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Calcium–Secretagogue Interaction in the Stimulation of Gastric Acid Secretion[1] (37784)

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Calcium is known to play a prime role in regulating permeability of biological membranes and transport of ions across their surfaces in various epithelia. Höber (1) has reviewed earlier literature on the relationship between the electrical resistance of tissues and the Ca^{2+} content of their bathing media. Mond (2) and later Gray and Adkinson (3) showed that Ca^{2+} affected both the secretory process and electrical parameters of the frog gastric epithelium. In recent years, Forte and Nauss (4) and Jacobson, Schwartz and Rehm (5) have carried out detailed investigations on the effects of removal of Ca^{2+} from the bathing media of bullfrog gastric mucosa with respect to the electrical, secretory and transmucosal flux properties. Their studies have indicated that replacement of Ca^{2+} in the nutrient bathing medium generally restored the normal mucosal functions, whereas only a partial reversal was obtained following inclusion of Ca^{2+} in the luminal bathing medium, suggesting poor permeability of the luminal surface to Ca^{2+}. On the basis of morphological studies at the electron microscope level, Sedar and Forte (6) have shown that Ca^{2+} is important primarily in maintaining the integrity of the junctional complex of the epithelial cells and have attempted to correlate the fine structural changes with the physiological data following Ca^{2+} removal.

Relatively little is known about the role of Ca^{2+}, if any, in the sequence of reactions leading to secretion of H^+ *per se* in situations where such junctional complexes may be reconstituted in the absence of complete repletion of cellular Ca^{2+}. The present communication describes such an attempt, and the effects of cyclic-AMP and theophylline on the Ca^{2+}-depleted gastric epithelia following incubation in the presence of luminal Ca^{2+}. The findings are discussed in terms of the relationship between the secretagogues, cyclic AMP and the role of Ca^{2+} in the mediation of acid secretion.

Materials and Methods. The experiments were carried out on *in vitro* preparations of gastric mucosae of *R. catesbeiana* isolated as described previously (7). The compositions of the bathing solutions used were as follows: the serosal side was bathed in a nutrient solution containing (m*M*): Na^+, 101; K^+, 4; Ca^{2+}, 1.8; Mg^{2+}, 0.8; Cl^-, 90; HCO_3^-, 18; PO_4^{2-}, 1; and glucose, 11; the luminal side was bathed in an unbuffered 120 m*M* NaCl solution. Histamine (0.1 m*M*) was added to the nutrient medium in most experiments, while pentagastrin (10^{-6} *M*) or acetylcholine (10^{-4} *M*) were used as secretagogues in others, and were present throughout the experimental period. Both solutions were bubbled with a gas mixture containing 95% O_2–5% CO_2. The H^+ secretory rate was measured by the pH stat method of Durbin and Heinz (8) and the luminal contents were titrated by addition of 15 m*M* NaOH with the stat setting at 4.5. The exposed mucosal area was 1.8 cm^2 throughout. Transmucosal potential difference (PD) was monitored by two matched saline-agar bridges connected

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via calomel electrodes to a Kontron recorder. The steady state transepithelial resistance was calculated from the change in PD in response to a 20 μA current applied for at least 2 min through another pair of saline–agar electrodes.

The mucosal Ca^{2+} depletion was accomplished in early experiments by replacement of the normal with Ca^{2+}-free nutrient solution and repeated washouts of the epithelium with the latter solution at 30 min intervals. However, the time required to reduce H^+ secretion to zero and the PD to a minimum value was of the order of 3–4 hr. In subsequent studies, EDTA was included in Ca^{2+}-free nutrient solution to facilitate Ca^{2+} depletion; EDTA was removed by at least 3 additional washes with Ca^{2+}-free and EDTA-free nutrient solution prior to further experimental manipulation of the epithelium.

Transmucosal fluxes of sucrose from nutrient to the secretory side (N→S) were measured as follows. Mucosae were depleted of Ca^{2+} to the stage of zero acid secretion and then sucrose-^{14}C (sp act 20 mCi/mmole) was added to the nutrient bathing medium. Luminal solutions were withdrawn at intervals of 20 min and replaced with fresh solutions. The first three consecutive replacements were carried out with Ca^{2+}-free luminal bathing medium, while the subsequent replacements included 1 m*M* Ca^{2+}. After 5 additional collections, 1.8 m*M* Ca^{2+} was added to the nutrient bathing medium and the collection process continued for 4 more periods. Aliquots of luminal media collected, were counted in a liquid scintillation counter. The data on sucrose flux is expressed as the ratio of counts appearing in the luminal bathing medium to the number of counts in the nutrient medium.

