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Electrophysiological Effects of Ca Ionophore A23187 on Frog Stomach (40398)

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Recently Candia *et al.* (1) found that the Ca ionophore A23187 decreased the electrical resistance, increased the permeability to Cl^- and stimulated active transport of Cl^- across the isolated frog cornea. These results were similar to those obtained with cyclic AMP, isoproterenol and epinephrine. They suggested that cyclic AMP and catecholamines increased Cl^- permeability by increasing intracellular Ca^{2+} concentrations and that Ca ionophore acted similarly since it also increased cellular Ca^{2+} concentrations (2–8). Other studies of Ca ionophore A23187 on specific tissues such as Bolton and Field (9) on the rabbit ileal mucosa and Frizzell (10) on the rabbit colon showed that the ionophore increased Cl^- secretion and the results in general, like in the case of Candia *et al.* (1), mimicked those obtained with cyclic AMP. They noted that the ionophore did not increase intracellular cyclic AMP and they also speculated that intracellular Ca^{2+} stimulates electrolyte secretion.

In the case of the frog gastric mucosa, the β agonists, isoproterenol and epinephrine, increased the electrical resistance (11), contrary to the effect observed in the frog cornea. Nevertheless, we decided to study the effect of ionophore A23187 on the frog gastric mucosa for the following two reasons. Firstly, the frog gastric mucosa like the frog cornea actively transports Cl^-. Secondly, it had previously been shown that Ca^{2+} appears to be essential for the normal functioning of the acid secretory mechanism (12, 13). The results of the present investigation with Ca ionophore A23187 are described herein. A preliminary report is given elsewhere (14).

Methods. Experiments were performed on gastric mucosae of Rana pipiens with an in vitro method described in detail elsewhere (15). Two pairs of electrodes were used, one for sending current across the mucosa and the other for measuring the PD. The resistance was obtained as the change in PD per unit of applied current. The H^+ secretory rate was determined by the pH stat method introduced by Durbin and Heinz (16). The pH of the secretory side was maintained at 4.90. In regular Cl^- preparations, the nutrient bathing solution contained (in m*M*): Na^+, 101; K^+, 4; Ca^{2+}, 1; Mg^{2+}, 0.8; Cl^-, 81; HCO_3^-, 25; phosphate, 1; and glucose, 10; and the secretory bathing solution contained: Na^+, 102; K^+, 4; Cl^-, 106. In regular Cl^--free preparations, the nutrient bathing solution contained (in mM): Na^+, 101; K^+, 4; Ca^{2+}, 1; Mg^{2+}, 0.8; SO_4^{2-}, 41.3; HCO_3^-, 25; phosphate, 1; glucose, 10; and sucrose, 40; and the secretory bathing solution contained: Na^+, 100; K^+, 4; SO_4^{2-}, 52; and sucrose, 64. In experiments involving increased concentrations of Ca^{2+} or Mg^{2+} in the nutrient solution, Ca^{2+} or Mg^{2+} replaced Na^+ and sucrose was added to maintain isosmolality. Both sides of the mucosa were gassed with 95% O_2 and 5% CO_2. Histamine was added to the nutrient solution to a concentration of 10^{-4} *M*. A stock solution of the ionophore A23187 was prepared by dissolving 1 mg of powder in 2 ml ethanol. When 0.1 ml of this solution was added to 10 ml of Ringer solution in the chamber, the concentration of A23187 was approximately 10^{-5} *M*.

In addition to the experiments described above, preliminary studies involving ^{36}Cl flux measurements were performed on the bull frog (*r. catesbiana*) under open-curcuit conditions. By using hemistomachs, Cl^- fluxes were simultaneously measured from the mucosal to serosal side and vice versa under control conditions and in the presence of 10^{-5} *M* A23187 in the nutrient solution. The radioactivity of ^{36}Cl from three 15-min samples obtained during the control period and three 15-min samples obtained following addition of the ionophore was measured in a liquid scintillation counter.

