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Ca Ionophore-Stimulated Ion Secretion in Rabbit Ileal Mucosa: Relation to Actions of Cyclic 3′,5′-AMP and Carbamylcholine

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Summary. Addition of the Ca ionophore, A23187 (0.5 μg/ml), to the serosal side of stripped rabbit ileal mucosa, produced changes in ion transport qualitatively identical with those produced by cyclic 3′,5′-AMP (cAMP) and theophylline: an increase in short-circuit current and resistance, net secretion of Cl due both to a decrease in the unidirectional mucosa (*m*) to serosa (*s*) flux and an increase in the (*s*) to (*m*) flux, and net secretion of Na due to a decrease in (*m*) to (*s*) flux. Measurements of intracellular cAMP level demonstrated no change following incubation with the ionophore. Removal of Ca from the serosal bathing medium diminished the effects of A23187 but did not impair the action of theophylline. Furthermore, removal of Ca from both the mucosal and serosal bathing media by replacing it with Sr completely abolished the p.d. response to A23187. These results suggest that the ionophore elicits its secretory actions by increasing Ca influx into the epithelial cells. In a similar way, carbamylcholine and serotonin, secretagogues known to have no effect on intracellular cAMP level in intestinal mucosa, were shown to be dependent on extracellular Ca to produce their full electrical response (although, in the case of carbamylcholine at least, Sr can substitute for Ca). In contrast, the secretagogues vasoactive intestinal peptide and prostaglandin E_1, which raise cAMP concentration in intestinal mucosa, do not appear to require external Ca. It is interesting to speculate that Ca is an intracellular mediator of intestinal ion and water secretion and that some intestinal secretagogues may act as Ca ionophores.

Cyclic 3′,5′-AMP (cAMP) and agents which increase its intracellular concentration, stimulate fluid secretion in mammalian small intestine (Field, 1974) as well as in several other epithelia. Certain intestinal secretagogues (cholinergic agonists, serotonin) do not, however, cause a sustained increase in intestinal mucosal cAMP level (Brasitus, Field & Kimberg, 1976; Isaacs, Corbett, Riley, Hawker & Turnberg, 1976). Since Ca has been shown to stimulate a variety of secretory processes, we have

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employed the divalent cation ionophore A23187 to examine the possibility that Ca may also act as a mediator of secretion in small intestine and that the above scretagogues may act as Ca ionophores.

Materials and Methods

Male, New Zealand rabbits, weighing 5–6 lb, were killed with ether and the distal ileum removed, stripped of muscle and mounted in Ussing chambers as previously described (Field, Fromm & McColl, 1971). Transmural potential difference (p.d.), short-circuit current (I_{sc}), total d-c conductance (G_t) and unidirectional fluxes of Na and Cl were also measured as previously described (Field *et al.*, 1971). Mucosa (*m*) to serosa (*s*) and (*s*) to (*m*) fluxes across short-circuited mucosa (J_{ms} and J_{sm}) were determined on paired tissues using ^{22}Na and ^{36}Cl. Experiments in which the electrical resistance of presumptively paired tissues differed during any flux period by more than 25% were rejected. In most experiments, fluxes were measured for two consecutive periods, with addition of ionophore or another agent immediately after the first period. The first period was begun 20 min after addition of radioisotope and approximately 60 min after mounting tissues *in vitro*. Fluxes were calculated from initial and final samples taken 30 min apart for the first period and 40 min apart for the second period. Fifteen min were allowed to elapse between first and second period flux measurements.

In most experiments, HCO_3-Ringer's solution (pH 7.4), containing 25 mM HCO_3 and bubbled with 5% CO_2 in O_2, was used to bathe the mucosal and serosal surface (Field *et al.*, 1971). Ca-free Ringer's solution was prepared by omitting the Ca and adding 0.2 mM ethyleneglycol-bis (*β*-aminoethyl ether)-N, N′-tetraacetic acid (EGTA). A modified Ringer's solution containing N-2-hydroxyethyl piperazine-N′-2′ethanesulfonic acid (HEPES) was used in those experiments in which effects of Ca and Sr were compared: Na, 114; K, 5; Mg, 2; Sr, 10; Cl, 129; HPO_4, 2; EGTA, 0.2 and HEPES, 40 mM (pH adjusted to 7.4 with KOH). The pH of this solution, when bubbled with 5% CO_2 in O_2 was 7.2. In all experiments, glucose (10 μmol/ml) was added to the serosal reservoir and an equimolar amount of mannitol to the mucosal reservoir. Viability of the tissues was tested at the end of each experiment by observing the change in p.d. produced by addition of glucose (20 μmol/ml) to the mucosal reservoir.

Intracellular cAMP levels were determined by the protein kinase binding assay of Gilman (1970) as previously described for intestinal mucosa (Field, Sheerin, Henderson & Smith, 1975). Ileal mucosa, weighing between 20 and 50 mg wet weight, was incubated with shaking at 37 °C in 2 ml of HCO_3-Ringer's solution. Flasks were individually gassed with 5% CO_2 in O_2. Experiments were terminated by quickly transferring tissues to cold 5% trichloroacetic acid.