Dibutyryl cyclic-AMP (DBcAMP) and theophylline, when present, were added to the nutrient bathing medium and the pH adjusted to 7.3.

Results. The effects of removal of Ca^{2+} on H^+ secretory rate, transmucosal PD and resistance are shown in Fig. 1. In all instances, washout of Ca^{2+} was continued until the cessation of H^+ secretion. The electrical and secretory parameters under these conditions followed a course similar to that described by Jacobson, Schwartz and Rehm (5). With the continued absence of Ca^{2+}, the pH of the unbuffered secretory solution gradually increased as is to be expected, due to passive bicarbonate diffusion from serosal to the luminal side. The normal functions of the epithelium can be restored almost completely on addition of Ca^{2+} to the serosal bathing medium up to 4 hr after cessation of acid secretion; following prolonged deprivation of Ca^{2+} however, the process of recovery was too slow and partial and in some cases not possible.

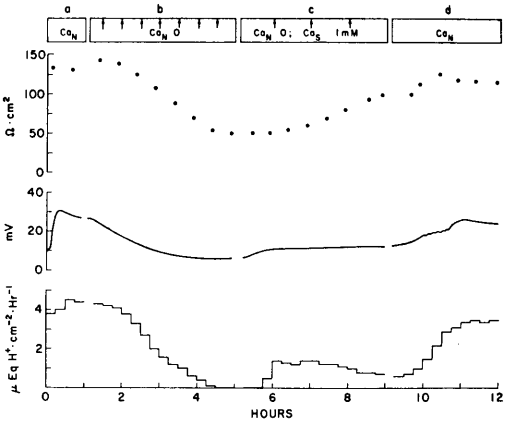

FIG. 1. Rate of acid secretion, PD and resistance of the gastric mucosa (a) in normal nutrient and secretory medium, (b) in the absence of Ca^{2+}, (c) in the presence of 1 mM Ca^{2+} in the luminal bathing medium, and (d) during recovery with normal nutrient medium. Upward arrows indicate washouts under appropriate conditions in this as well as subsequent illustrations.

Addition of Ca^{2+} (0.5–75 m*M*) to the luminal side of the mucosae depleted of Ca^{2+} as above led to concentration-dependent, small but significant increases in PD which reached steady state values within 1 hr. There was a similar but slower increase in transmucosal resistance (Table I). The effect on acid secretion was, however, variable. Of the 40 pairs of mucosal halves studied in the presence of 1.0 m*M* luminal Ca^{2+}, 18 did not secrete acid, 14 secreted between 0–30% of the initial control rates and 8 secreted between 30–60%. Repeated washout of the epithelium with Ca^{2+}-free nutrient and 1.0 m*M* Ca^{2+}-containing luminal bathing media at 30

TABLE I. Effect of Luminal Presence of Ca^{2+} on Electrical and Secretory Functions of Gastric Mucosae Depleted of Ca^{2+}.

Luminal Ca^{2+} (mM)	No. of mucosae	Mean PD (mV)	Resistance (Ω cm^2)	Secretory rate (μeQ H^+ cm^{-2} hr^{-1})
0	30[a]	6	35 ± 2	0
0.5	6[a]	6	46 ± 4	<0.1
1.0	15[a]	9	88 ± 2	0.9 ± 0.1
2.0	6[a]	12	96 ± 3	1.5 ± 0.3
75.0	3[a]	20	130 ± 10	2.2 ± 0.4
0	30[b]	30	161 ± 4	4.2 ± 0.2
0	30[c]	24	145 ± 4	3.4 ± 0.2

[a]Values for Ca^{2+}-depleted mucosae at 2 hr following addition of indicated concentrations of Ca^{2+} to the luminal side.

[b]Initial values in normal nutrient bathing medium.

[c]Values following recovery with normal nutrient bathing medium.

min intervals usually led to an eventual decline of the secretory rate in the mucosae of the last groups (Fig. 1) indicating the following possibilities: Either an initial paracellular and/or luminal plasma membrane leak of Ca^{2+} from the luminal side, or a significant amount of residual intracellular Ca^{2+} at the time of reconstitution of extracellular junctions may have been responsible for secretion in the presence of luminal Ca^{2+}. In none of the cases, the functional parameters of the epithelia were maximally restored in the presence of luminal Ca^{2+}, even at 75 m*M*; this was evident from additional increments in these parameters following addition of Ca^{2+} to the serosal bathing medium.

