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The Relationship between Free Cytosolic Calcium and Amylase Release in Rat Pancreatic Acini

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Abstract We have studied the effects of various pancreatic secretagogues on free cytosolic calcium ($[Ca^{2+}]_i$) and amylase release in dispersed rat pancreatic acini, to determine the role of $[Ca^{2+}]_i$ in stimulated enzyme secretion from the exocrine pancreas. Dispersed rat pancreatic acini were loaded with the new Ca^{2+}-sensitive fluorescent indicator, fura-2. Resting $[Ca^{2+}]_i$ was 110 ± 2 nM (a mean $\pm$ S.E.). Carbachol, caerulein, bombesin, and neuromedin B and C each caused a rapid increase in $[Ca^{2+}]_i$; maximal increases of 100 to 400–500 nM were reached within 20 s following the secretagogue addition, and this was followed by a return to a lower sustained level within 2 min. When enzyme secretion from the acini was monitored as a function of time using a perifusion system, secretagogue-induced amylase release took a biphasic pattern consisting of an initial burst phase for a several minutes and a second sustained phase during stimulation. Although sustained amylase secretion occurred at near resting $[Ca^{2+}]_i$, the peak $[Ca^{2+}]_i$ correlated with the amount of stimulated amylase release as well as with the initial release, during submaximal and maximal stimulation by these agents. At supramaximal concentrations of carbachol and caerulein, amylase release, but not the increase in $[Ca^{2+}]_i$, was attenuated. On the other hand, in response to supramaximal concentrations of bombesin, and neuromedin B and C, both the amount of amylase released and the peak $[Ca^{2+}]_i$ were similar to those obtained in response to maximal concentrations. From a standpoint of time course analysis of enzyme secretion, both the first burst phase and the second sustained phase were inhibited during stimulation by 10^{-3} M carbachol, compared with 10^{-5} M carbachol, while supramaximal stimulation by neuromedin C caused a pattern of amylase release similar to that produced by maximal stimulation. These data suggest that in pancreatic acinar cells an increase in $[Ca^{2+}]_i$ plays an important role in stimulus-secretion coupling; however, other factors may be indispensable in regulating enzyme secretion. Furthermore, it is suggested that there is a difference in the intracellular messenger system between carbachol and caerulein, and

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neurotransmitters belonging to the bombesin family, especially during supramaximal stimulations.

Key words: calcium, amylase, pancreas, bombesin, neuromedin.

The various secretagogues that act on pancreatic acinar cells are considered to stimulate enzyme secretion by activating one of two distinct processes (GARDNER and JENSEN, 1981). Some agents, such as secretin and VIP (vasoactive intestinal peptides) stimulate enzyme secretion by increasing cellular adenosine cyclic-3′,5′-monophosphate (cyclic AMP) (BISSONNETTE *et al.*, 1984). Other agents such as a) cholinergic agonists, b) hormones belonging to the cholecystokinin family, and c) some neuropeptides that are structurally related to bombesin, cause mobilization of intracellular calcium, which, after a series of presently undefined steps, results in enzyme secretion (SCHULTZ, 1980; GARDNER and JENSEN, 1981). The latter stimulus-secretion coupling is believed to involve an increase in the concentration of free cytosolic calcium ($[Ca^{2+}]_i$), and this has already been extensively reviewed (SCHULTS, 1980; WILLIAMS and HOOTMAN, 1986). Accurate measurement of $[Ca^{2+}]_i$ has, however, been difficult due to the lack of a reliable intracellular Ca^{2+} probe. Recently an intracellular fluorescent probe, quin2, was developed to measure $[Ca^{2+}]_i$ (TSIEN *et al.*, 1982). Studies using quin2 have allowed a direct demonstration of the fact that secretagogues which stimulate cholinergic or cholecystokinin receptors cause an elevation of $[Ca^{2+}]_i$ in pancreatic acini (OCHS *et al.*, 1985; PANDOL *et al.*, 1985; POWERS *et al.*, 1985). However, whether a stoichiometric relation exists between $[Ca^{2+}]_i$ and enzyme secretion remains controversial. Furthermore, the correlation between the kinetics of the changes in $[Ca^{2+}]_i$ with those of enzyme secretion has not been evaluated sufficiently.