All probability determinations were by Student's *t*-test for either paired or unpaired variates. Results in the text and Tables are expressed as means ±1 SE. A23187 was a gift of Dr. R.L. Hamill of Eli Lilly & Co. (Indianapolis, Ind.). Prostaglandin E_1 was a gift of Dr. U.F. Axen of the Upjohn Co. (Kalamazoo, Mich.). Vasoactive intestinal peptide was prepared by Prof. V. Mutt (Karolinska Institute, Stockholm, Sweden) and kindly supplied by Dr. J.E. Fischer (Massachusetts General Hospital, Boston, Mass.). Theophylline and carbamylcholine (carbachol) were obtained from Sigma Chemical Co. (St. Louis, Mo.). 5-Hydroxytryptamine (serotonin) was obtained as the oxalate salt (Sigma Chemical Co.). ^{22}Na, ^{36}Cl and [^{3}H]-cyclic AMP (36 C/mmol) were obtained from New England Nuclear (Boston, Mass.).

Results

Effects of Ionophore A23187 with Ca Present in both Mucosal and Serosal Bathing Solutions

With 1.25 mM Ca present in both mucosal and serosal bathing solutions, addition of A23187 to the serosal reservoir caused a rapid increase in transmural p.d. the time course of which is shown in Figure 1. The effects of varying concentrations of A23187 on p.d. are shown in Figure 2. The initial p.d. response was approximately half-maximal at 0.06 μg/ml and nearly maximal at 0.5 μg/ml. Addition of ionophore (0.1, 0.5 and 5.0 μg/ml) to the mucosal reservoir had no effect on the p.d. (data not shown).

Effects of the ionophore on steady-state Na and Cl fluxes, I_{sc} and G_t are shown in Table 1 A. Addition of 0.5 μg/ml ionophore to the serosal reservoir resulted in an increase in I_{sc}, a fall in G_t and net secretion of both Na and Cl (Table 1 Ai). Secretion of Cl was the result of both a decrease in J_{ms} and an increase in J_{sm} whereas net Na secretion resulted almost entirely from a decrease in J_{ms}. The residual ion flux ($J_r = I_{sc} - J^{Na}_{net} + J^{Cl}_{net}$) did not change significantly. Measurements were also made for a subsequent period (50–110 min after adding ionophore) in order to better evaluate the duration of the secretory response. Na and Cl secretion persisted unabated. The conductance of the tissues returned to control level, however, suggesting a delayed increase in passive permeability to ions. Whether this was due to the ionophore or simply to the passage of time is not clear from the present results but prior results suggest that the former is more likely (Sheerin & Field, 1977).

Cl fluxes were also determined following addition of 5.0 μg/ml ionophore (Table 1 Aii). Results were essentially the same as at the lower concentration, suggesting that 0.5 μg/ml produces a maximum response.

Throughout the present study, the ionophore was added in 50 μl methanol to 10 ml of solution in the serosal reservoir. In order to exclude a major effect of the methanol, fluxes were measured before and after its addition (five experiments).[1] Changes in I_{sc}, J^{Na}_{net} and J^{Cl}_{net} were -0.4 ± 0.26, 0.6 ± 0.51 and 0.7 ± 0.22, respectively, only the last of which was significant statistically. It is uncertain whether these minor changes

1 In these experiments, for reasons unrelated to the present study, the bathing media contained 60 rather than the usual 127 mM Cl (Cl replaced with 30 mM each of SO_4 and mannitol). This change does not in any way diminish the effect of A23187 (data not shown).

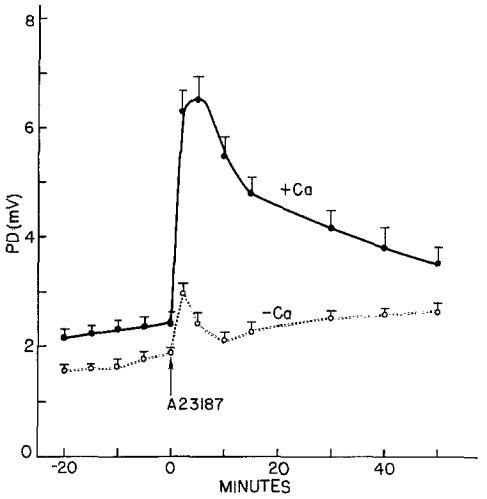

Fig. 1. Effect of A23187 on p.d. in presence and absence of serosal Ca. A23187 (0.5 μg/ml) was added to the serosal reservoir at time zero. Ca (1.25 mM) was always present in the mucosal bathing medium. Omission of Ca from the serosal medium (-Ca) was accompanied by addition of 0.2 mM EGTA. Each point is the mean of 12 observations in the presence and 18 observations in the absence of serosal Ca. Brackets indicate ± 1 SE

