Calcineurin Mediates Calcium-induced Potentiation of Adenylyl Cyclase Activity in Dispersed Chief Cells from Guinea Pig Stomach

FURTHER EVIDENCE FOR CROSS-TALK BETWEEN SIGNAL TRANSDUCTION PATHWAYS THAT REGULATE PEPSINOGEN SECRETION*

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In cholera toxin-treated gastric chief cells, incubation with a cholinergic agonist (carbamylcholine), a regulatory peptide (cholecystokinin), or a calcium ionophore (A23187) causes a dose- and time-dependent potentiation of cAMP levels. Because this augmented response is calcium/calmodulin-dependent, we hypothesized that it was mediated by calcineurin (protein phosphatase 2B). To test this hypothesis, we examined the actions of calcineurin inhibitors on secretagogue-induced potentiation of cAMP levels in guinea pig chief cells. Preincubation of cells with 0.1 μM FK-506 completely prevented carbachol-induced augmentation of cAMP levels and peptinogen secretion from choleratoxin-treated cells. Cyclosporin-A, another calcineurin inhibitor, also prevented the augmented cAMP response. FK-506 and cyclosporin inhibited augmentation of cAMP levels following treatment with cholecystokinin(26–33) and A23187, but not the smaller increase in cAMP following treatment with a phorbol ester that activates protein kinase C. Hence, the actions of calcineurin inhibitors were limited to secretagogues that increase cellular calcium. Rapamycin, an agent that competes with FK-506 for the immunophilin, FK binding protein 12, does not inhibit calcineurin. In the present study, preincubation with rapamycin did not prevent carbachol-induced augmentation of cAMP levels in choleratoxin-treated chief cells. However, a molar excess of rapamycin reversed the inhibitory actions of FK-506. These experiments provide further evidence that the actions of FK-506 on cholera toxin-treated gastric chief cells are caused by its inhibitory actions on calcineurin. FK-506 also inhibited potentiation of cAMP levels when carbachol was added to cells that were preincubated with forskolin, an agent that directly activates adenylyl cyclase. We conclude that, in gastric chief cells, calcineurin mediates cross-talk between the calcium/calmodulin and adenylyl cyclase signaling pathways.

Several years ago, we discovered a novel interaction between the calcium- and adenylyl cyclase-mediated signal transduction pathways in gastric chief cells (1). In these cells, preincubation with cholera toxin followed by a second incubation with agents that increase intracellular calcium concentration ([Ca2+]i) results in potentiation of pepsinogen secretion. Similar potentiation of enzyme secretion has been demonstrated in other exocrine tissues (e.g. pancreatic acini (2, 3)). However, in contrast to observations in other tissues (2, 3), in cholera toxin-treated chief cells, potentiation of enzyme secretion by agents that increase [Ca2+]i is mediated by an augmentation in cellular levels of cAMP (1).

We observed that in cholera toxin-treated chief cells, incubation with a cholinergic agonist (carbamylcholine (carbachol)), a regulatory peptide (cholecystokinin(26–33) (CCK)), or a calcium ionophore (A23187) caused an approximate 2-fold dose-dependent potentiation of cAMP levels. These agents did not alter cAMP levels in control cells that had not been treated with cholera toxin. The calcium-dependence of this effect was demonstrated by showing that potentiation of cAMP levels could be abolished by incubating cells in a calcium-free medium or in the presence of inhibitors of calmodulin (1, 4). Subsequently, we reported that phorbol esters that activate protein kinase C also potentiate cAMP levels in cholera toxintreated chief cells (4). However, when compared to agents that increase [Ca2+]i, phorbol esters are about 50% as efficacious in increasing levels of cAMP (4). Moreover, the additive response in cAMP levels that is observed when maximal concentrations of a phorbol ester and a calcium ionophore are combined indicates that these agents act by different mechanisms. Hence, we concluded that at least two different signaling pathways interact with adenylyl cyclase to augment cellular levels of cAMP (4).

