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ACTIVE SECRETION OF CALCIUM, SODIUM AND CHLORIDE BY ADULT RAT DUODENUM IN VITRO

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SUMMARY

Active secretion of Ca^{2+} is observed from the serosal to the mucosal surface across adult rat duodenum in vitro when absorptive Ca^{2+} flux is saturated by a high $[Ca^{2+}]$. Sodium and chloride are spontaneously secreted by this tissue with Cl secretion apparently accounting for about one-third of the short-circuit current when there is no absorptive co-transport of Na^{+}.

INTRODUCTION

Previous studies in this laboratory demonstrated that Ca^{2+} is actively transported in a serosal to mucosal direction across rat jejunum and ileum. This secretory process has been observed both in adult rats on a normal diet [1–3] and in growing rats that are vitamin D-deficient [2]. Calcium secretion can also be demonstrated across the ileum of growing rats raised on a diet high in Ca^{2+} content [3]. However, active Ca^{2+} secretion has not been observed across proximal duodenum under any of the preceding conditions [1–3].

In studies of Ca^{2+} transport across rat duodenum in vitro, Walling and Rothman found that both active Ca^{2+} absorption and unidirectional mucosal to serosal flux (J_{ms}) were saturable functions of incubation medium Ca^{2+} concentration, while serosal to mucosal flux (J_{sm}) was not [4, 5]. In contrast to Ca^{2+} absorption, active secretion and J_{sm} across rat ileum are not saturable processes [1, 3]. The present experiments were performed to determine whether active Ca^{2+} secretion could be demonstrated across proximal rat duodenum when transport was studied at a medium Ca^{2+} concentration that should saturate J_{ms} but not J_{sm} [4, 5].

Adult male Holtzman rats (weight $>$ 400 g) raised on a normal laboratory chow diet were used in this study. Ion fluxes were studied in vitro with a modified Ussing apparatus using a technique previously described in detail [1, 4], except that the serosal musculature was dissected from the intestine giving a preparation similar to that described by Binder and Rawlins [6]. The transmural PD was short-circuited using the method for compensating for fluid resistance described by Field et al. [7]. To prevent $CaCO_3$ precipitation which would occur at 25 mM Ca^{2+} if a bicarbonate

TABLE I

IN VITRO FLUXES OF CALCIUM AND SODIUM ACROSS STRIPPED ADULT RAT DUODENUM AT MEDIUM CALCIUM CONCENTRATIONS OF 1.25 AND 25 mM

PD short-circuited, HEPES buffer, O_2-gassing, pH 7.4, $[Na^+]$ = 133 mM, 11 mM D-glucose in both the mucosal and serosal solutions. Net fluxes measured on paired pieces of intestine for each n = 1, J_{net} = J_{ms} − J_{sm}, therefore positive values of J_{net} represent active absorption; negative values, active secretion.

Condition	n	Ca^{2+} fluxes, nequiv · cm^{-2} · h^{-1}			Na fluxes, μequiv · cm^{-2} · h^{-1}			s.c.c. μequiv · cm^{-2} · h^{-1}	G mΩ^{-1} · cm^{-2}
		J_{ms}	J_{sm}	J_{net}	J_{ms}	J_{sm}	J_{net}		
1.25 mM $CaCl_2$ − 36 mM choline Cl	5	49.3 ± 6.6	51.0 ± 5.4	1.7 ± 9.4[1]	6.3 ± 0.3	7.1 ± 0.4	−0.8 ± 0.3[4]	2.3 ± 0.3	24.5 ± 1.0[5]
25 mM $CaCl_2$	8	491.8 ± 20.9	904.8 ± 51.8	−413.0 ± 45.6[2]	3.8 ± 0.2[3]	5.3 ± 0.3[3]	−1.5 ± 0.4[4]	2.7 ± 0.3	19.0 ± 0.8

[1] No net calcium flux when the $[Ca^{2+}]$ = 1.25 mM, P > 0.9.
[2] Active calcium secretion when the $[Ca^{2+}]$ = 25 mM, P < 0.001.
[3] Both sodium J_{ms} and J_{sm} were lower at 25 mM calcium, P < 0.001 and 0.005, respectively.
[4] Active secretion of sodium at both 1.25 and 25 mM calcium, P < 0.05 and 0.01, respectively.
[5] Intestinal conductance was greater at 1.25 than at 25 mM Ca^{2+}, P = 0.005.

buffer were used, a 10 mM *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer with 100 % O_2 gassing was employed. Control tissues were perfused with solutions containing 1.25 mM $CaCl_2$ and 36 mM choline chloride so that osmolarity and Cl^- concentrations would be similar to those in the presence of 25 mM $CaCl_2$. To provide an indication of any deleterious effect due to the high Ca medium, Na transport and short-circuit current (s.c.c.) were measured.