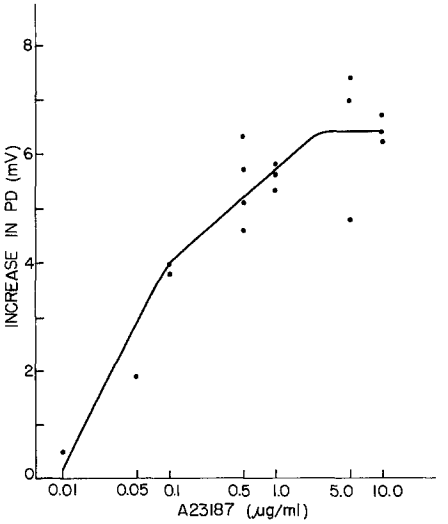

Fig. 2. p.d. response as a function of concentration of A23187. A23187 was added to the serosal reservoir and the resulting peak change in p.d. was recorded. Each point is a result for a single tissue

Table 1. Effects of Ca ionophore A23187 on ion fluxes across rabbit ileal mucosa

	Time period (min)	J_{ms}^{Na}	J_{sm}^{Na}	J_{net}^{Na}	J_{ms}^{Cl}	J_{sm}^{Cl}	J_{net}^{Cl}	I_{sc}	J_r	G_t
A. Ca present in *m* and *s*										
(i) +0.5 μg/ml A23187 (6)										
baseline	60–90	10.7±0.89	10.6±1.14	0.2±0.56	10.9±0.79	10.6±0.98	0.3±0.64	1.9±0.17	2.1±0.33	22.6±1.46
+A23187	105–145	9.2±0.48[a]	11.2±0.71	−1.9±0.70[b]	9.8±0.54	11.8±0.63	−2.0±0.50[b]	2.7±0.28[b]	2.7±0.48	19.9±1.10[b]
	145–205	11.0±0.63	12.6±0.74[a]	−1.6±0.74[b]	11.3±0.75	13.5±0.96[b]	−2.2±1.18[a]	2.6±0.20[b]	1.8±0.57	23.8±1.45
(ii) +5.0 μg/ml A23187 (4)										
baseline	60–90	–	–	–	9.8±0.25	9.3±0.50	0.5±0.70	2.1±30	–	23.0±0.35
+A23187	105–145	–	–	–	9.0±0.77	10.7±0.90	−1.7±1.41	3.4±0.30[b]	–	19.4±0.88[b]
B. Ca present in *m* only; +0.5 μg/ml A23187 (9)[c]										
baseline	60–90	12.9±0.70	12.6±0.51	0.2±0.61	10.0±0.56	7.9±0.26	2.1±0.48	1.5±0.10	3.4±0.38	24.6±0.71
+A23187	105–145	12.2±0.67	12.8±0.55	−0.6±0.71	10.2±0.50	9.7±0.37[b]	0.6±0.60[a]	2.1±0.10[b]	3.3±0.57	23.1±0.75[b]

Ion fluxes (including I_{sc}) are in μEquiv/hr cm^2 and conductance (G_t) is in mmhos/cm^2; J_r refers to the residual ion flux ($I_{sc}-J_{net}^{Na}+J_{net}^{Cl}$). Time period refers to time after tissues were mounted *in vitro*. Values are means±1 SE; number of animals used for flux measurements are in parentheses. A23187 added to serosal bathing medium immediately after first flux period.

[a] Different from baseline, $p \leqq 0.05$.

[b] Different from baseline, $p < 0.01$.

[c] EGTA (0.2 μmol/ml) added to serosal bathing solution.

were due to the methanol or to the passage of time. Since directed oppositely to those that follow addition of A23187, however, these changes cannot be held responsible for any portion of the changes which developed after addition of ionophore.

Dependence of the Ionophore Effects on Extracellular Ca

We found that it was not possible to omit Ca from both mucosal and serosal bathing solutions since, when this was done, the resistance of the tissue fell sharply, indicating disruption of the structural integrity of the mucosa. As shown in Fig. 1, when Ca was removed only from the serosal bathing solution, the initial p.d. response to the ionophore was greatly reduced. In the absence of serosal Ca, A23187 reduced net Cl absorption, increased I_{sc} and decreased G_t (Table 1 B), but these changes were significantly smaller than those observed in the presence of serosal Ca.

The changes that persisted after Ca was omitted from the serosal medium may have been due to Ca entry into the lateral intercellular spaces from the mucosal medium, which still contained Ca. To evaluate this possibility, we bathed tissues in a modified Ringer's solution in which Sr (10 mM) was substituted for Ca on the mucosal side and neither Sr nor Ca were present on the serosal side. In these solutions, tissue resistance remained constant and tissue viability, as measured by the p.d. response to glucose, was unimpaired. Addition of A23187 did not alter p.d. even transiently. If Ca was added back to the serosal side, however, addition of ionophore produced a substantial increase in p.d. (peak increment $= +5.5 \pm 25$ mV; four experiments).