In the present study, we determined the effects of various pancreatic secretagogues on $[Ca^{2+}]_i$ using fura-2 (1-(2-(5′-carboxyoxazol-2′-yl)-6-amino-5′-methylphenoxy)-ethane-N,N,N′,N′-tetraacetic acid, K salt), an improved Ca^{2+} indicator with an increased brightness of fluorescence (GRYNKIEWICZ *et al.*, 1985), and on the release of amylase, in dispersed rat pancreatic acini. Particular interest was taken in obtaining dose-response curves for each agent and in following the time course of each response. Our results demonstrate the important role of the secretagogue-stimulated increase in $[Ca^{2+}]_i$ on enzyme secretion, the involvement of other factors in addition to changes in $[Ca^{2+}]_i$ in stimulus-secretion coupling in pancreatic acini, and indicate differences in the intracellular regulating system between carbachol and caerulein, and the neuropeptides which belong to the bombesin family, when they are applied at supramaximal concentrations.

MATERIALS AND METHODS

Materials. Fura-2/AM (the acetoxymethyl derivative of fura-2), HEPES (N-

2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid), and EGTA (ethyleneglycol-bis(β-aminoethyl ester)-tetraacetic acid) were obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan; collagenase type III 125 U/mg from Worthington Diagnostic Systems, Inc., Freehold, NJ; bovine serum albumin (fraction V), soybean trypsin inhibitor (type 1-S), and carbachol from Sigma Chemical Co., St. Louis, MO, U.S.A. The Eagle MEM minimum essential amino acids and vitamins were obtained from Nissui Pharmaceutical Co., Ltd., Tokyo; Tris and EDTA (ethylene-diaminetetraacetic acid) from Nakarai Chemicals, Ltd., Kyoto; Triton X from Yoneyama Yakuhin Kogyo Co., Ltd., Osaka; and caerulein was from Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan. Synthesized bombesin, neuromedin B, neuromedin C (although neuromedin C is identical in structure to gastrin-releasing peptide-(18-27) (GRP-(18-27)), the term "neuromedin C" has been used in this manuscript, instead of GRP-(18-27), for comparison with neuromedin B (MINAMINO *et al.*, 1984 a, b)), secretin (porcine), and VIP (porcine) were obtained from Peptide Institute Inc., Minoh, Osaka, Japan; synthesized GRF (growth hormone-releasing factor; rat hypothalamus) from Peninsula Laboratories, Inc., Belmont, CA, U.S.A.; and Phadebas amylase test from Shionogi Co., Ltd., Osaka, Japan.

Except where indicated, the incubation solution was Krebs-Ringer-HEPES buffer containing 10 mM HEPES, 120 mM NaCl, 4.7 mM KCl, 1 mM KH_2PO_4, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 15 mM glucose, 0.1% (w/v) bovine serum albumin, 0.01% (w/v) soybean trypsin inhibitor, and Eagle MEM essential amino acids and vitamins, adjusted with NaOH to pH 7.4 equilibrated with 100% O_2.

Methods. Preparation of dispersed acini: Dispersed pancreatic acini were prepared by enzymatic digestion of rat pancreas similar to the method of AMSTERDAM and JAMIESON (1972, 1974). Briefly, the pancreas was obtained from an unfasted male Wistar King albino rat (approximately 300 g). The pancreatic tissue was injected by means of a 27-gauge needle with a standard medium containing digestive enzyme, 170 U/ml collagenase type III. The injected pancreatic tissue was incubated in this medium for 15 min and shaken at approximately 80 cycles/min in a water bath at 37°C. The medium was replaced by fresh Krebs-Ringer-HEPES buffer with no Ca^{2+} and no Mg^{2+}, but containing 2 mM EDTA for 10 min. This was followed by a second digestion period with the standard medium containing 200 U/ml collagenase type III for 30–40 min. During the digestion steps, the tissue was continuously gassed with 100% O_2. Pancreatic acini were then mechanically dissociated by sequential passage through four pipettes of decreasing orifice into the standard medium containing 0.1% (w/v) soybean trypsin inhibitor and filtered through one layer of medical gauge. The suspension of dispersed acini was layered over the standard medium containing 4% (w/v) bovine serum albumin and centrifuged for 3 min at 20 *g*. The pellet was then washed three times with a standard medium and the pancreatic acini were resuspended in the standard medium.

Fura-2 loading and measurement of $[Ca^{2+}]_i$: Dispersed acini from one animal were suspended in 10 ml of the standard medium containing 1 μM fura-2/AM,

incubated at 37°C for 30 min. These fura-2-loaded acini were then washed twice, and resuspended in the standard medium.