Results. Electrophysiological effects of Ca ionophore A23187 in Cl^- solutions. As Fig. 1 shows, at the 10-min mark, Ca ionophore was

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added to the regular Cl^- nutrient solution to a concentration of 10^{-5} *M*. Shortly thereafter, the resistance decreased and the H^+ secretory rate increased. The PD decreased a small extent, that is, the nutrient side of the mucosa became somewhat less positive relative to the secretory side. At the 40-min mark, the gastric mucosa was washed with regular solutions without ionophore. The resistance and H^+ secretory rate returned to near control levels.

Table I presents the effects of A23187 in the Cl^- nutrient solution at a concentration of 10^{-5} *M* in the presence of varying concentrations of Ca^{2+} in that solution. It compares the electrophysiological parameters 30 min after the addition of ionophore in the nutrient solution with the control values just before the addition. In the absence of Ca^{2+} and without ionophore, the resistance and PD are very low and the H^+ secretory rate is zero as described elsewhere (12, 13). Ca ionophore in the Cl^- nutrient solution produced a further decrease in resistance ($\Delta R = -34 \pm 11\ \Omega\ cm^2$) and maintained the H^+ secretory rate at zero. The effects of A23187 on 0.04, 1.0 and 3.0 m*M* Ca^{2+} in the Cl^- nutrient solution were quite similar in that there were significant decreases in resistance and significant increases in H^+ secretory rate. The PD either did not change significantly or decreased very little. With 8 m*M* Ca^{2+} in the nutrient solution, the resistance still decreased significantly but the H^+ secretory rate did not increase significantly. As Table I also shows, 10^{-6} *M* A23187 in a regular nutrient solution gave decreases in resistance and increases in

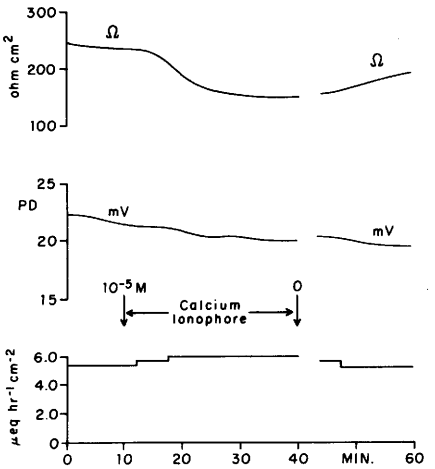

FIG. 1. Effect of Ca ionophore A23187 in Cl^- nutrient solution on resistance, PD and H^+ secretory rate versus time. At the 10-min mark 10^{-5} *M* A23187 was added to the nutrient solution and at the 40-min mark A23187 was removed.

TABLE I. EFFECTS OF Ca IONOPHORE A23187 IN Cl^- SOLUTIONS WITH VARYING Ca^{2+} CONCENTRATIONS ON RESISTANCE, PD, AND H^+ SECRETORY RATE OF FROG GASTRIC MUCOSA.[a]