Calcineurin (also referred to as protein phosphatase-2B), a calcium/calmodulin-dependent serine/threonine protein phosphatase, appears to play a role in signal transduction in a variety of tissues (for reviews, see Refs. 5 and 6). As with other phosphatases which had once been thought to play only a constitutive role in preparing substrates for the actions of kinases, it is now believed that calcineurin is an active component of signaling cascades (7, 8). Examination of the actions of calcineurin has been facilitated by the discovery of relatively specific inhibitors. These include cyclosporin-A, a cyclic undecapeptide produced by the fungus Tolypocladium inflatum, and FK-506, a macrolide antibiotic obtained from the bacterium Streptomyces tsukubensis. The inhibitory actions of these agents on calcineurin are dependent on binding to endogenous immunophilins, cyclophilin, and FK-binding protein 12.

1 The abbreviations used are: [Ca2+]i, intracellular calcium concentration; carbachol, carbamylcholine; CCK, cholecystokinin(26–33); FKBP12, FK-506 binding protein 12; ACTH, adrenocorticotropic hormone; PMA, phorbol 12-myristate 13-acetate; and IBMX, 3-isobutyl 1-methylxanthine.
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Using these inhibitors and other methods, investigators have reported that calcineurin regulates neutrophil chemokinesis (13, 14), neuronal filopodial retraction (15), pancreatic acinar enzyme secretion (16), and, parotid gland zymogen granule fusion (17). We have observed that FK-506 inhibits calcium- and carbachol-induced pepsinogen secretion from permeabilized and intact chief cells, respectively (18). Regarding our observations in gastric chief cells, Baukal et al. (19) reported that calcineurin mediates a potentiating interaction involving adenyl cyclase in bovine adrenal glomerulosa cells. In glomerulosa cells, angiotensin II potentiates the increase in cAMP caused by adrenocorticotropic (ACTH). Preincubation of glomerulosa cells with cyclosporin A or FK-506 inhibits angiotensin-induced potentiation of the increase in cAMP caused by ACTH (19).

Based on the above findings and the observation that augmentation of cholera toxin-induced increases in cAMP is calcium/calmodulin-dependent, we hypothesized that this potentiated response was mediated by calcineurin. In the present study, we tested this hypothesis by examining the actions of calcineurin inhibitors on secretagogue-induced potentiation of cAMP levels in cholera toxin-treated gastric chief cells.

EXPERIMENTAL PROCEDURES

Materials—Male Hartley guinea pigs (150–175 g) were obtained from Camm Research Laboratory (Camm Research Lab Animals, Wayne, NJ), collagenase (type I), bovine albumin (fraction V), 3-isobutyl-1-methylxanthine (IBMX), carbamylcholine (carbachol), phorbol 12-myristate 13-acetate (PMA), and EGTA from Sigma; cholera toxin, papain, and A23187 from Calbiochem (La Jolla, CA); basal medium (Eagle’s) amino acids (100 times concentrated) and essential vitamin solution (100 times concentrated) from Grand Island Biological, Grand Island, NY; Percoll from Pharmacia Biotech Inc.; 125I-albumin from ICN; cholecystokinin(26–33) (CCK) from Peninsula Labs. (Belmont, CA); and, cAMP-2′-O-succinyl-[125I]-iodotyrosine methyl ester and cAMP antiserum (preconjugated to a second antibody) from Du Pont NEN (Boston, MA). Cyclosporin A and FK-506 were kindly provided by Sandoz Pharmaceuticals (NJ) and Fujisawa Pharmaceutical Corp. (Osaka, Japan), respectively.