The effect of addition of 1 m*M* luminal Ca^{2+} on transmucosal sucrose flux is shown in Fig. 2. The data indicate that high N→S sucrose flux across Ca^{2+}-depleted mucosa declines rapidly and reaches almost steady state values within 60–80 min following addition of luminal Ca^{2+}. However, restoration of Ca^{2+} to the nutrient bathing medium caused a further small and reproducible decline, the significance of which is not known. It is possible that given sufficient time sucrose fluxes would reduce to values comparable to those observed with Ca^{2+} in the nutrient bathing medium.

Since prolonged washouts of the epithelia with the Ca^{2+}-free nutrient medium were necessary to reduce acid secretion to zero, EDTA (0.5–5 m*M*) was used to accelerate Ca^{2+} removal. It was noted during these experiments that exposure to EDTA for more than 1 hr usually impaired subsequent recoveries of secretory and electrical functions on restoration of Ca^{2+} to the nutrient bathing medium. These observations are in agreement with those of Forte and Nauss (4). The following procedures which permitted near normal recoveries were therefore routinely used to deplete mucosal Ca^{2+}. All mucosae were allowed to reach steady state secretion and PD. They were then exposed to

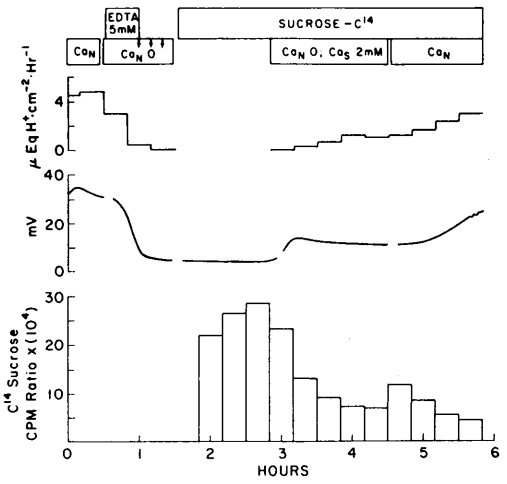

FIG. 2. Sucrose-C^{14} flux (N→S) following EDTA depletion of mucosal Ca^{2+}, and addition of 2 mM luminal Ca^{2+}. Also shown is the effect on the flux ratio (see text) of subsequent addition of 1.8 m*M* Ca^{2+} to the nutrient medium for comparative purposes.

Ca^{2+}-free nutrient solution containing 0.5 m*M* EDTA for 1 hr or 5 m*M* EDTA for 30 min. At the end of this period, the secretion usually declines to near zero and the PD reaches its minimum value. Any residual secretion was abolished by at least three serial washes of the epithelium with EDTA- and Ca^{2+}-free nutrient solution at 10 min intervals. Recoveries of the mucosal functions with normal nutrient solution following such treatments were similar to those shown in Fig. 1.

Effect of DBcAMP. These experiments were carried out with paired mucosal halves depleted of Ca^{2+} as described above, and incubated with 1 m*M* luminal Ca^{2+}. At the end of 1 hr following addition of Ca^{2+} to the luminal side, DBcAMP was added to one mucosal half while the other half was used as a control. DBcAMP (1 to 10 m*M*) at this time elicits a concentration-dependent increase in H^+ secretory rate with a short lag phase of 5 to 10 min. Steady state secretions are reached within 30 min at each concentration and are shown in Fig. 3. With the onset of acid secretion, the average PD of 9 mV gradually declines to 6 mV with little change

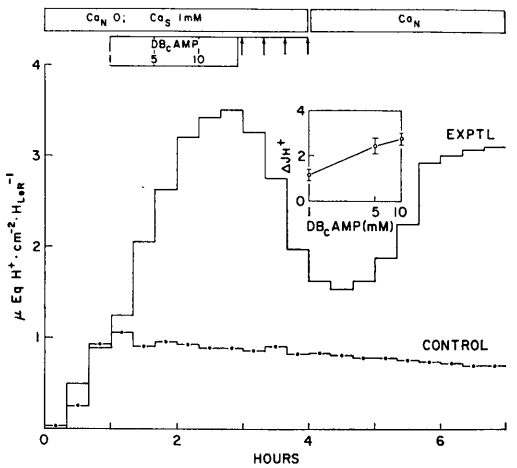

FIG. 3. Effect of DBcAMP on H^+ secretion by Ca^{2+} depleted mucosa following preincubation for 1 hr in presence of 1 m*M* luminal Ca^{2+}. DBcAMP was added only to one mucosal half, indicated as "exptl" while the other half was used as "control." (The data during preliminary period of Ca^{2+} depletion in the absence of EDTA is not included). Insert shows the increase in acid secretory rate over basal rate as a function of DBcAMP concentration.

