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Sodium, Potassium and Chloride Intracellular Concentrations in the Intestine of the Freshwater Turtle *Mauremys caspica*

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Sodium, potassium and chloride intracellular concentrations were measured. Sodium replacement from the bathing medium induced a significant decrease in chloride intracellular concentration. Conversely, chloride replacement from the bathing medium induced a significant decrease in sodium intracellular concentration. These results suggest the existence of a coupled NaCl transport system.

The presence of 10^{-3} M ouabain in the bathing medium increased Na^+ intracellular concentration while decreasing that of K^+. Cyanide (10^{-3} M) produced a significant increase in Na^+ intracellular concentration, whereas that of K^+ was not affected by this agent.

The existence of a coupled electroneutral NaCl transport mechanism located in the apical membrane of epithelial cells has been well established in preparations of mammalian (18), amphibian (1) and teleost small intestine (10), mammalian gallbladder (4, 11), amphibian gallbladder (7, 20) and the proximal tubules of amphibian kidney (22).

The aim of the present paper was to measure Na^+, K^+ and Cl^- intracellular concentrations in the intestine of the freshwater turtle *Mauremys caspica* and relate intracellular concentrations to the ability of the intestine to transport NaCl.

Materials and Methods

Freshwater turtles *Mauremys caspica* were used in the present study. Turtles were obtained from a local dealer. Animals were doubly pithed. The shell was opened and a segment of intestine was excised. The segment was opened along the mesenteric border and stripped of its external muscle layers. Histological examination of sections prepared from segments of stripped intestine showed that the mucosa remained intact and no damage was produced on it, due to the stripping procedure.

The composition of the bathing medium was (in mM): NaCl, 100; KH_2PO_4, 0.8; K_2HPO_4, 2.3; Ca-gluconate, 1.8; mannitol, 21; pH was 7.2. In Na^+-free medium, Tris (Tris-hydroximethylaminomethane) was substituted for Na^+. Tris-Cl was added in an amount sufficient to bring the Cl^- concentration to 100 mM. The mannitol content was varied to maintain the same osmolarity in all solutions. In Cl^--free medium, Na-gluconate was substituted for NaCl. These solutions were gassed with O_2.

KCN and ouabain (g-strophantin) were obtained from Merck.

Apparent extracellular space (AES) was estimated using polyethylene-glycol (MW$\simeq$4,000) labelled with trace amounts of poly-([^{14}C]-ethylene-glycol) (New England Nuclear). The following procedure was adopted: segments of stripped intestine were incubated at room temperature in 5 ml of Ringer solution. Following the time of incubation, the wet weight of the tissue was obtained. The tissue was dried at 100° C overnight and the dry weight was determined.

The dry tissue was extracted in 0.1 M HNO_3, as previously described (2). Aliquots of the extract and of the bathing medium were taken for counting in a Packard series 2425 liquid scintillation counter.

Na^+, K^+ and Cl^- intracellular concentrations (C_{Na}, C_K and C_{Cl}) were determined in a parallel set of experiments. The same procedure as that followed to estimate AES was performed. The time of incubation was 90 min. Aliquots of the extract were analyzed for Na^+, K^+ and Cl^- concentration. Na^+ and K^+ were determined by atomic absorption (Perkin Elmer, 372) and Cl^- by a colourimetric method (15). Samples were read on an spectrophotometre (Zeiss PMQ 3).

Results

Data presented in figure 1 show that the extracellular space equilibrates with the marker after 60 min of incubation. The shape of the curve displayed in figure 1 suggests that the marker used ([^{14}C]-PEG, 4,000) does not enter the intracellular compartment and that is not metabolized by the tissue. In these experiments a mean value of 14 $\pm$ 3 per cent of the wet weight of the tissue was obtained for AES in 6 different animals. In a parallel set of 4 experiments AES was measured following a 60 min incubation period under the different experimental conditions in which Na^+, K^+ and Cl^- intracellular concentrations were to be measured.