For measurement of $[Ca^{2+}]_i$, fura-2 fluorescence was measured in 2 ml of resuspended acini solution held in a quartz cuvette in a Hitachi 650-40 fluorescence spectrophotometer, with settings to 340 nm excitation (slit width, 5 nm) and 490 nm emission (slit, 10 nm). The final cell concentration was 10^6 cells/ml. During the measurement of fluorescence, the cell suspension was continuously stirred, and maintained at 37°C by means of a thermostatted jacket. Maximal fura-2 fluorescence (F_{max}) and minimal fluorescence (F_{min}) were measured following the addition of Triton X (0.2%) and EGTA (10 mM) plus Tris base (20 mM) respectively. $[Ca^{2+}]_i$ was calculated from the equation $[Ca^{2+}]_i = K_d(F-F_{min})/(F_{max}-F)$ (TSIEN *et al.*, 1982), using a K_d value, effectively a dissociation constant for Ca^{2+}-fura-2, of 224 nM (GRYNKIEWICZ *et al.*, 1985). The background autofluorescence of non-fura-2-loaded acini was much less than the resting fluorescence level of fura-2-loaded acini. The baseline fluorescence of fura-2-loaded acini (i.e., resting $[Ca^{2+}]_i$) was linear for at least 20 min, suggesting that no photobleaching or cell damage had occurred in the cuvette. For the most part, fluorescence measurements were completed within 10 min after the samples were placed in the fluorometer.

Amylase release: Amylase release was measured from fura-2-loaded and from control acini, both being incubated at 37°C for 5 or 30 min in a closed container with 100% O_2. Amylase release in the test vials was measured on duplicate samples and calculated as the percentage of the total content present in the acini at the beginning of the incubation that was released into the extracellular medium during incubation. Amylase activity was assayed by the method of CESKA *et al.* (1969) using the Phadebas reagent.

To examine the time course of enzyme secretion in detail, we employed a perifusion system of dispersed rat pancreatic acini as described previously by IMAMURA *et al.* (1983) in our laboratory. Pancreatic acini placed on a filter (5 μm pore size, SMWP, Millipore Corp., Bedford MA, U.S.A.) in a perifusion chamber were perifused with a medium through tubing, using a pump. Perifusate was collected every minute using a fraction collector. The acini in the perifusion chamber were held in a water bath at 37°C, and the perifusion medium was gassed with 100% O_2 continuously. Amylase activity of the perifusion fraction samples was expressed as a percentage of the total initial content.

RESULTS

Effect of fura-2 loading on amylase release from dispersed rat pancreatic acini

Fura-2 loading did not alter the acinar cell's viability as measured by trypan blue exclusion (over 95%). The response of amylase release from both fura-2-loaded and control acini to increasing concentrations of the secretagogues we used were superimposable (results not shown).

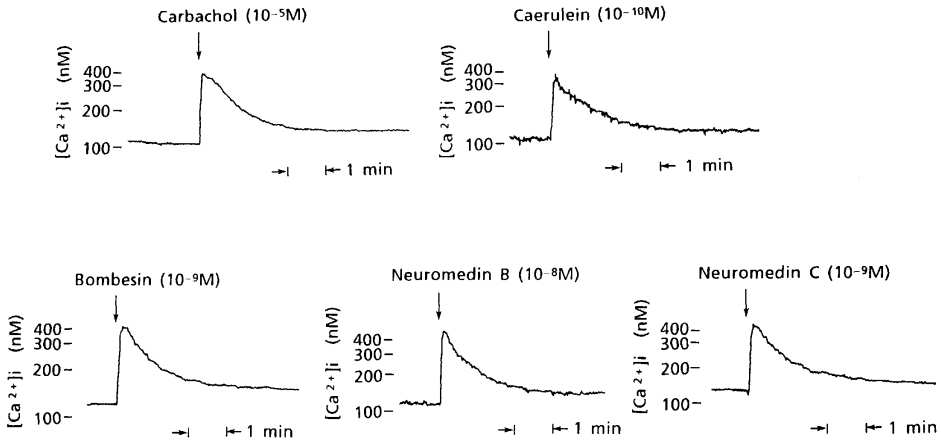

Fig. 1. Effect of carbachol, caerulein, bombesin, neuromedin B, and neuromedin C on $[Ca^{2+}]_i$ in rat pancreatic acini. The addition of the secretagogues is indicated by the arrows. Each recording is of a single experiment representative of at least 3 others.