Tissue Preparation and Standard Incubation Solution—Dispersed chief cells from guinea pig stomach were prepared as described previously (20). The composition of the cell suspension was determined by light microscopy using morphological criteria (20). As in previous studies (20–22), chief cells constituted at least 85% of the total cell population and trypan blue exclusion was greater than 95%. The cells were washed and resuspended in standard incubation solution containing 24.5 mM HEPES (pH 7.4), 98 mM NaCl, 6 mM KCl, 2.5 mM KH2PO4, 1 mM MgCl2, 11.5 mM glucose, 5 mM sodium fumarate, 5 mM sodium pyruvate, 5 mM sodium glutamate, 1.5 mM CaCl2, 2 mM glutamine, 0.1% (w/v) bovine serum albumin, 1% (v/v) amino acid mixture, and 1% (v/v) essential vitamin mixture. IBMX (100 μM) was added to the incubation solution when changes in cellular cAMP were determined. The standard incubation solution was equilibrated with 100% O2, and all incubations were performed with 100% O2 as the gas phase.

Determination of Pepsinogen Secretion—Pepsinogen secretion was determined as described previously (21) using 125I-albumin substrate and expressed as the percentage of total cellular pepsinogen that was released into the medium during the incubation.

Measurement of cAMP—Cellular cAMP was determined by radioimmunoassay using the procedure described previously (22). The concentration of chief cells in the incubate was adjusted to maintain cAMP on the linear portion of the standard curve.

Statistics—Statistical evaluation was performed using Student’s t test on paired values, or ANOVA followed by Dunnett’s test when comparing multiple values to a single control. p values < 0.05 were considered significant.

RESULTS

As illustrated by the open symbols in Fig. 1A, over the course of a 2-h incubation in the presence of IBMX, an inhibitor of cyclic nucleotide phosphodiesterase, chief cell cAMP levels remained relatively constant at approximately 2–5 pmol/106 cells. In cells incubated with 0.1 μM choleratoxin, there was a steady increase in cAMP to approximately 40 pmol/106 cells by 90 min, after which a plateau was attained with little, if any, further increase in cAMP. In control cells, the addition of 100 μM carbachol at 90 min did not alter cellular cAMP, whereas, in cholera toxin-treated cells, a further increase in cAMP was observed within 15 min of adding the cholinergic agonist and by 32 min was more than 2-fold greater than values observed with choleratoxin alone. In the absence of cholera toxin, addition of the calcineurin inhibitor FK-506 did not alter levels of cAMP. Although in this and some other experiments, the addition of FK-506 appeared to decrease choleratoxin-induced levels of cAMP (compare solid circles to diamonds in Fig. 1A) these changes were not significant (p > 0.05, Student’s t test). Nevertheless, as shown by the solid triangles (partially ob-
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TABLE I
Lack of effect of cyclohexamide on carbachol-induced augmentation of cAMP levels in cholera toxin-treated chief cells

| Additions                     | CAMP (pmol/10^6 cells) |
|-------------------------------|------------------------|
| None                          | 29.4 ± 3.3             |
| Carbachol (100 μM)            | 31.5 ± 3.8             |
| Cholera toxin, 0.1 μM, cyclohexamide | 24.0 ± 3.4             |
| Cholera toxin, 0.1 μM FK-506, cyclohexamide | 25.1 ± 1.2             |

*Indicates results that are significantly greater than those obtained in the absence of carbachol (p < 0.05, Student's t test).

![Graph showing actions of FK-506 on pepsinogen secretion from chief cells](image)

**Fig. 2. Actions of FK-506 on pepsinogen secretion from chief cells that were preincubated with or without cholera toxin and then stimulated with carbachol.** Dispersed chief cells were first incubated for 90 min at 37 °C alone, with 0.1 μM cholera toxin, with 0.1 μM FK-506, or a combination of cholera toxin and FK-506. Cells were then incubated for an additional 30 min at 37 °C with or without 100 μM carbachol. Pepsinogen secretion was determined as a percentage of total cellular pepsinogen released during the second 30-min incubation. In each experiment each value was determined in duplicate and results given are means ± S.E. from three separate experiments. * Indicates that response with carbachol and FK-506 is significantly less than that with carbachol alone (p < 0.05, Student's t test). ** Indicates that the response with carbachol and cholera toxin is significantly greater than the sum of the actions of carbachol and cholera toxin acting alone (indicated by horizontal bar) (p < 0.05, Student's t test).