Does cAMP Mediate the Action of A23187?

Since the effects of the ionophore are qualitatively similar to, although perhaps smaller in magnitude than, the effects of cAMP and agents that increase its intracellular concentration (Field, 1974), we wished to know what role, if any, cAMP played in the action of the ionophore. A23187 has recently been shown to raise cAMP concentration in rat pancreas (Karl, Zawalich, Ferrendelli & Matschinsky, 1975). We therefore investigated the effect of the ionophore on intestinal mucosal cAMP concentration. As shown in Figure 3, the ionophore (0.5 μg/ml) had no effect on cAMP concentration for at least 40 min after its addition.

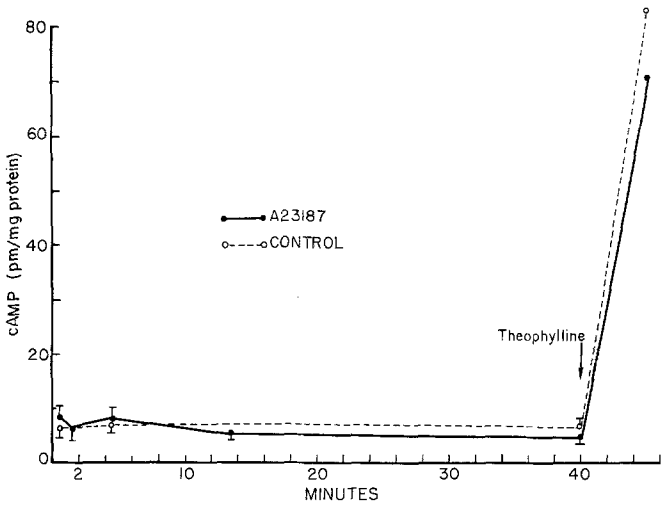

Fig. 3. Effect of A23187 on cAMP concentration. A23187 (0.5 μg/ml) was added to the incubation flask at time zero and theophylline (5 μmol/ml) was added at 40 min. The ionophore solvent was added to control flasks. Each point represents the mean of four observations for four complete and separate experiments. Brackets indicate ± 1 SE. The SE's after theophylline, which are not shown, were large (34.1 in the presence of and 49.9 in the absence of A23187) due to variable responses to theophylline among animals. There was little variability of response, however, between ionophore-treated and control tissues from the same animal (SE = 14.3% of the mean response)

Also, the ionophore did not interfere with the effect of theophylline on cAMP concentration.

The action of cAMP can also be dissociated from that of the ionophore by comparing their actions in the presence and absence of serosal Ca. In the presence of serosal Ca (Table 2A), theophylline stimulated secretion of both Na and Cl, the latter effect being the greater. These results are similar to those previously reported for theophylline (Field, 1974; Sheerin & Field, 1975) and are also similar to those shown in Table 1 for A23187. As can be seen from Table 2B, theophylline produced identical changes in the absence of serosal Ca. Thus, unlike the ionophore, the action of cAMP appears not to depend on extracellular Ca.

Adding both the ionophore and theophylline produced no effect beyond that caused by theophylline alone (Table 2C). Thus, theophylline appears to stimulate secretion maximally, its effects not being further augmented by independently increasing Ca influx into the epithelial cell.

Table 2. Effects of theophylline in the presence and absence of serosal Ca and A23187

	Time period (min)	J_{ms}^{Na}	J_{sm}^{Na}	J_{net}^{Na}	J_{ms}^{Cl}	J_{sm}^{Cl}	J_{net}^{Cl}	I_{sc}	J_r	G_t
(a) Ca present on both sides (11)										
Baseline	60–90	11.1±0.66	10.8±0.75	0.3±0.49	8.8±0.66	8.0±0.37	0.8±0.70	1.8±0.21	2.2±0.72	23.4±1.01
+theo	105±145	8.8±0.60[b]	10.8±0.47	2.0±0.51[b]	6.6±0.39[b]	11.4±0.39[b]	−4.8±0.44[b]	4.8±0.19[b]	1.8±0.50	18.7±0.65[b]
(b) Ca present only on mucosal side (6)										
baseline	60–90	13.0±1.2	12.7±0.44	0.3±1.16	9.6±1.06	8.0±0.90	1.5±1.61	1.6±0.31	2.9±0.17	23.4±0.76
+theo	105–145	9.2±0.44[a]	11.5±0.72	−2.3±0.67	7.2±0.57[a]	10.4±1.13[b]	−3.1±1.31[a]	4.5±0.47[b]	3.7±1.22	18.8±1.06[b]
(c) Ca present on both sides; A23187 (0.5 μg/ml) added to serosal side (4)										
baseline	60–90	8.6±1.45	11.5±1.75	−2.9±0.44	7.1±1.01	11.1±1.11	−4.0±0.55	3.7±0.29	2.6±0.73	21.9±2.27
+theo	105–145	9.2±0.67	11.1±0.83	−2.0±0.87	7.2±0.55	12.6±1.09	−5.4±1.09	5.3±0.48[a]	1.9±0.30	21.0±1.90

Values are means±1 SE; number of animals in parantheses. Theophylline (5 μmol/ml) and A23187 added to serosal bathing solutions. EGTA (0.2 μmol/ml) added to Ca-free medium.

