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Electrical Properties and Fluxes of Na^+ and Cl^- Across Lizard Intestine

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Electrical parameters and unidirectional Na^+ and Cl^- fluxes were determined *in vitro* across the duodenum, ileum and colon of lizard *(Gallotia galloti)*. Electrical potential difference (PD) and short circuit current (Isc) were low in the three segments studied, whilst tissue conductance (Gt) was high. A net active transport of Na^+ and Cl^- was observed in the three segments. Net Na^+absorption was higher across duodenum and ileum than across the colon, while net Cl^- absorption was similar in duodenum, ileum and colon. Ouabain virtually abolished Isc, PD and net Na^+ and Cl^- fluxes in all the segments. Amiloride abolished net Cl^- flux in duodenum, ileum and colon, whereas net Na^+ flux was abolished in colon but decreased in duodenum and ileum. PD and Isc were not affected by the presence of the diuretic.

Key words: Na^+ transport, Cl^- transport, Lizard, Intestine.

Previous investigations have described the importance of the absorption and secretion of ions, such as Na^+, K^+, Cl^- and HCO_3^- across the various regions of the intestine for the normal nutrition of all vertebrates. The intimate nature and mechanisms of these processes have mainly been studied in colon (9, 23-25) and ileum (8, 17, 18) of mammals. Relatively few such studies have, however, been made in reptiles (1, 2, 4, 12).

Segmental differences of electrolyte transport have already been demonstrated for rat colon (6, 10, 24) and for guinea pig colon (7). Na^+ transport and electrical properties across rabbit caecum, proximal and distal colon *in vitro* were investigated (5). Recently GRUBB *et al.* (11) compared the electrical potential difference (PD), short-circuit current (Isc) and tissue conductance (Gt) using classical divided Ussing-chamber techniques, across duodenum, jejunum, ileum and colon (or rectum) of the domestic fowl *(Gallus domesticus)*. The purpose of the present study was: To compare PD, Isc and Gt across the duodenum, ileum and colon of the lizard

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(Gallotia galloti), to establish where tight and leaky segments are located; and to compare the individual Na^+ and Cl^- transport and to determine whether the characteristics of Na^+ and Cl^- transport differ in the duodenum, ileum and colon.

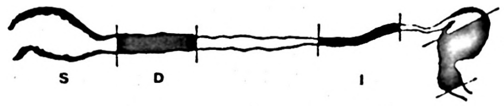

Fig. 1. *Schematic drawing of the lizard* (Gallotia galloti) *small and large intestine.*
Shaded areas represent the segments on which measurements were performed. (S = Stomach, D = Duodenum, I = Ileum, C = Colon).

Materials and Methods

Experiments were performed on male and female *Gallotia galloti* lizards (mean body weight 30-40 g). Animals were killed by spinal transection.

Different intestine segments were used: the first centimetre of duodenum, ileum segments extending anteriorly 1 cm from the origins of the colon, and the whole colon segment of around 1 cm in length (fig. 1). After removal of the segments, they were rinsed several times with Ringer solution.

The tissues were mounted in Ussing chambers, with an exposed surface of 0.21 cm^2 and bathed on each side with 4.0 ml of solution. Less than five min was required to mount the tissue after the animals were killed. The Ringer solution contained in mmoles/l: 140 Na^+, 124 Cl^-, 21 HCO_3^-, 5.4 K^+, 2.4 HPO_4^-, 0.6 $H_2PO_4^-$, 1.2 $Mg^=$, 1.2 Ca^+, 5 glucose and had a pH of 7.4. It was oxygenated by a gas lift (95 % O_2 and 5 % CO_2), and warmed to 30°C. The glucose was added only to solution bathing the serosal surface. Under these conditions, tissues could be maintained for 3-4 h, and their viability was verified by a stable Isc and Gt.

The methods for measuring the electrical parameters were essentially the same as described by BADIA *et al.* (2) The tissue was continuously short circuited with the aid of an automatic computer controlled voltage clamp device (AC-Microclamp, Aachen, W. Germany).

Series resistance of the bathing solution was determined before the experiment and then corrected automatically. All clamps were microcomputer-controlled and the electrical parameters open circuit potential difference (PD), short circuit current (Isc) and tissue conductance (Gt) were recorded by a digital printer at 1 min intervals. In order to examine the effect of different concentrations of amiloride (Sigma) on the Isc, this inhibitor was added to the mucosal bathing solution in small volumes (100 µl). The final concentration of amiloride in the mucosal solution varied from 10^{-7} to 10^{-3} M. A standard concentration was added at 10 min intervals throughout the experiments.