Secretable by the solid circles) in Fig. 1A, preincubation of cells with FK-506 plus cholera toxin completely prevented carbachol-induced potentiation of cAMP levels.

Fig. 1B shows the dose-response curve for carbachol-induced potentiation of the CAMP response in cholera toxin-treated chief cells. The actions of the cholinerceptor agonist were detectable with 1 and maximal with 100 μM carbachol. This is the same range of concentrations that causes an increase in intracellular calcium and stimulates pepsinogen secretion (L, 20, 21). Preincubation of chief cells with 0.1 μM FK-506 plus cholera toxin completely prevented carbachol-induced potentiation of the CAMP response (Fig. 1B).

To determine whether protein synthesis was necessary to mediate potentiation of CAMP levels in cholera toxin-treated cells, we examined the actions of cyclohexamide, an inhibitor of protein synthesis. As shown in Table I, in cholera toxin-treated cells, the addition of 100 μg/ml cyclohexamide did not alter the actions of carbachol or the inhibitory effects of FK-506. Hence, protein synthesis is not required for carbachol-induced potentiation of CAMP levels in cholera toxin-treated cells.

To determine whether the addition of a calcineurin inhibitor would also prevent carbachol-induced potentiation of pepsinogen secretion from cholera toxin-treated chief cells, we examined the effects of FK-506 on cells that were preincubated for 90 min with or without cholera toxin and then incubated for an additional 30 min with or without carbachol (Fig. 2). In cells that had been incubated without cholera toxin for 90 min, pepsinogen secretion during the second 30-min incubation was increased approximately 4-fold by the addition of 100 μM carbachol (from 2.0 ± 1.4% with no additions to 7.9 ± 0.4% with carbachol, mean ± S.E. from three experiments). Secretion during the second incubation from cells that were preincubated for 90 min with 0.1 μM cholera toxin and then incubated with no additions for an additional 30 min was the same as that from cells preincubated without cholera toxin. However, potentiation of pepsinogen secretion was observed when cholera toxin-treated cells were incubated for an additional 30 min with carbachol. That is, during the 30-min incubation following the addition of carbachol to cholera toxin-treated cells, pepsinogen secretion was more than twice the sum of secretion observed with carbachol and cholera toxin acting alone (see horizontal line in Fig. 2).

Although pepsinogen secretion seemed diminished when FK-506 was added to control or cholera toxin-treated cells (Fig. 2), these changes were not significant (p > 0.05, Student's paired t test). In contrast, FK-506 significantly decreased secretion caused by carbachol alone or by carbachol plus cholera toxin. Secretion from cholera toxin-treated cells that were incubated with carbachol plus FK-506 was not significantly different from the calculated additive value for the effects of carbachol and cholera toxin acting alone (horizontal line in Fig. 2). These results indicate that calcineurin plays a role in mediating carbachol-induced pepsinogen secretion and potentiation of secretion in cells that are treated with carbachol plus cholera toxin. Hence, in cholera toxin-treated chief cells, addition of a calcineurin inhibitor prevents potentiation of both the CAMP and secretory response when cells are incubated for an additional period with carbachol.

Because previous studies (4) indicated that other agonists could augment the CAMP response in cholera toxin-treated chief cells, we examined the effect of FK-506 on the actions of these agents. As illustrated in Fig. 3, in addition to carbachol, a regulatory peptide (CCK), a calcium ionophore (A23187), and a phorbol ester (PMA) increased CAMP in cholera toxin-treated cells. These agonists did not alter CAMP in cells that were not pretreated with cholera toxin (data not shown). With the exception of the modest response caused by PMA, augmentation in CAMP levels observed with these agonists in cholera toxin-treated cells was prevented by incubation with FK-506. These results indicate that a variety of agents that increase chief cell calcium concentration can augment CAMP levels in cholera toxin-treated chief cells. Likewise, these actions can be prevented by adding a calcineurin inhibitor. In contrast, PMA, an agent that does not increase cell calcium concentration, causes only a minimal increase in CAMP levels that is not altered by preincubation with FK-506 (Fig. 3).