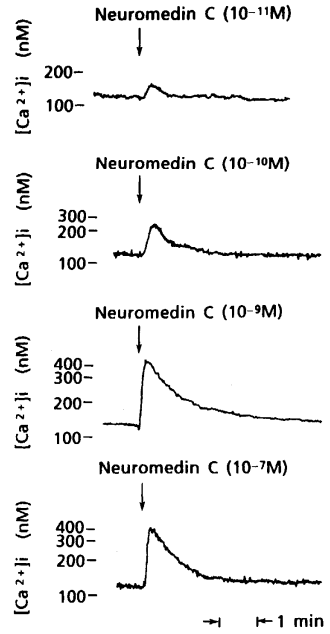

Fig. 2. Effect of neuromedin C at various doses on $[Ca^{2+}]_i$ in rat pancreatic acini. The addition of the secretagogues is indicated by the arrows. Each recording is of a single experiment representative of at least 3 others.

Effect of secretagogues on $[Ca^{2+}]_i$ in dispersed rat pancreatic acini

In a large series of experiments, the mean resting $[Ca^{2+}]_i$, calculated from the fluorescence of fura-2, was 110 ± 2 nM (a mean $\pm$ S.E., $n=321$). Carbachol, caerulein, bombesin, neuromedin B and C each induced a rapid increase of $[Ca^{2+}]_i$ (Fig. 1). The length of time it took for $[Ca^{2+}]_i$ to reach its peak level was only a few seconds. When the secretagogues were applied at low concentrations, the time required for $[Ca^{2+}]_i$ to attain its peak level was longer, but less than 20 s, and the peak value of $[Ca^{2+}]_i$ was smaller (Fig. 2). After reaching a peak value, this fluorescence decreased gradually to a plateau level near to the resting level within 2 min, and thereafter this level was maintained.

Secretin (5×10^{-7} M), VIP (10^{-9} M), or GRF (10^{-7} M), all of which are believed to cause enzyme secretion by increasing cellular cyclic AMP but not by mobilizing cellular Ca^{2+}, had no effect on $[Ca^{2+}]_i$. Recently, TRIMBLE *et al.* (1986) claimed that secretin raised $[Ca^{2+}]_i$ when applied at concentrations higher than 10^{-8} M. This is not supported by the data reported in this study.

Time course of secretagogue-stimulated amylase release in perifusion of dispersed rat pancreatic acini

When acini were stimulated by 10^{-5} M carbachol, the secretory pattern was biphasic consisting of an initial burst release (initial secretion) followed by a return to a sustained level (sustained secretion). Withdrawal of carbachol resulted in a return of the amylase release to the basal rate (Fig. 3A). The 10^{-9} M neuromedin C (Fig. 3C) caused a similar biphasic pattern of amylase release. When stimulated by 10^{-3} M carbachol, both the first release phase and second phase were inhibited, compared with those produced by 10^{-5} M carbachol (Fig. 3B). The 10^{-7} M neuromedin C caused a pattern of amylase release similar to that of 10^{-9} M neuromedin C (Fig. 3D).

Dose-response curves for secretagogue-stimulated amylase release in dispersed rat pancreatic acini

Amylase release over 5 min (initial secretion) or 30 min ("total" secretion, which means the sum of initial and sustained enzyme release) in response to increasing concentrations of the secretagogues in fura-2-loaded acini is illustrated in the upper panels in Fig. 4.

(A) Total enzyme secretion. With increasing concentrations of carbachol, amylase release increased, became maximal with 10^{-5} M carbachol, and then decreased as the secretagogue concentration was increased to 10^{-3} M. Caerulein also caused submaximal amylase stimulation when it was applied at supramaximal concentrations. The concentration of caerulein which produced maximal amylase release was 10^{-10} M. Caerulein had the same efficacy as carbachol in stimulating amylase release. The concentration of bombesin and neuromedin C that produced maximum amylase release was 10^{-9} M. However, neuromedin B produced maximal amylase release when given at a concentration of 10^{-7} M; i.e., 100-fold higher