The control value obtained in this group was 10 $\pm$ 3. This value does not differ significantly ($p > 0.2$) from the control value obtained in the other group. The difference obtained between control values must be related to variability among animals of the same species. None of the experimental conditions studied induced

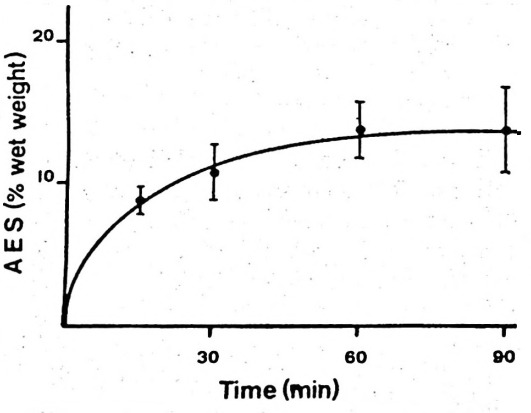

Fig. 1. *Apparent extracellular space (AES, expressed as per cent of tissue wet weight) in intestinal mucosa of* Mauremys caspica *after incubating the tissue for 15, 30, 60 and 90 min with [^{14}C]-PEG-4,000.*
Mean values ± S.E.M. in 6 animals are shown.

significant changes in AES except the presence of 10^{-3} M CN^- in the bathing medium, which induced AES to change significantly ($p<0.05$) from control value to 19 ± 3 per cent of the tissue wet weight. When all control values (10 experiments) were averaged, an AES mean value of 12 ± 3 per cent of the tissue wet weight was obtained. This value was considered as a good estimate of the extracellular space, and Na^+, K^+, Cl^- and water content of the tissue were corrected for this value to obtain C_{Na}, C_K, C_{Cl} and cell water in the different experimental conditions presented in this paper, except when CN^- (10^{-3} M) was present in the bathing medium. The experimentally obtained AES value for this condition was corrected in the same proportion as above.

Table I shows the values obtained for C_{Na}, C_K and C_{Cl} in control conditions and in Na^+-free and Cl^--free media. Replacement of Na^+ by Tris induced a significant ($p<0.05$) decrease in C_{Cl}. Conversely, replacement of Cl ions by gluconate induced a significant decrease ($p<0.01$) in C_{Na}. Neither Na^+ nor Cl^- replacement induced any change in C_K. These results are consistent with the presence of a coupled NaCl transport in this preparation.

Ouabain, a specific inhibitor of Na^+/K^+ pump, was tried to see its effects on C_{Na} and C_K (table II). As a consequence of the action of this agent, C_{Na} increases ($p<0.05$) and C_K decreases ($p<0.05$).

Table I. *Cell water and intracellular Na^+, K^+ and Cl^- concentration (C_{Na}, C_K and C_{Cl}) in Na^+-free or Cl^--free media.*
Mean values ± S.E.M. are given. Number of experiments 9. Paired t-test was used.

	Cell water (per cent wet weight)	C_{Na} (mM)	C_K (mM)	C_{Cl} (mM)
Control	70±1	48±4	98±4	62±5
Na^+-free	68±1	19±3***	102±4	45±5*
Cl^--free	69±1	37±4**	93±5	13±3**

* $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Table II. *Effect of ouabain and cyanide (CN^-) on cell water, C_{Na} and C_K.*
Mean values ± S.E.M. are given. Number of experiments 9. Paired t-test was used.

	Cell water (per cent wet weight)	C_{Na} (mM)	C_K (mM)
Control	71±1	57±4	98±2
Ouabain (10^{-3} M)	71±1	69±4*	71±4*
CN^- (10^{-3} M)	64±2***	94±12**	92±5

* $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Ouabain did not change cell water content. Cyanide, a metabolic inhibitor, was tried at 10^{-3} M concentration (table II). CN^- elicited a significant ($p<0.01$) increase in C_{Na} but left C_K unaltered. In addition, CN^- induced a significant ($p<0.05$) decrease in cell volume.

Discussion

Accurate and precise measurement of extracellular space is a crucial point in order to get reliable values of intracellular concentrations. It has been found that inulin and PEC-4,000 yield the same values for AES and that these two extracellular markers reach equilibrium within the tissue water in rat jejunum (8) and in rabbit ileum (19). Therefore, ^{14}C-PEG-4,000 was used as an extracellular marker in the present experiments. The value obtained in this work for AES (12 % wet weight) is in close agreement with the 16 % of total tissue water reported for rat jejunum (8) and it is slightly different from the 19 % wet weight reported for *Necturus* intestine (6). The AES value presented in this work differs markedly from the 27 % value reported by WRIGHT (23) for the intestine of *Testudo graeca*. However, mannitol was used by WRIGHT (23) as an extracellular marker. Studies made in bullfrog small intestine (2), in rabbit gallbladder (5) and rabbit ileum (19) have shown that mannitol is not a