To verify that the actions of FK-506 on chief cell CAMP were caused by inhibition of calcineurin, we examined the actions of cyclosporin-A, another inhibitor of this phosphatase. As shown in Fig. 4A, in cells that had not been preincubated with cholera toxin, carbachol, and CCK did not alter basal CAMP levels. Likewise, neither FK-506 nor cyclosporin-A altered basal CAMP levels. In contrast, as shown in Fig. 4B, cholera toxin incubation for 90 min increased CAMP levels to approximately 35 pmol/10^6 cells. The addition of carbachol and CCK at 90 min...
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Forskolin alone caused an approximate 8-fold increase in cellular cAMP. The addition of carbachol almost doubled the cAMP response. As observed with cholera toxin-treated cells, in forskolin-treated cells, the addition of FK-506 blocked carbachol-induced potentiation of cAMP levels.

DISCUSSION

Calcineurin (protein phosphatase-2B) plays a role in signal transduction in a variety of secretory cells (5–8). In preliminary studies, we have demonstrated that, in gastric chief cells, inhibition of calcineurin decreases calcium-mediated pepsinogen secretion and dephosphorylation of cytoskeletal phosphoproteins (18, 23).

In the present study, we show that, in gastric chief cells, calcineurin mediates an interaction between the calcium and adenylyl cyclase signaling systems that results in augmentation of cAMP levels and potentiation of cAMP levels and secretion caused by agents that increase cellular calcium concentration. Evidence that this calcium/calmodulin-dependent serine/threonine phosphatase plays an important role in mediating these potentiating interactions includes the

increased cAMP levels to 70 and 60 pmol/10⁶ cells, respectively, after an additional 30-min incubation. This augmentation of cAMP levels was prevented by incubation with either FK-506 or cyclosporin-A supporting the hypothesis that these actions are mediated by calcineurin.

Next, we compared the dose-response curves for the abilities of FK-506, cyclosporin A, and okadaic acid, a protein phosphatase 1 and -2A inhibitor, to inhibit carbachol-induced augmentation of cAMP in cholera toxin-treated cells. As shown in Fig. 5, the order of potency for these inhibitors was FK-506 > cyclosporin > okadaic acid. Whereas FK-506 and cyclosporin are efficacious in the nanomolar range, okadaic acid concentrations less than 0.3 μM have no effect on the augmented response. It has been reported previously that at micromolar concentrations, the actions of okadaic acid become less specific and the agent can inhibit calcineurin (10).

To validate further the use of FK-506 and cyclosporin A as specific calcineurin inhibitors, we examined the actions of rapamycin. Like FK-506, rapamycin, an antibiotic produced by the bacterium Streptomyces hygroscopicus, binds to FKBP12 (9–11). However, unlike the FK-506-FKBP12 complex, the rapamycin-FKBP12 complex does not inhibit calcineurin. Because FK-506 and rapamycin compete for FKBP12, a molar excess of rapamycin should diminish the ability of FK-506 to inhibit calcineurin (9–11). As shown in Fig. 6, rapamycin alone did not alter cAMP levels in cholera toxin-treated cells. Whereas, incubation with FK-506 prevented carbachol-induced potentiation of cAMP levels, rapamycin had no effect. Moreover, rapamycin reversed the inhibitory effects of FK-506. In contrast, rapamycin did not alter the inhibitory effects of cyclosporin A on carbachol-induced potentiation of cAMP levels. This result is expected because unlike FK-506 or rapamycin which compete for FKBP12, cyclosporin A binds to a different immunophilin, cyclophilin (9–11). These data provide evidence that the actions of FK-506 demonstrated in these studies are specific and mediated by inhibition of calcineurin by the FK-506-FKBP12 complex.