Calcium and Na^+ fluxes were measured simultaneously using $^{45}Ca^{2+}$ and $^{22}Na^+$ as tracers. Samples were counted in Bray's solution [8] in an Intertechnique model SL-40 multi-channel liquid scintillation spectrometer. When Ca^{2+}, Na^+ and Cl^- fluxes were studied simultaneously, $^{22}Na^+$ was assayed both in a Nuclear Chicago gamma counter and by liquid scintillation techniques, and efficiency and overlap corrections were used to determine the $^{36}Cl^-$ and $^{45}Ca^{2+}$ activities from the liquid scintillation measurements.

The data in Table I show that while there was no net Ca^{2+} flux across the duodenum when the medium Ca^{2+} concentration was 1.25 mM, there was a large active secretion of Ca^{2+} by this tissue when the level was raised to 25 mM. Active Ca^{2+} secretion was apparently observed at 25 mM because this 20-fold rise in concentration increased J_{ms} only by a factor of 10 while J_{sm} increased in proportion to the change in Ca^{2+} concentration (Table I). An unexpected finding was active Na^+ secretion of similar magnitude at both Ca^{2+} concentrations (Table I). Since both unidirectional Na^+ fluxes and intestinal conductance were decreased at 25 mM Ca^{2+} (Table I), it seems likely that intestine is affected by high divalent cation concentration in a manner similar to that reported for rabbit gallbladder epithelium [9]. Possible changes in intracellular Ca^{2+} activity that might have resulted from the 25 mM Ca^{2+} concentration were not measured. However, because the magnitude of both active Na^+ secretion and the s.c.c. were unchanged at this high Ca^{2+} level (Table I), according to these indicators it appears that cellular metabolism and some intracellular transport processes were unaffected by any change in Ca^{2+} that did occur.

Since the mucosal surface of the duodenum is negatively charged relative to the serosa, a net serosal to mucosal movement of Na^+ could not produce the observed s.c.c. Additional experiments were performed in a second group of animals in which Cl^- as well as Na^+ and Ca^{2+} fluxes were measured in order to better evaluate the source of duodenal s.c.c. Glucose in solutions perfusing the mucosal surface was replaced by D-mannitol to eliminate any Na^+-hexose co-transport component of Na^+ J_{ms} and to provide better conditions for demonstrating Na^+ secretion. When Na^+, Ca^{2+}, and Cl^- fluxes were measured simultaneously across stripped rat duodenum under these conditions, active secretion of Cl^- as well as Na^+ was observed together with a small absorption of Ca^{2+} (Table II). While chloride secretion exceeded Na^+ secretion, the sum of these ion fluxes did not account for the entire s.c.c. (Table II). Unlike the experiments in Table I, these tissues were incubated in a HCO_3^--buffered solution [1] making it reasonable to suggest that, as is the case for rabbit ileum [10], bicarbonate ion secretion may account for the residual ion flux (Table II).

The present data indicate that under certain conditions the existence of active Ca^{2+} secretion across rat duodenum can be readily demonstrated. In addition, this tissue spontaneously secretes Na^+ and Cl^- with the net flux of the latter species apparently accounting for about one-third of the s.c.c. when there is no co-transportable substrate (hexose or amino acid) in the mucosal solution (Table II). Sodium

TABLE II

FLUXES OF CALCIUM, SODIUM AND CHLORIDE ACROSS STRIPPED ADULT RAT DUODENUM IN VITRO

Fluxes determined from paired pieces of duodenum from the same animal for each n. HCO_3^--buffer, 95% O_2/5% CO_2 gassing. 20 mM D-mannitol in mucosal solutions. 20 mM D-glucose in serosal solutions. J_{net} J_{ms} J_{sm} with positive values representing active absorption, negative values net secretion.