suitable extracellular marker, because it does not equilibrate within tissue water and its use tends to overestimate the extracellular space determination. AES for serosal side of rat jejunum obtained with sucrose is larger than that obtained with PEG-900 or PEG-4,000 (9). The difference obtained when markers of different molecular weight are used has been related to a sweeping-away effect in the lateral intercellular spaces (5). In the light of these considerations a 12 % of tissue wet weight may be considered as a good estimate of the extracellular space of *Mauremys caspica*. Although this value might be somewhat underestimated, this error would not affect our main conclusions.

The mean values of C_{Na}, C_K and C_{Cl} found in these experiments under control conditions agree reasonably well with those reported for *Testudo graeca* (23). A well known property is that the interior of intestinal cells in electrically negative (1, 13, 21, 23). This fact together with the low C_{Na} obtained in these experiments suggests that Na^+ enters the epithelial cell down its electrochemical potential gradient. Replacement of Na^+ by Tris induced a decrease in C_{Cl}. Conversely, replacement of Cl^- by gluconate induced a decrease in C_{Na}. These results may be considered as evidence of the existence of a NaCl coupled transport located at the apical membrane of the epithelial cell. Recently, this transport system has been widely studied, and it appears to be a fundamental mechanism in cells of leaky epithelia (12).

When Na^+ was replaced from the bathing medium C_{Na} dropped dramatically. However, C_{Na} value in this condition (19 ± 3 mM) differs significantly from zero. This intracellular Na^+ concentration may be cytoplasmic Na^+ that has not leaked out of the cell, or may be the fraction of intracellular Na^+ that is «bound» or sequestered in one or more cellular regions (14) or a combination of both possibilities. C_K was not affected by sodium removal from the bathing medium. This suggests that at least a part of the C_{Na} measured under this experimental condition is free in the cytoplasm and that Na^+/K^+ pump is still active at this low C_{Na}. When Cl^- was replaced from the bathing medium, a low but significant C_{Cl} was found (13 ± 3 mM). Whether or not this C_{Cl} is due to intracellular Cl^- ions that are free or bound in the cytoplasm cannot be elucidated from the present experiments.

Low sodium and high potassium concentrations have been reported for a wide variety of animal cells (14). The source of this asymmetry in Na^+ and K^+ distribution across cell membranes has been related to the ubiquitous ATPase Na^+/K^+ pump. Results presented in table II confirm this view. Ouabain, an specific inhibitor of Na^+/K^+ pump, induced an increase in C_{Na} and a decrease in C_K.

In addition to the well known action of cyanide in inhibiting cell metabolism it has been recently reported that cyanide hyperpolarizes apical and basolateral membranes of epithelial cells (3). As a result of this hyperpolarization Na^+ and K^+ ions will enter into the cell. This effect may explain the high C_{Na} (94 ± 12 mM) obtained under CN^- poisoning. The effect of the hyperpolarization and the effect of inhibiting Na^+/K^+ pump are added to build up that C_{Na} value. In contrast, C_K will increase due to the membrane hyperpolarization, but C_K will tend to decrease due to metabolic inhibition of Na^+/K^+ pump. These two different tendencies may cancel each other. The final result is that C_K was not affected by CN^- poisoning of the tissue. As a result of metabolic inhibition the epithelial cell swells (16, 17). However, data presented in table II suggest that cells shrink under CN^- poisoning. We do not have any explanation for this finding. Further studies must be made to clarify the nature of this cell shriking under CN^- poisoning.

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Resumen

Se miden las concentraciones intracelulares de Na^+, K^+ y Cl^-. La supresión de Na^+ en la solución de incubación provoca una disminución significativa en la concentración intracelular de Cl^-. De forma recíproca, la supresión de Cl^- en la solución de incubación produce una disminución significativa en la concentración intracelular de Na^+. Estos resultados sugieren la existencia de un sistema acoplado de NaCl.

La presencia de ouabaína 10^{-3} M en la solución de incubación produce un incremento en la concentración intracelular de Na^+ y una disminución en la concentración intracelular de K^+. El cianuro (10^{-3} M) incrementa significativamente la concentración intracelular de Na^+; no obstante, la concentración intracelular de K^+ no se alteró en presencia de este agente.

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