Nut. sol. Ca^{2+} conc. (m*M*)	No. of expts.	R $\Omega - cm^2$	ΔR $\Omega - cm^2$	PD mV	ΔPD mV	$\dot{H}$ $\mu eq\ h^{-1}\ cm^{-2}$	$\Delta\dot{H}$ $\mu eq\ h^{-1}\ cm^{-2}$
			10^{-5} *M* A23187 nutrient solution				
0	3	80 ± 16[b]	−34 ± 11 ($P < 0.05$)	0.7 ± 1.2	0.6 ± 1.4 ($P > 0.10$)	0 ± 0	0 ± 0 ($P = 0$)
0.04	8	344 ± 106	−101 ± 62 ($P < 0.01$)	28.4 ± 5.3	−0.6 ± 4.5 ($P > 0.50$)	2.69 ± 1.28	0.92 ± 1.03 ($P < 0.05$)
1.0	7	249 ± 75	−69 ± 33 ($P < 0.01$)	29.5 ± 3.7	−1.5 ± 1.4 ($P < 0.05$)	4.77 ± 0.36	0.41 ± 0.33 ($P < 0.01$)
3.0	8	271 ± 83	−58 ± 21 ($P < 0.01$)	29.1 ± 5.7	−0.1 ± 1.9 ($P > 0.80$)	3.40 ± 0.46	0.41 ± 0.33 ($P < 0.01$)
8.0	8	292 ± 121	−34 ± 33 ($P < 0.05$)	25.7 ± 6.3	0.3 ± 2.1 ($P > 0.50$)	3.64 ± 1.30	0.23 ± 0.83 ($P > 0.20$)
			10^{-6} *M* A23187 nutrient solution				
1.0	6	387 ± 107	−46 ± 30 ($P < 0.02$)	27.2 ± 3.3	0.4 ± 2.8 ($P > 0.50$)	3.10 ± 0.87	0.65 ± 0.52 ($P < 0.05$)
			10^{-5} *M* A23187 secretory solution				
1.0	4	326 ± 43	−45 ± 16 ($P < 0.01$)	29.5 ± 3.4	2.8 ± 1.8 ($P > 0.05$)	3.87 ± 0.80	0.70 ± 0.27 ($P < 0.02$)

[a] Ca ionophore was added to nutrient solution.

[b] Values are means ± standard deviations. The columns R, PD, and $\dot{H}$ refer to the control values with the specified Ca^{2+} concentration in the nutrient solution given in column 1. The columns ΔR, ΔPD, and $\Delta\dot{H}$ refer to the changes in the parameters 30 min after the addition of A23187 to the nutrient solution.

H^+ secretory rate comparable to 10^{-5} *M* A23187 in the same nutrient solution.

It is to be noted, as the last line of Table I indicates, that the addition of Ca ionophore on the secretory side to a concentration of 10^{-5} *M* produced a decrease in resistance and an increase in H^+ secretory rate similar to these effects of Ca ionophore added on the nutrient side.

Effect of Ca ionophore on ^{36}Cl fluxes in Cl^- solutions. In five preliminary experiments, under open-circuit conditions, the Cl^- flux from the nutrient to secretory side in the presence of Ca^{2+} ionophore in the nutrient solution compared to the same flux in the absence of ionophore increased by 1.31 ± 1.05 (mean ± SD) $\mu eq\ h^{-1}cm^{-2}$ ($P < 0.05$). Similarly the Cl^- flux from the secretory to the nutrient side increased by 0.48 ± 0.22 $\mu eq\ h^{-1}cm^{-2}$ ($P < 0.01$). The net flux from the nutrient to secretory side increased by 0.84 ± 1.15 $\mu eq\ h^{-1}cm^{-2}$ ($P > 0.10$). In the last case, the net increase in flux did not change significantly since in one out of five experiments, the net change from nutrient to secretory side was negative.

Electrophysiological effects of Ca ionophore A23187 in Cl^--free solutions. As Fig. 2. shows, at the 10-min mark, Ca ionophore was added to a Cl^--free (SO_4^{2-}) nutrient solution to a concentration of 10^{-5} *M*. In this case, the resistance increased, the magnitude of the PD decreased and the H^+ secretory rate decreased. Washing both sides with regular SO_4^{2-} solutions without ionophore did not restore the parameters to control or near control levels. In general, as shown in Table II, the resistance did not change significantly but the magnitude of the PD and the H^+ secretory rate both decreased significantly. Similar experiments with 10^{-6} *M* A23187 in the nutrient solution gave a small but significant increase in resistance and no significant changes in the other two parameters (Table II).