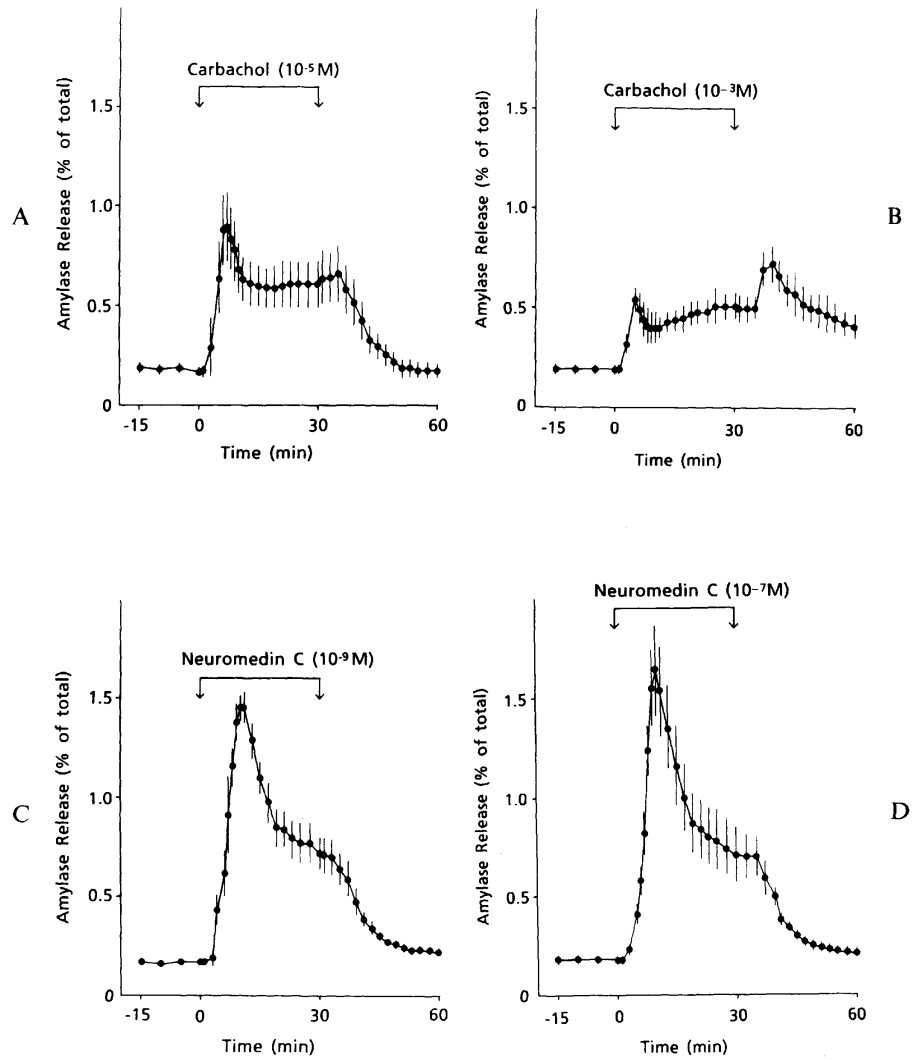

Fig. 3. Time course of amylase release in response to 30-min perifusion with 10^{-5} M carbachol (A), 10^{-3} M carbachol (B), 10^{-9} M neuromedin C (C), and 10^{-7} M neuromedin C (D) in perifusion of rat pancreatic acini. Amylase release for 1 min is expressed as a percentage of total amylase content. Each value represents the mean ± S.E. of 4–9 individual experiments.

concentration than for bombesin or neuromedin C. When bombesin and the structurally related peptides, neuromedin B and C, were applied at supramaximal concentrations, there was little attenuation of amylase release. Although bombesin and the bombesin-related peptides neuromedin B and C had different potencies for stimulating amylase release, they were almost equal in efficacy, and the con-