Unidirectional Na^+ and Cl^- fluxes were measured on randomly paired tissues under short circuit conditions. Four µci Na^{22} and Cl^{36} (New England Nuclear) were added either to the mucosal or the serosal side, and after an equilibration period of 20 min 0.2 ml samples were withdrawn in 20 min intervals from both sides. Unidirectional fluxes were calculated using standard equations (20).

The net residual ion flux (J^{R}_{net}) was calculated from the difference in Isc and net Na^+ (J^{Na}_{net}) and Cl^- (J^{Cl}_{net}) fluxes (J^{R}_{net} = Isc- (J^{Na}_{net} - J^{Cl}_{net}).

Results are given as the mean ± standard error of the mean (SEM). Significances of differences were tested using a two-tailed Student's-test. Paired or unpaired tests were used.

Results

Segmental differences of electrical properties under control conditions. — The

Table I. *Effects of ouabain (10^{-3} M) and amiloride (10^{-3} M) on electrical parameters of the 3 intestinal segments of the lizard* (Gallotia galloti).
Values represent means ± S.E.M, n = number of experiments. *, #, Statistically different (P<0.01, P<0.001) respectively from control.

	n	PD (mV)	Isc ($\mu A/cm^2$)	Gt (mS xcm^2)
Duodenum				
Control	(18)	2.05 ± 0.37	32.17 ± 4.80	14.07 ± 1.86
Amiloride	(18)	2.21 ± 0.19	29.49 ± 3.00	13.20 ± 1.99
Control	(16)	2.30 ± 0.28	31.48 ± 2.98	14.60 ± 1.20
Ouabain	(16)	0.60 ± 0.24#	13.40 ± 4.55*	26.00 ± 2.08#
Ileum				
Control	(17)	0.79 ± 0.09	21.71 ± 2.60	24.72 ± 1.43
Amiloride	(17)	0.68 ± 0.08	20.37 ± 3.20	29.62 ± 1.91
Control	(20)	0.98 ± 0.13	28.95 ± 4.55	27.74 ± 2.38
Ouabain	(20)	0.08 ± 0.02#	2.60 ± 0.90#	31.50 ± 2.81
Colon				
Control	(12)	0.30 ± 0.37	8.22 ± 1.20	18.17 ± 1.21
Amiloride	(12)	0.56 ± 0.26	6.70 ± 0.80	17.40 ± 3.24
Control	(15)	0.42 ± 0.21	6.98 ± 0.90	16.30 ± 1.82
Ouabain	(15)	0.10 ± 0.24	0.30 ± 0.20#	28.20 ± 1.92#

three segments of lizard intestine showed some differences in their basic electrical properties. The values of electrical parameters are presented in table I. All three segments exhibited a transmural PD and the serosal side was usually electropositive, compared to the mucosal side. There were, however, regional differences in the magnitude of this PD, which was smallest in the colon and greatest across the doudenum. Similar characteristics were recorded for Isc, which was very low across the colon and highest in the duodenum. The Isc in ileum fell between the values in the duodenum and colon. The ileum exhibited the greatest electrical conductance, while duodenum and colon showed a much lower conductance.

Unidirectional fluxes of Na^{22} and Cl^{36} across the intestines. — Unidirectional measurement and net sodium and chloride fluxes across lizard duodenum, ileum and colon under short-circuit conditions (tables II, III) showed that the three intestinal segments actively absorb sodium and chloride. The fluxes of both Na^+ and Cl^- in either direction, J_{ms} and J_{sm} were higher across the ileum than across the duodenum and colon. Duodenum and ileum had greater Na-transporting capacities than the colon. The net transport of chloride was similar across the three segments. These results show that the doudenum and ileum of *Gallotia galloti* absorb sodium and chloride in a ratio of approximately 2:1, while the colon absorbs sodium and chloride in approximately equivalent amounts. The transport of these two ions accounts for Isc in duodenum, ileum and colon, whereas the net residual flux was approximately equal to zero (tables II, III). It is suggested that if any movements of other unmeasured ions

Table II. *Effects of amiloride (10^{-3} M) on unidirectional fluxes of Na^+ and Cl^- across various segments of the intestine of lizard.*
Ion fluxes and Isc are given in $\mu Eq/cm^2 \times h$. Measurements were made before and after the addition of amiloride to the mucosal bathing solution. Values represent means ± S.E.M. Figures in parentheses denote number of experiments. *, #, significance difference ($p<0.01$, $p<0.001$) respectively from control.