Electrophysiological effects of Ca ionophore A23187 in Cl^- solutions containing Mg^{2+} or Ba^{2+}. A concentration of 5 m*M* Mg^{2+} in the nutrient solution did not affect the action of A23187 to any significant extent in decreasing the resistance and increasing the H^+ secretory rate. However, as Table III shows, the addition of 10^{-5} *M* Ca ionophore to a nutrient solution containing 1 m*M* Ca^{2+} and 20 m*M* Mg^{2+} resulted in no significant changes in the three parameters.

The experiments with 1 m*M* Ba^{2+} in the nutrient solution are of particular interest. Previous work showed that 1 m*M* Ba^{2+} in the nutrient solution increases the resistance markedly but decreases the H^+ secretory rate

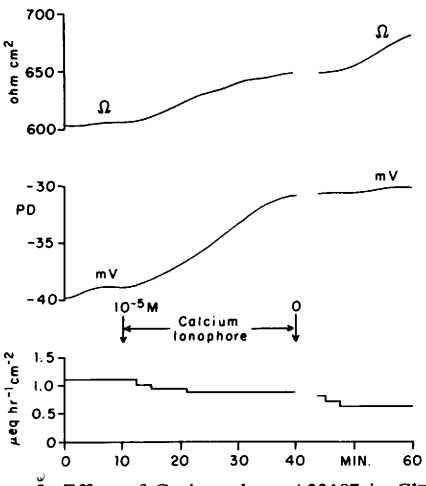

FIG. 2. Effect of Ca ionophore A23187 in Cl^--free (SO_4^{2-}) nutrient solution on resistance, PD and H^+ secretory rate versus time. At the 10-min mark 10^{-5} *M* A23187 was added to the nutrient solution and at the 40-min mark A23187 was removed.

TABLE II. EFFECTS OF Ca IONOPHORE A23187 IN Cl^--FREE SOLUTIONS CONTAINING 1 m*M* Ca^{2+} ON RESISTANCE, PD AND H^+ SECRETORY RATE OF FROG GASTRIC MUCOSA.[a]

No. of Expts.	R Ω − cm²	ΔR Ω − cm²	PD mV	ΔPD mV	$\dot{H}$ $\mu eq\ h^{-1}\ cm^{-2}$	$\Delta\dot{H}$ $\mu eq\ h^{-1}\ cm^{-2}$
			10^{-5} *M* A23187			
7	555 ± 80	32 ± 52 ($P > 0.10$)	−36.1 ± 6.0	7.2 ± 2.6 ($P < 0.01$)	1.21 ± 0.49	−0.22 ± 0.22 ($P < 0.05$)
			10^{-6} *M* A23187			
5	657 ± 55	58 ± 40 ($P < 0.05$)	−24.2 ± 3.9	−0.2 ± 2.6 ($P > 0.80$)	0.87 ± 0.20	0.04 ± 0.06 ($P > 0.10$)

[a] Same considerations as Table I. 1 m*M* Ca^{2+} in nutrient solution.

TABLE III. EFFECTS OF 10^{-5} *M* Ca IONOPHORE A23187 IN Cl^- SOLUTIONS CONTAINING Mg^{2+} OR Ba^{2+} ON RESISTANCE, PD AND H^+ SECRETORY RATE OF FROG GASTRIC MUCOSA.[a]

Nutr. sol. conc. (m*M*)	No. of Expts.	R $\Omega - cm^2$	ΔR $\Omega - cm^2$	PD mV	ΔPD mV	$\dot{H}$ $\mu eq\ h^{-1}\ cm^{-2}$	$\Delta\dot{H}$ $\mu eq\ h^{-1}\ cm^{-2}$
Ca^{2+}, 1; Mg^{2+}, 20	8	369 ± 110	−4 ± 40 ($P > 0.50$)	20.5 ± 8.9	−0.7 ± 2.7 ($P > 0.20$)	2.42 ± 1.73	0.03 ± 50 ($P > 0.80$)
Ca^{2+}, 1; Ba^{2+}, 1	6	593 ± 79	11 ± 56 ($P > 0.50$)	19.8 ± 5.3	2.0 ± 1.9 ($P < 0.05$)	2.95 ± 0.81	0.48 ± 0.28 ($P < 0.01$)

