efecto alguno — incluso a altas concentraciones —, en presencia de bicarbonato. Se determina una ATPasa dependiente de aniones (Cl^- o $SO_4^=$) diferente de la ATPasa Na^+, K^+. Esta ATPasa dependiente de aniones se estimula por el bicarbonato.

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