[a] Different from baseline, $p<0.05$.

[b] Different from baseline, $p<0.01$.

Table 3. Effects of secretagogues on p.d. in the presence and absence of serosal Ca

Agent	Side(s) containing Ca	n	Peak p.d. response (mV)	Integrated p.d. response (mV·min)
A23187 (0.5 μg/ml)	*m* only	5	0.5±0.10	19.9±4.25
	m+*s*	5	4.4±0.27[a]	75.7±3.66[a]
carbachol (10^{-4}M)	*m* only	10	3.4±0.58	6.1±0.77
	m+*s*	6	5.5±0.44[b]	17.5±1.63[a]
serotonin (10^{-4}M)	*m* only	7	4.0±0.38[a]	11.4±1.57[a]
	m+*s*	7	5.8±0.24[a]	20.7±2.12[a]
PGE_1 (0.5×10^{-4} M)	*m* only	6	4.6±0.47	54.9±11.77
	m+*s*	6	4.7±0.57	78.4±12.57
VIP (0.5 μg/ml)	*m* only	4	6.9±0.63	140.6±10.48
	m+*s*	4	8.6±0.51	152.7±14.50
theophylline (5 mM)	*m* only	5	4.3±0.34	129.3±7.80
	m+*s*	5	4.4±0.32	137.4±11.40

Values are means±1 SE; n refers to the number of animals. EGTA (0.2 μmol/ml) was added to Ca-free medium. All agents were added to the serosal bathing medium. The peak p.d. response is the difference between the baseline p.d. and maximum p.d. within 5 min after adding secretagogue. The integrated p.d. response is the above-baseline area of the p.d. tracing for 30 min after adding secretagogue.

[a] Different from "*m* only", $p<0.01$.

[b] Different from "*m* only", $p<0.05$.

p.d. Responses to other Secretagogues: Dependence on Divalent Cations

Of the hormones and neurotransmitters that stimulate intestinal secretion, only VIP and the prostaglandins have clearly been shown to produce sustained increases in intestinal mucosal cAMP concentration (Kimberg, Field, Gershon & Henderson, 1974; Schwartz, Kimberg, Sheerin, Field & Said, 1974). Cholinergic stimulation and serotonin produce secretory responses which are not associated with increases in cAMP concentration (Brasitus *et al.*, 1976; Donowitz & Charney, 1976; Hubel, 1976; Isaacs *et al.*, 1976; Kisloff & Moore, 1976).

To further explore the similarities between these two agonists and A23187, we determined the p.d. responses to carbachol, serotonin, PGE_1 and VIP in both the presence and absence of serosal Ca. Table 3 shows both the initial, peak increments in p.d. and the integrated increments in p.d., the latter of which takes into account the duration of the response, as well as its amplitude. Neither peak nor integrated p.d. responses to PGE_1, VIP and theophylline were significantly affected by omission

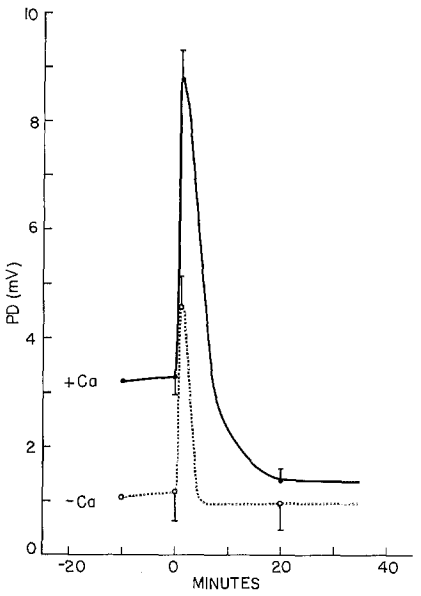

Fig. 4. Effect of carbachol on p.d. in presence and absence of serosal Ca. Carbachol. (0.1 μmol/ml) was added to the serosal reservoir at time zero. Ca (1.25 mM) was always present in the mucosal bathing medium. Omission of Ca from the serosal medium (-Ca) was accompanied by addition of 0.2 mM EGTA. Each point is the mean of 10 observations in the presence and six observations in the absence of serosal Ca. Brackets indicate ± 1 SE

of serosal Ca. In contrast, both peak and integrated responses to A23187, carbachol and serotonin were diminished by omission of Ca from the serosal bathing medium. The ratio of integrated p.d. responses in the absence and presence of serosal Ca was nearly the same for carbachol (0.35) as for A23187 (0.26). There was less similarity in the ratio of peak p.d. responses, the peak response in the absence of serosal Ca being much smaller with A23187 than with carbachol.