[a] Same considerations as Table I. Column I gives Ca^{2+} and Mg^{2+} or Ba^{2+} in nutrient solution.

to a small extent (17). The increase results primarily from Ba^{2+} blocking K^+ on the nutrient membrane (18) and to some degree Cl^- on this membrane (19). As shown in Table III, Ba^{2+} increased the resistance markedly and, in this case, the addition of A23187 in the presence of 1 m*M* Ba^{2+} did not decrease the resistance. However, the ionophore produced a significant increase in the H^+ secretory rate despite the presence of Ba^{2+}.

Discussion. In the frog gastric mucosa in Cl^- solutions, both Cl^- and H^+ are actively transported across the secretory membrane into the lumen (15). Under open-circuit conditions, the Cl^- conductance constitutes a return pathway for the secretion of H^+. Therefore, the decrease in resistance, if it occurred in part on the Cl^- conductance pathway (20) of the secretory membrane, would facilitate the secretion of H^+. This fact would explain the increase in H^+ secretory rate with a decrease in resistance observed under the influence of the Ca ionophore A23187.

In the present case, like that of Candia *et al.* (1), A23187 in Cl^- media decreased the electrical resistance. Frizzell (10) obtained a decrease in resistance in the rabbit ileum, but Bolton and Field (9) obtained an increase in resistance in the rabbit colon. The decrease in resistance, as already mentioned, could account for an increased H^+ secretory rate. However, in Cl^--free solutions bathing the frog gastric mucosa, the ionophore caused some inhibitory effects on H^+ secretion. Similarly, a lack of response to the ionophore on the frog cornea was observed in the absence of Cl^-.

From these results and the introductory remarks, one might be tempted to attribute the increased H^+ secretory rate to an increased Cl^- conductance resulting from an increased Ca^{2+} cellular concentration. From work done with c-AMP and theophylline which stimulate H^+ secretion, it has been found that in the presence of these substances there is an increase in Ca^{2+} efflux from isolated amphibian oxyntic cells. Based on these data, Sachs et al. (21) suggest that intracellular Ca^{2+} may play a critical role in gastric secretion. However, the decrease in resistance due to the ionophore in Ca^{2+}-free solutions suggests either a direct effect of the Ca^{2+} ionophore on conductance, or, perhaps in this case, an effect through a release of cellular Ca^{2+} from internal stores.

Is the direct or indirect effect of the ionophore strictly on the Cl^- conductance or does it affect the active Cl^- transport as well? The preliminary studies involving Cl^- flux measurements on the bullfrog under open-circuit conditions suggest increased Cl^- conductances and a possible increase in active Cl^- transport. Further flux measurements are contemplated to confirm these data.

The results with Ba^{2+} support and amplify the suggestion that the ionophore affects the Cl^- conductances (20). With 1 m*M* Ba^{2+} in the nutrient solution, the resistance markedly increased. Addition of ionophore with 1 m*M* Ba^{2+} present in the nutrient solution did not decrease the resistance but the H^+ secretory rate increased comparable to the increases observed in the absence of Ba^{2+}. Since Ba^{2+} acts mainly on the nutrient membrane, in its presence the resistance of the nutrient membrane is much larger than the resistance of the secretory membrane (18). Therefore, while a decrease in resistance by the ionophore on the nutrient membrane should be evident, a decrease in the Cl^- resistance on the secretory membrane could be obscured by the high resistance due to Ba^{2+} on the nutrient membrane. These data add further support to the hypothesis that A23187 acts, directly or indirectly, on the conductance of the secretory membrane.