To localize the site of calcineurin action in augmenting the cAMP response, we examined the actions of forskolin, a plant alkaloid that acts directly on adenylyl cyclase to stimulate an increase in cAMP. Table I shows the effects of forskolin, with or without carbachol, on cAMP levels in dispersed chief cells.
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The specificity of FK-506 and cyclosporin A for calcineurin is demonstrated by the observation that rapamycin, a competitor for FKBP12, blocks the inhibitory actions of FK-506 but not those of cyclosporin A. Although high concentrations of okadaic acid inhibited augmentation of cAMP levels, it is likely that this is caused by the previously reported promiscuity of this agent at micromolar concentrations (10). Moreover, in in vivo systems it is not possible to control completely the stoichiometry of these interactions. That is, although one can add a known quantity of the inhibitor to the cell suspension, one does not know how much calcineurin is present. Hence, if chief cells contain relatively small amounts of calcineurin, nonspecific actions of high concentrations of okadaic acid are more likely (10).

Dissection of the locus of action of calcineurin in mediating augmentation of chief cell cAMP levels is possible from the observations reported here. This action is not likely to be caused by a change in receptor number or affinity for the various agonists used in this study. This is evident because carbachol and CCK interact with different classes of receptors (24), and A23187 is a divalent cation ionophore. Likewise, an action on cholera toxin receptors is unlikely because the augmented response occurs 90 min after the cells were first exposed to the toxin and cAMP levels have reached a plateau. An action of calcineurin on cyclic nucleotide phosphodiesterases is unlikely because these studies were performed in the presence of IBMX, an inhibitor of these enzymes. We have shown previously that adding IBMX facilitates experiments by increasing the amount of cAMP in these cells, but that its presence is not required for observing augmentation of cAMP levels (1). The observation that cyclohexamide does not alter the actions of carbachol or FK-506 provides evidence that the augmented response is not caused by the actions of calcineurin on gene expression. Hence, based on these exclusions, the most likely target for calcineurin in mediating augmentation of cAMP levels is adenylyl cyclase. This hypothesis is supported by the observation that carbachol-induced potentiation of cAMP in cells treated with forskolin, an agent that acts directly on adenylyl cyclase, is also blocked by preincubation with FK-506. Calcineurin may act directly on an isofrom of adenylyl cyclase or it could activate a signaling cascade in which another phosphatase or kinase is activated to act on the cyclase. The adenylyl cyclases are a fast growing family with as many as 10 isofroms (25–27). These isofroms differ with respect to regulation by guanine nucleotide-binding proteins, calcium/calmodulin, and protein kinase C (24–26). Based on analysis of these differences when adenylyl cyclase isofroms are expressed in HEK 293 cells (25), investigators have separated the isofroms into three groups. Group I, consisting of isofroms I, III, and VIII, is stimulated by increases in calcium/calmodulin (28–30). Nonetheless, matters are complicated further by the observation that adenylyl cyclase isofrom III may be stimulated by calcium/calmodulin in vitro, but inhibited by these agents in vivo (31). This latter observation raises the possibility of a

TABLE II

| Additions                  | cAMP (pmol/10⁶ cells) |
|---------------------------|-----------------------|
| None                      | 9.7 ± 0.8             |
| Carbachol (100 μM)        | 15.7 ± 2.0 NS         |
| FK-506, 0.1 μM            | 8.6 ± 1.0             |
| Forskolin, 1 μM           | 51.8 ± 5.1            |
| Forskolin + FK-506        | 40.4 ± 7.0            |

NS, value with carbachol not significantly different than control (p < 0.05, Student's t-test).

b Indicates that result is significantly greater than that obtained in the absence of carbachol (p < 0.05, Student's t-test).

\[ Ca^{2+} \] cholera toxin-treated cells is caused by agents like carbachol, CCK, and A23187 that increase cellular CAMP in cholera toxin-treated cells. The following observations: (a) augmentation of CAMP levels in cholera toxin-treated cells is caused by agents like carbachol, CCK, and A23187 that increase \[ Ca^{2+} \] (Refs. 1, 4, and the present paper); (b) the CAMP response requires the presence of extracellular calcium (1); (c) inhibitors of calmodulin, like trifluoperazine or calmidazolium, block the augmented response (1, 4); and, (d) preincubation of cells with inhibitors of calcineurin, like FK-506 and cyclosporin A, prevents augmentation of the CAMP and secretory responses (present paper).