It is worth noting that 0.1 mM carbachol produces an initial increase in p.d. followed by a subsequent decline below the baseline (*see* Fig. 4). This biphasic response, which was also observed occasionally but not consistently upon addition of 5 μg/ml of A23187, developed only in the presence of serosal Ca. This delayed response to carbachol reduced the p.d. to the lower level routinely observed in the absence of serosal Ca (Fig. 4) and appears to correlate with an enhancement of NaCl absorption (Tapper, Powell & Morris, 1976). It is probably responsible for the small magnitude of the integrated p.d. response to carbachol.

To further compare the actions of carbachol to that of A23187, p.d. responses to these agents were determined in both Sr- and Ca-

Table 4. Comparison of p.d. responses to A23187 and carbachol in Sr- and Ca-containing bathing media

Mucosal medium	Serosal medium	Integrated p.d. response (mV·min)	
		+A23187	+carbachol
Sr	–	0(6)	16.8±3.80(6)
Sr	Ca	20.2±3.53(4)	20.4±3.51(4)
Sr	Sr	0(4)	18.7±3.53(6)
Ca	–	0.4±0.4(4)	5.0±1.43(4)
Ca	Sr	0(4)	25.3±0.40(4) [a]
Ca	Ca	38.2±1.92(4)	13.2±1.53(4)

Values are means±1 SE; number of experiments in parentheses. Tissues bathed in HCO_3-free, HEPES-buffered medium (*see* Materials and Methods for composition); Sr and Ca concentrations were 10 and 1.25 mM, respectively. *See* legend to Table 3 for further details.
[a] $p<0.01$.

containing Ringer's solution. As shown in Table 4, a significant p.d. response to A23187 developed only in the presence of serosal Ca.[2] In contrast, the p.d. response to carbachol could be sustained by mucosal or serosal Sr as well as by serosal Ca. This suggests that the secretory process can be stimulated by Sr as well as by Ca and that carbachol increases cell membrane permeability to both cations.[3] The fact that Sr will not substitute for Ca in generating a p.d. response to A23187 is consistent with the known affinities of the ionophore for various divalent cations [Mn > > Ca ≧ Mg > > Sr > Ba; *see* Reed (1972)].

In summary, although there are particular differences, the increases in p.d. caused by both carbachol and A23187 occur only in the presence of certain extracellular divalent cations, suggesting that both stimulate secretion by increasing cellular uptake of one or more of these cations. In contrast, the secretory response to agents which increase intracellular cAMP concentration is independent of these cations.

2 The small p.d. response to A23187 that occurred in HCO_3-buffered Ringer's solution in the presence of mucosal Ca only (*see* Table 3) was not observed in HEPES-buffered Ringer's solution under the same conditions. The reasons for this difference is not known. In contrast, under these conditions, carbachol produced the same small increase in p.d. in both HCO_3- and HEPES-buffered solutions. It is possible that carbachol has a limited capacity to release Ca from an intracelluler store.

3 Sr can also substitute for Ca in the stimulation of fluid secretion by serotonin in insect salivary gland (Prince & Berridge, 1973) and of fluid absorption by angiotensin in rat colon (Munday, Parsons, Poat & Smith, 1973).

Discussion

Addition of the Ca ionophore A23187 to the serosal side of isolated rabbit ileal mucosa elicits a secretory response qualitatively identical with, although perhaps smaller in magnitude to that elicited by theophylline and other agents which increase intracellular cAMP. Unlike theophylline, however, the ionophore does not increase mucosal cAMP concentration and its action is dependent on extracellular Ca.

Similar observations have been made with several other secretory epithelia: secretion of histamine by mast cells (Foreman, Mongar & Gomperts, 1973), potassium by rat parotid (Selinger, Eimerl & Schramm, 1974), fluid by blowfly salivary gland (Prince, Rasmussen & Berridge, 1973), catecholamines by cat adrenal (Garcia, Kirpekar & Prat, 1975), enzymes by rodent pancreas (Eimerl, Savion, Heichal & Selinger, 1974; Williams & Lee, 1974) and fluid by rabbit lacrimal gland (Phopramool & Tangkrisanavinont, 1976) can all be elicited by the ionophore more readily in the presence than in the absence of external Ca. Direct evidence that A23187 increases Ca uptake has been presented for isolated rat (Kondo & Schulz, 1976) and guinea pig (Christophe, Frandsen, Conlon, Krishna & Gardner, 1976) pancreatic acinar cells and blowfly salivary gland (Prince *et al.*, 1973).