As to the Mg^{2+} experiments, we note that excess Ca^{2+}, 8 m*M* in the nutrient solution

containing 0.8 m*M* Mg^{2+}, reduced the effect of the Ca^{2+} ionophore on the H^+ rate but still decreased the resistance. Also, excess Mg^{2+}, 20 m*M* in the nutrient solution containing 1 m*M* Ca^{2+}, completely abolished the effectiveness of the ionophore. It remains to determine the effect of Ca^{2+} and Mg^{2+} in other proportions to decide whether Mg^{2+} competes with Ca^{2+} for ionophore activated sites.

Summary. The Ca ionophore A23187 at a concentration of 10^{-6} *M* in a Cl^- nutrient solution containing 1 m*M* Ca^{2+} or at a concentration of 10^{-5} *M* in a Cl^- nutrient solution containing 0.04, 1 or 3 m*M* Ca^{2+} decreased the resistance and increased the H^+ secretory rate of the frog gastric mucosa of Rana pipiens. Similar effects were obtained with 10^{-5} *M* ionophore in secretory solutions. The ionophore exhibited inhibitory effects in Cl^--free media. These results together with those involving Ca^{2+}-free Cl^- solutions and Ba^{2+} studies suggested that the ionophore increased Cl^- conductance, particularly of the secretory membrane.

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1. Candia, O. A., Montoreano, R., and Podos, S. M., Amer. J. Physiol. **233,** F94 (1977).
2. Caswell, A. H., and Pressman, B. C., Biochem. Biophys. Res. Commun. **49,** 292 (1972).
3. Foreman, J. C., Mongar, J. L., and Gomperts, B. D., Nature (London) **245,** 249 (1973).
4. Garcia, A. G., Kirpekar, S. M., and Prat, J. C., J. Physiol. (London) **244,** 253 (1975).
5. Holland, D. R., Steinberg, M. I., and Armstrong, W., McD., Proc. Soc. Expt. Biol. Med. **148,** 1141 (1975).
6. Reed, P. W., and Lardy, H. A., J. Biol. Chem. **247,** 6970 (1972).
7. Scarpa, A., and Inesi, G., Fed. Eur. Biochem. Soc. Letters **22,** 273 (1972).
8. Selinger, Z., Eimerl, S., and Schramm, M., Proc. Nat. Acad. Sci. U.S.A. **71,** 128 (1974).
9. Bolton, Jennifer E., and Field, M., J. Membrane Biol. **35,** 159 (1977).
10. Frizzell, R. A., J. Membrane Biol. **35,** 175 (1977).
11. Farmer, J. M., Carrasquer, G., Holloman, T. L., and Schwartz, M., Proc. Soc. Expt. Biol. Med. **157,** 256 (1978).
12. Jacobson, A., Schwartz, M., and Rehm, W. S., Amer. J. Physiol. **209,** 134 (1965).
13. Schwartz, M., Kashiwa, H. K., Jacobson, A., and Rehm, W. S., Amer. J. Physiol. **212,** 241 (1967).
14. Collins, C. H., Carrasquer, G., Holloman, T. L., and Schwartz, M., Fed. Proc. **37,** 651 (1978).
15. Rehm, W. S., Amer. J. Physiol. **203,** 65 (1962).
16. Durbin, R. P., and Heinz, E., J. Gen. Physiol. **41,** 1035 (1958).
17. Schwartz, M., Pacifico, A. D., MacKrell, T. N., Jacobson, A., and Rehm, W. S., Proc. Soc. Exp. Biol. Med. **127,** 223 (1968).
18. Pacifico, A. D., Schwartz, M., MacKrell, T. N., Spangler, S. G., Sanders, S. S., and Rehm, W. S., Amer. J. Physiol. **216,** 536 (1969).
19. Rangachari, P. K., Amer. J. Physiol. **228,** 511 (1975).
20. Sanders, S. S., Shoemaker, R. L., and Rehm, W. S., Amer. J. Physiol. **234,** E298 (1977).
21. Sachs, G., Spenney, J. G., and Rehm, W. S., *in* "Gastrointestinal Physiology II" (R. K. Crane, ed.), Vol. 12, p. 135. University Park Press, Baltimore (1977).

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