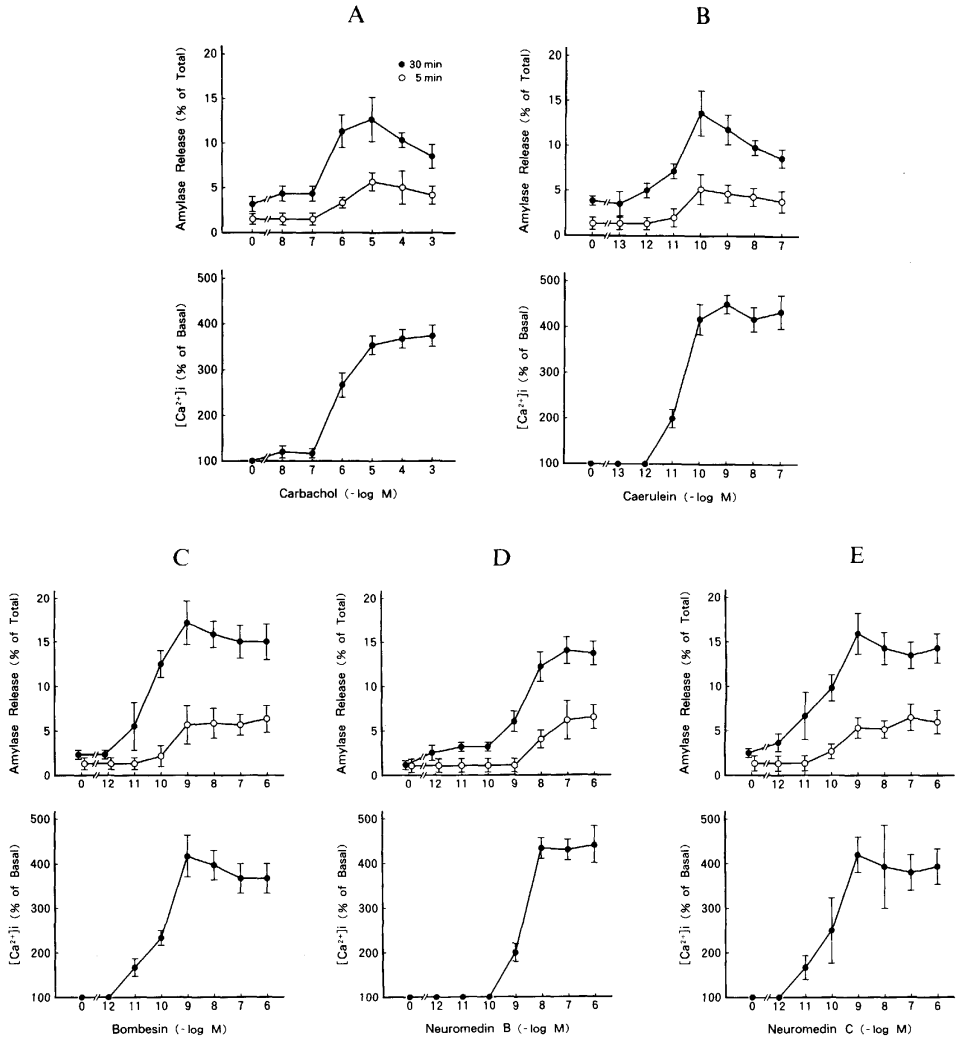

Fig. 4. Dose-dependent effect of carbachol (A), caerulein (B), bombesin (C), neuromedin B (D), and neuromedin C (E) on amylase release (upper panels; ● 30-min release; ○ 5-min release) and $[Ca^{2+}]_i$ (lower panels) in fura-2-loaded rat pancreatic acini. Amylase release is presented as a percentage of total cell amylase released in 5 or 30 min with secretagogue stimulation. Change in $[Ca^{2+}]_i$ is presented as the maximal percent increase that occurred above resting levels of $[Ca^{2+}]_i$ following addition of secretagogues. Each point represents the mean ± S.E. of 4–8 experiments.

figurations of their dose-response curves were similar. When given at maximally effective concentrations, the increase in amylase release caused by bombesin and neuromedin B or C was slightly larger than that caused by carbachol or caerulein.