in mucosal resistance. Repeated washout of DBcAMP with luminal Ca^{2+} present throughout reduces the secretory rate within 1 hr, indicating that the DBcAMP-induced secretion may not result from any appreciable cellular Ca^{2+} uptake. Similar results are obtained whether pentagastrin (10^{-6} *M*) or acetylcholine (10^{-4} *M*) is used as a secretagogue in lieu of 0.1 m*M* histamine. These observations suggest that the presence of Ca^{2+} in the nutrient-facing membrane may be obligatory to the secretagogue-mediated enhancement of cellular cAMP levels. In the absence of this Ca^{2+}, exogenous DBcAMP achieves stimulation of H^+ secretion as has been shown.

The above response to DBcAMP however, depends upon the time interval elapsing between the addition of luminal Ca^{2+} and the time of addition of DBcAMP; an interval of 4 hr significantly reduces the response. Also, preliminary treatment of the epithelium with 0.5 m*M* EDTA to accelerate Ca^{2+} depletion usually elicited reduced H^+ secretory responses to DBcAMP while with 5 m*M* EDTA, the stimulation is completely abolished irrespective of the time of addition of DBcAMP. The lack of response to DBcAMP under these conditions is suggestive of additional post-cAMP stage participatory role of Ca^{2+} in the sequence of reactions leading to acid secretion. This speculation is based on the assumption that the prolonged absence of Ca^{2+} in the nutrient bathing medium or the use of EDTA, removes such Ca^{2+} from the cellular compartments as may be necessary for cAMP-induced secretion and also the fact that the secretory and electrical functions of such mucosae are returned to near normal values following restoration of Ca^{2+} to the nutrient bathing medium.

Effect of theophylline. Paired mucosal halves, as in DBcAMP experiments, were used in these studies. Luminal Ca^{2+} (1 m*M*) was added to both mucosal halves following Ca^{2+} depletion, and one half was used as a control while to the other half, 10 m*M* theophylline was added. All mucosae responded to theophylline in terms of acid secretion within 15 to 30 min irrespective of whether EDTA was used or not during Ca^{2+} depletion. Furthermore unlike the observations with DBcAMP,

the time interval elapsing between addition of luminal Ca^{2+} and of theophylline was of little consequence. Removal of theophylline by repeated washouts with Ca^{2+}-containing luminal bathing solutions and Ca^{2+}-free nutrient solutions led to a decline of acid secretion, suggesting that theophylline and not Ca^{2+} was responsible for acid secretion (Fig. 4).

Discussion. The absence of a measured H^+ secretory rate subsequent to removal of Ca^{2+} from the mucosal bathing media has raised the following question namely, does the acid production continue, but is neutralized by passive movement of HCO_3^- from the nutrient to the secretory solution? Or is Ca^{2+} also required in the production of acid *per se?* The role played by Ca^{2+} in maintaining the integrity of the junctional complexes and the consequences of lack of Ca^{2+} have been described by Forte and Nauss (4), and Sedar and Forte (6). However, Rehm and collaborators (9) showed on the basis of histochemical work that removal of external Ca^{2+} results in the loss of Ca^{2+} from the tubular cells. According to their studies, while addition of Ca^{2+} to the nutrient side closes intercellular spaces, it also caused Ca^{2+} to enter the tubular cells thus rendering it difficult to assess the role of cellular Ca^{2+} in acid production. Based on such cytochemical evidence they further noted that "addition of 1–2 m*M* Ca^{2+} to the secretory side closes the intercellular spaces, but because of the relative impermeability of the plasma membrane on this side, Ca^{2+} generally does not penetrate into the cell readily." The data presented in this communication is generally in agreement with their findings and have been used to explore further the role of cellular Ca^{2+} in acid secretion.

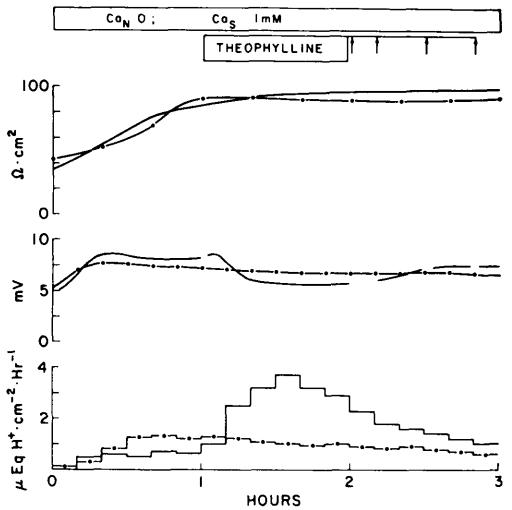

FIG. 4. Theophylline (10 m*M*) stimulation of acid secretion in presence of 1 m*M* luminal Ca^{2+} following preliminary Ca^{2+} depletion. Continuous lines are for experimental halves, while chain link lines are for the control halves to which theophylline was not added.