The recent preliminary report by Frizzell (1976) comparing the effects of A23187 and cAMP on ion transport in isolated rabbit colon is especially relevant to the present study. Thus, both agents produced identical secretory responses and, in further agreement with the present results for the ileum, A23187 did not increase cAMP concentration in the colon and its action was dependent on extracellular Ca whereas the action of cAMP was not. One interesting difference between these studies is that A23187 is effective when added to the medium bathing the mucosal surface of the colon whereas in the ileum it is effective only when added to the serosal medium.

The relation between cAMP and Ca in the stimulation of secretory processes is unclear. Cyclic AMP does not appear to increase the flux of external Ca into epithelial cells (Kondo & Schulz, 1976), but it may release Ca from an intracellular site, thereby increasing activity of the ion in the cytoplasm. Consistent with this hypothesis is the finding that cAMP increases Ca efflux from dispersed guinea pig pancreatic cells (Christophe *et al.*, 1976) and rabbit colonic mucosa (Frizzell, 1976). Thus, an increase in cytoplasmic Ca could underlie the secretory response to

cAMP as well as to A23187. Direct demonstration of such an increase is presently lacking, however.

The present study suggests an alternative, adenylate cyclase-independent means by which hormones and neurotransmitters may stimulate secretion in the small intestine, namely, by altering the permeability of intestinal epithelial cell membranes to Ca. It is likely on the basis of the evidence presented that cholinergic agonists and perhaps also serotonin affect the intestine in this way. Carbachol has recently been shown to enhance Ca uptake by isolated rat pancreatic acinar cells (Kondo & Schulz, 1976) and serotonin, which stimulates secretion in the blowfly salivary gland, has been shown to increase Ca uptake in that organ (Prince, Berridge & Rasmussen, 1972).

The possible role of cyclic 3′,5′-GMP (cGMP) in Ca ionophore and cholinergic agonist-evoked intestinal secretion deserves brief comment. Both the Ca ionophore A23187 (Ferrendelli, Rubin & Kinscherf, 1975; Van Sande, Decoster & Dumont, 1975; Christophe *et al.*, 1976) and cholinergic agonists (Schultz, Hardman, Schultz, Baird & Sutherland, 1973; Clyman, Blacksin, Sandler, Manganiello & Vaughan, 1975; Christophe *et al.*, 1976) have been shown to increase cGMP concentration in a number of tissues. Immediately pertinent to the present study, carbachol has been shown to increase cGMP in rabbit ileal mucosa (Brasitus *et al.*, 1976). It is unlikely, however, that cGMP has a stimulatory role in intestinal secretion, since α-adrenergic agonists, which also increase intestinal mucosal cGMP (Brasitus *et al.*, 1976), inhibit HCO_3 secretion (Dietz & Field, 1973) and enhance absorption of NaCl (Field & McColl, 1973).

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References

Brasitus, T.A., Field, M., Kimberg, D.V. 1976. Intestinal mucosal cyclic GMP: Regulation and relation to ion transport. *Am. J. Physiol.* **231**:275

Christophe, J.P., Frandsen, E.K., Conlon, T.P., Krishna, G., Gardner, J.D. 1976. Action of cholecystokinin, cholinergic agents and A23187 on accumulation of guanosine 3′:5-monophosphate in dispersed guinea pig pancreatic acinar cells. *J. Biol. Chem.* **251**:4640

Clyman, R.I., Blacksin, A.S., Sandler, J.A., Manganiello, V.C., Vaughan, M. 1975. The role of calcium in regulation of cyclic nucleotide content in human unbilical artery. *J. Biol. Chem.* **250**:4718

Dietz, J., Field, M. 1973. Ion transport in rabbit ileal mucosa. IV. Bicarbonate secretion. *Am. J. Physiol.* **255:**858

Donowitz, M., Charney, A.N. 1976. Effect of chronic elevation of blood serotonin on intestinal transport. *Gastroenterology* **70:**A-22/880

Eimerl, S., Savion, N., Heichal, O., Selinger, Z. 1974. Induction of enzyme secretion in rat pancreatic slices using the ionophore A23187 and calcium. *J. Biol. Chem.* **249:**3991

Ferrendelli, J.A., Rubin, E.H., Kinscherf, D.A. 1975. Calcium ionophore A23187 elevates levels of cyclic GMP in incubated brain slices. *Fed. Proc.* **34:**231

Field, M. 1974. Intestinal secretion. *Gastroenterology* **66:**1063

Field, M., Fromm, D., McColl, I. 1971. Ion transport in rabbit ileal mucosa. I. Na and Cl fluxes and short-circuit current. *Am. J. Physiol.* **220:**1388

Field, M., McColl, I. 1973. Ion transport in rabbit ileal mucosa. III. Effects of catecholamines. *Am. J. Physiol.* **225:**852

Field, M., Sheerin, H.E., Henderson, A., Smith, P.L. 1975. Catecholamine effects on cyclic AMP levels and ion secretion in rabbit ileal mucosa. *Am. J. Physiol.* **229:**86