(n = 7)	Steady-state ion fluxes (μequiv · cm^{-2} · h^{-1})		
	J_{ms}	J_{sm}	J_{net}
Calcium (1.25 mM)	0.036 ± 0.002	0.032 ± 0.001	+ 0.004 ± 0.002[1]
Sodium (144 mM)	6.1 ± 0.2	8.8 ± 0.6	2.7 ± 0.5[2]
Chloride (127 mM)	5.1 ± 0.3	8.5 ± 0.6	3.5 ± 0.5[3, 4]

s.e.c. = 2.5 ± 0.2 μequiv · cm^{-2} · h^{-1}, serosal surface positive
$Na^+ J_{net} + Cl^- J_{net}$ = 0.8 ± 0.3 μequiv · cm^{-2} · h^{-1}
Residual ion flux = 1.7 ± 0.3 μequiv · cm^{-2} · h^{-1}
Conductance = 20.5 ± 0.8 mΩ^{-1} · cm^{-2}

[1] Active absorption of calcium, $P < 0.05$.
[2] Active secretion of sodium, $P < 0.005$.
[3] Active secretion of chloride, $P < 0.005$.
[4] $Cl^- J_{net} > Na^+ J_{net}$ on a paired basis, $P < 0.02$.
[5] Residual ion flux was significant, $P < 0.005$.

secretion has been reported across rabbit jejunum [11], and unstimulated Cl^- secretion of a similar magnitude to that observed across duodenum (Table II) has been observed across rat jejunum [12, 13]. Since Na^+ and Cl^- are absorbed by both rat [12, 14] and rabbit ileum [12, 15], the present results, together with the data from jejunum [12, 13] suggest that the polarity of net Na^+ and Cl^- fluxes changes from secretion toward absorption between proximal and distal small bowel. This hypothesis is supported by the in vivo observations of Schedl et al. who found that blood to lumen fluxes of Na^+ and Cl^- were greatest in rat duodenum and decreased in the lower small intestine [16].

In contrast to Na^+ and Cl^- movements, Ca^{2+} secretion is greatest in the distal small intestine [1–3], and only when the Ca^{2+} absorptive process is saturated by high substrate levels can active secretion be observed across duodenum (Table I). In addition to the active Ca^{2+} absorption process that appears to be primarily regulated by metabolites of vitamin D [2, 17], the present results show rat duodenum also contains an active Ca^{2+} secretory process. The existence of Na^+ and Ca^{2+} secretory processes in this tissue may make it incorrect to assume that Na^+, Ca^{2+}-ATPase [18] and Ca^{2+}-ATPase [19, 20] activities isolated from rat duodenum are associated with absorption rather than with secretion of Ca^{2+} or Na^+.

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REFERENCES

1 Walling, M. W. and Kimberg, D. V. (1973) Am. J. Physiol. 225, 415–422
2 Walling, M. W., Favus, M. J. and Kimberg, D. V. (1974) J. Biol. Chem. 249, 1156–1161
3 Walling, M. W. and Kimberg, D. V. (1974) Am. J. Physiol. 226, 1124–1130
4 Walling, M. W. and Rothman, S. S. (1969) Am. J. Physiol. 217, 1144–1148
5 Walling, M. W. and Rothman, S. S. (1970) J. Biol. Chem. 245, 5007–5011
6 Binder, H. J. and Rawlins, C. L. (1973) Am. J. Physiol. 225, 1232–1239
7 Field, M., Fromm, D. and McColl, I. (1971) Am. J. Physiol. 220, 1388–1396
8 Bray, G. (1960) Anal. Biochem. 1, 279–285
9 Wright, E. M., Barry, P. H. and Diamond, J. M. (1971) J. Membrane Biol. 4, 331–357
10 Dietz, J. and Field, M. (1973) Am. J. Physiol. 225, 858–861
11 Fromm, D. (1973) Am. J. Physiol. 224, 110–116
12 Taylor, A. E., Wright, E. M., Schultz, S. G. and Curran, P. F. (1968) Am. J. Physiol. 214, 836–842
13 Munck, B. G. (1970) Biochim. Biophys. Acta 203, 424–433
14 Curran, P. F. (1960) J. Gen. Physiol. 43, 1137–1148
15 Schultz, S. G. and Zalusky, R. (1964) J. Gen. Physiol. 47, 567–584
16 Schedl, H. P., Wilson, H. D. and Miller, D. L. (1968) Proc. Soc. Exp. Biol. Med. 129, 511–515
17 DeLuca, H. F. (1973) Kidney Int. 4, 81–88
18 Birge, S. J., Gilbert, H. R. and Avioli, L. V. (1972) Science 176, 168–170
19 Martin, D. L., Melancon, M. J., Jr. and DeLuca, H. F. (1969) Biochem. Biophys. Res. Commun. 35, 819–823
20 Kowarski, S. and Schachter, D. (1973) J. Clin. Invest. 52, 2765–2773