The concentration-dependent DBcAMP stimulation of H^+ secretion by Ca^{2+}-depleted mucosae incubated in 1 m*M* luminal Ca^{2+} suggests a possible role of this ion in the mediation of secretagogue effects *via* cAMP. The results were similar irrespective of the secretagogue used and indicate that the presence of Ca^{2+} intracellularly or in the nutrient-facing membrane may be obligatory to the expression of secretagogue action. The small PD which declines further on addition of DBcAMP under these conditions and also the decline of secretion following DBcAMP washout suggest that DBcAMP and not increased permeability of the epithelium to Ca^{2+} is responsible for the stimulation of secretion. Similar evidence for Ca^{2+}–hormone interactions at the cell membrane in the expression of various physiological functions have been presented by other investigators recently (11, 12).

The lack of response to DBcAMP following use of 5 m*M* EDTA for preliminary Ca^{2+} depletion, despite normal recoveries of such mucosae following restoration of Ca^{2+} to the nutrient bathing medium, suggests a Ca^{2+} requirement in the post-cAMP stage in the sequence of reactions leading to acid secretion. This is further supported by the observation that the response to DBcAMP declines with increasing interval elapsing between the time of addition of luminal Ca^{2+} and that of DBcAMP. One would expect these results if EDTA were responsible for excessive depletion of intracellular Ca^{2+} or if prolonged absence of serosal Ca^{2+} led to a further loss of this cation from cellular compartment. Such a loss is to be expected in

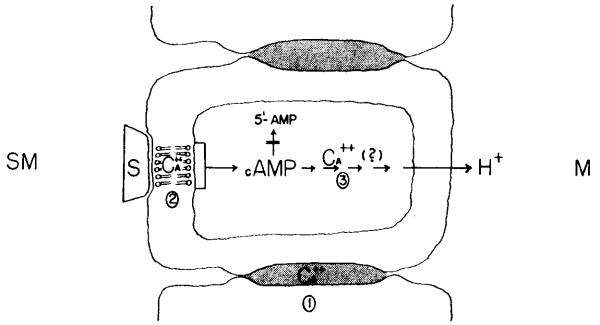

FIG. 5. Diagrammatic illustration of established and postulated Ca^{2+} compartments related to acid secretion. Evidence for the junctional complex Ca^{2+} compartment (1) is well established. Two additional compartments, one in the submucosal (SM) facing membrane (2) required for secretatogue (S) action and a possible additional intracellular compartment (3) are indicated.

view of the low permeability of the luminal-facing membrane to Ca^{2+}, relative to the nutrient-facing membrane (10).

The theophylline stimulation of Ca^{2+}-depleted mucosae exposed to 1 m*M* Ca^{2+} is to be expected in view of the results obtained with DBcAMP. However it is difficult to explain the positive response to the theophylline in EDTA-treated mucosae, if indeed there is a requirement of Ca^{2+} in the post-cAMP stages for stimulation of acid secretion.

In general, the present studies suggest the existence of cellular Ca^{2+} compartments related to the process of acid secretion, in addition to those in the junctional complexes. These are illustrated in a tentative scheme depicted in Fig. 5. A direct approach to the study of such compartments using labeled Ca^{2+} and the techniques of compartmental analysis appears to be difficult, if not impossible at the present time.

Summary. Gastric epithelia were depleted of Ca^{2+} in the continued presence of secretagogues by removing this cation from the bathing media and repeated washouts with or without EDTA until acid secretion was reduced to zero. Under these conditions, both the transmucosal PD and resistance declined. Incubation of these epithelia in presence of 1 m*M* Ca^{2+} in the luminal bathing medium resulted in a partial reversal of PD and resistance with initiation of some secretion, in contrast to near complete reversal obtained following restoration of Ca^{2+} to the serosal bathing medium. Dibutyryl cyclic-AMP or theophylline enhance acid secretion in these circumstances without complete reversal of PD and resistance. These observations suggest that cellular Ca^{2+} may play a direct role in the production of H^+ *per se* by mediating the secretagogue effect in raising intracellular cyclic-AMP levels, in addition to its established role in the maintenance of integrity of junctional complexes which are essential for the luminal segregation of H^+ in the secretory process.

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