The specificity of FK-506 and cyclosporin A for calcineurin is demonstrated by the observation that rapamycin, a competitor for FKBP12, blocks the inhibitory actions of FK-506 but not those of cyclosporin A. Although high concentrations of okadaic acid inhibited augmentation of CAMP levels, it is likely that this is caused by the previously reported promiscuity of this agent at micromolar concentrations (10). Moreover, in in vivo systems it is not possible to control completely the stoichiometry of these interactions. That is, although one can add a known quantity of the inhibitor to the cell suspension, one does not know how much calcineurin is present. Hence, if chief cells contain relatively small amounts of calcineurin, nonspecific actions of high concentrations of okadaic acid are more likely (10).

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Fig. 5. Dose-response curves for the actions of FK-506, cyclosporin A, and okadaic acid on cellular CAMP in cholera toxin-treated chief cells incubated with carbachol. Dispersed chief cells were first incubated for 90 min at 37°C with 0.1 μM cholera toxin plus the indicated concentrations of phosphatase inhibitors. Cells were then incubated for an additional 30 min at 37°C with no additions or 100 μM carbachol. Results are expressed as a percentage of maximal CAMP, that is the CAMP level observed with 100 μM carbachol. Results are expressed as a percentage of maximal CAMP, that is the CAMP level observed with 100 μM carbachol. In each experiment each value was determined in duplicate and results given are means ± S.E. from three separate experiments.

Fig. 6. Actions of rapamycin on carbachol-induced augmentation of CAMP levels in cholera toxin-treated cells. Dispersed chief cells were first incubated for 90 min at 37°C with 0.1 μM cholera toxin alone or with 300 μM rapamycin, 30 nM FK-506, rapamycin plus FK-506, 100 nM cyclosporin A, or rapamycin plus cyclosporin A. Cells were then incubated for an additional 30 min at 37°C alone or with 100 μM carbachol. In each experiment each value was determined in duplicate and results given are means ± S.E. from three separate experiments. * indicates value with carbachol that is significantly greater than corresponding value without carbachol (p < 0.05, Student's t-test).
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dynamic interaction wherein calcineurin activates an isofrom of adenyl cyclase that was previously inactivated by calmodulin kinase II-mediated phosphorylation.

Interaction with a different isofrom of adenyl cyclase may explain the different pattern of action observed in the present studies with PMA, an activator of protein kinase C. The modest PMA-induced augmentation in the cAMP response, that is not explained by actions of protein kinase C in gastric chief cells it will be necessary to determine which adenyl cyclase isofroms are expressed in these cells and to examine directly the actions of calcium/calmodulin and calcineurin on phosphorylation of these isofroms and on other potential substrates.

In conclusion, in gastric chief cells, calcineurin regulates a form of cross-talk between the calcium/calmodulin and adenylyl cyclase signaling systems that results in augmentation of cAMP production and pepsinogen secretion. As has been hypothesized for the brain (32) and other organs (19), such potentiating interactions may serve an important purpose by providing "fine-tuning" and/or amplification of secretory responses. Redundancy in signaling pathways may also provide an evolutionary advantage should one pathway fail or be blocked. Finally, in the pathophysiological state of infection with Vibrio cholera, the potentiating interactions shown here may worsen the clinical manifestations of gastrointestinal hypersecretion. Cholinergic antagonists, inhibitors of other agents that stimulate the calcium/calmodulin signaling pathway, and possibly inhibitors of calcineurin may ameliorate this disease.

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