Foreman, J.C., Mongar, J.L., Gomperts, B.D. 1973. Calcium ionophore and movement of calcium ions following the physiological stimulus to a secretory process. *Nature (London)* **245:**249

Frizzell, R.A. 1976. Ion transport by rabbit colon: Effects of cyclic AMP and calcium ionophore. *Physiologist* **19:**200

Garcia, A.G., Kirpekar, S.M., Prat, J.C. 1975. A calcium ionophore stimulating the secretion of catecholamines from the cat adrenal. *J. Physiol. (London)* **244:**253

Gilman, A.G. 1970. A protein binding assay for adenosine 3′,5′-cyclic monophosphate. *Proc. Nat. Acad. Sci. USA* **67:**305

Hubel, K.A. 1976. Intestinal ion transport: Effect of morepinephrine, pilocarpine and atropine. *Am J. Physiol.* **231:**252

Isaacs, P.E.T., Corbet, C.L., Riley, A.K., Hawker, P.C., Turnberg, L.A. 1976. *In vitro* behavior of human intestinal mucosa. The influence of acetyl choline on ion transport. *J. Clin. Invest.* **58:**535

Karl, R.C., Zawalich, W.S., Ferrendelli, J.A., Matschinsky, F.M. 1975. The role of Ca^{2+} and cyclic adenosine 3′:5′-monophosphate in insulin release induced *in vitro* by the divalent cation ionophore A23187. *J. Biol. Chem.* **250:**4575

Kimberg, D.V., Field, M., Gershon, E., Henderson, A. 1974. Effects of prostaglandins and cholera enterotoxin on intestinal mucosal cyclic AMP accumulation. *J. Clim. Invest.* **53:**941

Kisloff, B., Moore, E.W. 1976. Effects of serotonin on rabbit jejunal and ileal H_2O and electrolyte transport. *Gastroenterology* **70:**A-44/902

Kondo, S., Schultz, I. 1976. Calcium ion uptake in isolated pancreas cells induced by secretagogues. *Biochim. Biophys. Acta* **419:**76

Munday, K.A., Parsons, B.J., Poat, J.A., Smith, D.J. 1973. The effect of divalent cations on angiotensin stimulation of fluid transport by rat colon. *J. Physiol. (London)* **232:**89

Pholpamool, C., Tangkrisanavinont, V. 1976. A calcium ionophore-induced secretion in rabbit lacrimal gland *in vitvo*. *Life Sci.* **19:**381

Prince, W.T., Berridge, M.J. 1973. The role of calcium in the action of 5-hydroxytryptamine and cyclic AMP on salivary glands. *J. Exp. Biol.* **58:**567

Prince, W.T., Berridge, M.J., Rasmussen, H. 1972. Role of calcium and adenosine 3′:5′-cyclic monophosphate in controlling fly salivary gland secretion. *Proc. Nat. Acad. Sci. USA* **69:**533

Prince, W.T., Rasmussen, H., Berridge, M.J. 1973. The role of calcium in fly salivary gland secretion analysed with the ionophore A23187. *Biochim. Biophys. Acta* **329:**98

Reed, P.W. 1972. A23187: A divalent cation ionophore. *Fed. Proc.* **31:**432

Schultz, G., Hardman, J.G., Schultz, K., Baird, C.E., Sutherland, E.W. 1973. The importance of calcium ions for the regulation of guanosine 3′:5′-cyclic monophosphate levels. *Proc. Nat. Acad. Sci. USA* **70:**3889

Schwartz, C.J., Kimberg, D.V., Sheerin, H.E., Field, M., Said, S.I. 1974. Vasoactive intestinal peptide stimulation of adenylate cyclase and active electrolyte secretion in intestinal mucosa. *J. Clin. Invest.* **54:**536

Selinger, A., Eimerl, S., Schramm, M. 1974. A calcium ionophore simulating the action of epinephrine on the α-adrenergic receptor. *Proc. Nat. Acad. Sci. USA* **71:**128

Sheerin, H.E., Field, M. 1975. Ileal HCO_3 secretion: Relationship to Na and Cl transport and effect of theophylline. *Am. J. Physiol.* **228:**1065

Sheerin, H.E., Field, M. 1977. Ileal mucosal cyclic AMP and Cl secretion: Serosal *vs.* mucosal addition of cholera toxin. *Am. J. Physiol.* (*in press*)

Tapper, E.J., Powell, D.W., Morris, S.M. 1976. Effect of high dose carbachol on intestinal electrolyte transport. *Gastroenterology* **70:**A-84/942

Van Sande, J., Decoster, C., Dumont, J.E. 1975. Control and role of cyclic 3′,5′-guanosine monophosphate in the thyroid. *Biochem. Biophys. Res. Commun.* **62:**168

Williams, J.A., Lee, M. 1974. Pancreatic acinar cells: Use of a Ca^{++} ionophore to separate enzyme release from the earlier steps in stimulus-secretion coupling. *Biochem. Biophys. Res. Commun.* **60:**542