		J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{net}	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}	Isc	J^{R}_{net}
Duodenum									
Control	(10)	5.50±0.57	3.21±0.30	2.29±0.20	6.27±0.36	5.11±0.47	1.16±0.18	1.18±0.18	0.05±0.19
Amiloride	(10)	4.40±0.46	3.40±0.31	1.00±0.17#	5.21±0.28*	5.32±0.12	-0.11±0.09*	1.06±0.11	-0.05±0.12
Ileon									
Control	(10)	11.25±0.68	8.60±0.43	2.65±0.25	12.11±0.59	10.97±0.32	1.14±0.21	0.82±0.09	-0.69±0.13
Amiloride	(10)	9.38±0.57	7.67±0.35	1.71±0.20*	10.86±0.92	10.37±0.70	-0.01±0.38#	0.80±0.14	-0.92±0.19
Colon									
Control	(16)	3.71±0.38	2.65±0.16	1.06±0.10	6.48±0.83	5.52±0.39	0.96±0.23	0.26±0.04	0.16±0.07
Amiloride	(16)	2.90±0.19	2.82±0.14	0.08±0.06#	5.81±0.42	5.70±0.42	-0.09±0.01#	0.31±0.02	0.14±0.06

Table III. *Effects of ouabain (10^{-3} M) on unidirectional fluxes of Na^+ and Cl^- across various segments of lizard intestine.*
Ion fluxes and Isc are given in $\mu Eq/cm^2 \times h$. Measurements were made before and after the addition of amiloride to the mucosal bathing solution. Values represent means ± S.E.M. Figures in parentheses denote number of experiments. *, #, significance difference ($p<0.01$, $p<0.001$) respectively from control.

		J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{net}	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}	Isc	JR_{net}
Duodenum									
Control	(12)	5.60±0.40	3.10±0.61	2.50±0.21	6.32±0.48	5.02±0.81	1.30±0.27	1.14±0.11	-0.06±0.14
Ouabain	(12)	4.71±0.50	4.70±0.72	0.01±0.26#	4.96±0.48	4.78±0.79	0.18±0.26#	0.45±0.17#	0.62±0.20
Ileon									
Control	(10)	10.08±0.74	7.19±0.56	2.89±0.29	12.30±0.68	11.02±0.60	1.48±0.28	1.08±0.17	-0.35±0.21
Ouabain	(10)	8.26±0.55	8.20±0.20	0.06±0.18#	11.32±0.51	11.15±0.50	0.17±0.22	0.06±0.12#	0.17±0.15
Colon									
Control	(14)	3.37±0.34	2.84±0.23	1.03±0.10	7.82±0.79	6.53±0.53	1.29±0.27	0.27±0.06	0.53±0.09
Ouabain	(14)	2.10±0.20#	2.62±0.18	-0.50±0.07#	5.58±0.62*	5.38±0.33	0.20±0.18*	0.02±0.02#	0.72±0.05

across the tissues occur, then they must be transported by an electroneutral mechanism.

Effects of amiloride and ouabain on Isc. — In a first experimental series, different doses of amiloride were added to the mucosal bathing solution, to determine the effects on Isc. The tissue was first incubated until relatively stable, usually about 30 min, and after a 20 min control period any spontaneous increase or decrease in Isc was recorded. Amiloride was then added to the mucosal compartment and after a new plateau was reached the Isc was recorded. The different doses of amiloride (10^{-7} M-10^{-3} M) did not cause any change in the Isc across the duodenum, ileum and colon (data not shown).

In a second experimental series (table II), 10^{-3} M amiloride was used to characterize the effect on Na^+ and Cl^- fluxes. Amiloride decreased the mucosal to serosal sodium fluxes in duodenum, ileum and colon and the net sodium flux was significantly reduced in duodenum and ileum and completely abolished in colon. Amiloride decreased the mucosal to serosal flux of Cl^- in the three segments, but a significant difference was only found for the duodenum (table II). These changes caused an abolishing of the net fluxes of Cl^-. Amiloride did not affect PD, Gt and Isc across the intestine (table I). Ouabain (Merck) abolished the PD and decreased the Isc, but increased the Gt in all the segments except in ileum (table I). The net fluxes of sodium and chloride were virtually abolished (table III).

Discussion

The data reported here show that the various regions of the intestine of the lizard *Gallotia galloti* exhibit differences in their electrical parameters. The doudenum had the highest PD and Isc. The colon exhibited the lowest PD and Isc but the conductance was similar to that observed in the duodenum. The Isc and PD in ileum fell between the values in duodenum and colon but the conductance was higher than in duodenum and colon, which was associated with the highest measured unidirectional ion fluxes of all fluxes measured in the different intestinal zones. The three segments present in general low PD and Isc and high conductance. These results show that the epithelium of the duodenum, ileum and colon of *Gallotia galloti* can be considered as leaky. These segments have about the same conductance as other more leaky epithelia such as small intestine and proximal colon of mammals (5, 8, 16). The high conductance observed in the colonic epithelium of this animal species differs from those reported for other colonic epithelia, such as human colon (15), turtle colon (13, 14) and those of the lizards *Amphibolorus ornatus, A. inermis* and *Trachiglosaurus rugosus* (4) which have been regarded as tight epithelia. These epithelia display high tissue resistance (R_t>500 $\Omega \times cm^{-2}$) and potential differences that are due to the electrogenic absorption of sodium. These electrical characteristics are clearly distinct from that displayed by the colon of *G. galloti*, in which the PD and Isc are near zero.

The relative importance of the different segments of the vertebrate intestine in maintaining salt balance is not clear. In mammals, net transport of sodium and chloride from mucosa to serosa has mainly been studied in ileum (8) and distal colon (9, 22, 23). Recent studies (5) performed in four segments of rabbit large intestine showed remarkable differences in their basic electrical properties and Na^+-transport. Net sodium absorption was higher in caecum than in proximal colon and higher in this than in distal colon. In avian intestine it has been suggested (21) that the posterior parts of the intestine, the caecum, colon and coprodeum may play a major role in fluid and sodium absorption, while the anterior small intestine is principally involved in calcium and potassium absorption. Recent studies (11) suggest that the ileum may also contribute significantly to the process of sodium absorption. In reptilian intestine no data are available about segmental differences in electrical properties and sodium transport. The data reported here show that the sodium and chloride ions are actively transported in all segments studied. This is clear from the presence of a considerable sodium and chloride net flux under the short-circuit conditions. Measurements of both unidirectional and net fluxes (table II, III) indicate that the absorption of these ions occurs in a stoichiometric ratio of 2:1 in duodenum and ileum while in colon the stoichiometric ratio was 1:1. These observations suggest that the doudenum, ileum and colon may contribute significantly to the process of maintaining salt balance.

The addition of ouabain to the serosal side abolished Isc and net sodium flux in all segments. It is very likely that as in other intestinal epithelia (5, 19) a basolateral Na^+/K^+ pump is responsible for the sodium extrusion out of the cells. On the other hand, as the addition of ouabain also abolished the net chloride transport in all the segments, a possible mechanism of dependence of Cl^- transport on the Na^+-K^+ pump appears to be present. The electrochemical gradient created by the Na^+/K^+ pump appears to energize the influx of chloride across the said membrane.

The addition of amiloride (10^{-3}M) to the mucosal solution abolished the net chloride flux and reduced the net sodium flux across the doudenum and ileum. PD and Isc were not significantly reduced when the diuretic was added. These results are in agreement with those suggesting that sodium chloride entry in lizard ileum cells is mediated by a dual system of Na^+/H^+ and Cl^-/HCO_3^- exchange (2) the Na^+/H^+ antiport being sensitive to amiloride as has been described for a wide variety of epithelia (3). On the other hand, these results contrast with those described for tight epithelia in which amiloride addition abolishes Isc and net sodium flux (9). The reason for this is unclear at the moment but it is not thought to be a matter of concentrations since the results show that no dose of the amiloride induces any change in the Isc. Studies performed in caecum and proximal parts of the colon of rabbit large intestine (5) showed that these epithelia are leaky and that the action of amiloride was incomplete or had no effect. These observations support the hypothesis that amiloride action in leaky epithelia may be different from that in tight epithelia. Experiments to clarify this hypothesis must be performed in different leaky epithelia.

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Resumen

Se determinan *in vitro*, a través del duodeno, íleon y colon de lagarto *Gallotia galloti* los parámetros bioeléctricos y flujos unidireccionales de Na^+ y Cl^-. La diferencia de potencial y la corriente de cortocircuito son bajas en los tres segmentos, mientras que la conductancia tisular es alta. El transporte neto de sodio y cloruro muestra que la absorción de sodio es más alta a través del duodeno e íleon que en el colon, mientras que el cloruro se absorbe con similar intensidad en los tres segmentos intestinales. La ouabaina abole la intensidad de corriente, la diferencia de potencial y los flujos netos de sodio y cloruro en todos los segmentos. El amiloride suprime el flujo neto de cloro en duodeno, íleon y colon. El flujo neto de sodio se anula en el colon, mientras que en duodeno e íleon solo disminuye. La diferencia de potencia y la intensidad de corriente de cortocircuito no se ven afectadas por la presencia del diurético.

Palabras clave: Transporte de Na^+, Transporte de Cl^-, Lagarto, Intestino.

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