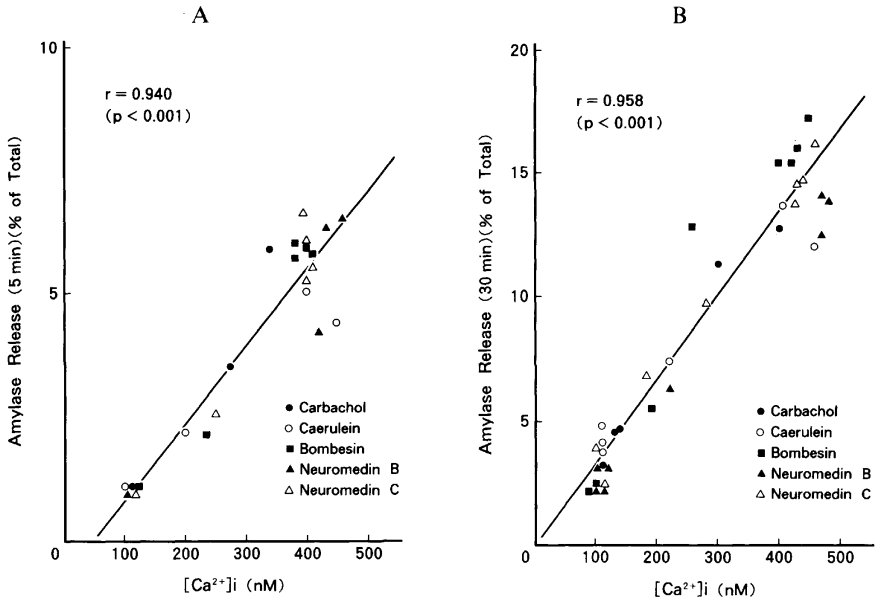

Fig. 5. Relation between amylase release and $[Ca^{2+}]_i$. Amylase release for 5 min (initial release) or 30 min (total release) and $[Ca^{2+}]_i$ of fura-2-loaded rat pancreatic acini were measured following addition of various concentrations of carbachol (●), caerulein (○), bombesin (■), neuromedin B (▲), and neuromedin C (△). Each point represents the mean ($n=4$–8) of both of these parameters. The solid line is drawn according to a linear-regression analysis based on points for all of the secretagogues except for the supramaximal concentrations of carbachol and caerulein (i.e., 10^{-4}, 10^{-3} M carbachol, and 10^{-8}, 10^{-7} M caerulein). A: $n=24$; $Y=0.015X-0.598$; correlation coefficient $r=0.940$ ($p<0.001$). B: $n=35$; $Y=0.034X-0.148$; $r=0.958$ ($p<0.001$).

(B) Initial enzyme secretion. A similar pattern of dose-response curves for initial amylase release was indicated.

Dose-response curves of secretagogue-stimulated $[Ca^{2+}]_i$ increase in dispersed rat pancreatic acini

With increasing concentrations of the secretagogues, the initial peak $[Ca^{2+}]_i$ became maximum, and then remained relatively unchanged when supramaximal concentrations were applied (Fig. 4, lower panels). The concentrations of the secretagogues required to produce maximal increase in $[Ca^{2+}]_i$ were 10^{-4} M carbachol, 10^{-9} M caerulein, 10^{-9} M bombesin, 10^{-9} M neuromedin C, or 10^{-8} M neuromedin B. After an initial dose-dependent increase in $[Ca^{2+}]_i$ from approximately 100 nM (resting $[Ca^{2+}]_i$), Ca^{2+} level returned to a plateau at approximately 100–150 nM, whose dose-dependency was less clear.

When the dose responses for amylase release and peak $[Ca^{2+}]_i$ are compared, a feature becomes apparent. The peak $[Ca^{2+}]_i$ induced by the secretagogues exhibits a dose dependency that is correlated to that of both "initial" (5 min) (Fig. 5A) and "total" (30 min) amylase release (Fig. 5B), except at supramaximal concentrations of carbachol and caerulein, i.e., over 10^{-4} M and over 10^{-9} M respectively.

DISCUSSION

It is known that after a secretagogue has coupled to the cell surface receptors of pancreatic acinar cells, there are at least two distinct intracellular transduction pathways that are involved in stimulus-secretion coupling (GARDNER and JENSEN, 1981). Some agents such as secretin, VIP, and GRF act to cause pancreatic enzyme secretion by increasing cellular cyclic AMP (BISSONNETTE *et al.*, 1984; PANDOL *et al.*, 1984). Other agents such as cholinergic agonists and cholecystokinin act by causing a phospholipase C-mediated breakdown of membrane phosphoinositides and changes in $[Ca^{2+}]_i$ (PUTNEY *et al.*, 1983; ORCHARD *et al.*, 1984; RUBIN, 1984; RUBIN *et al.*, 1984). Recent studies suggest that the changes in $[Ca^{2+}]_i$ are caused by inositol 1,4,5-trisphosphate, a phosphoinositide breakdown product that releases calcium from the rough endoplasmic reticulum store into the cytoplasm (STREB *et al.*, 1983, 1984). The release of calcium from the rough endoplasmic reticulum results in an increase in $[Ca^{2+}]_i$, and this rise in $[Ca^{2+}]_i$ in turn stimulates exocytosis. But whether a stoichiometric relationship exists between $[Ca^{2+}]_i$ and enzyme secretion in pancreatic acini remains controversial. OCHS *et al.* (1985) demonstrated a stoichiometric relationship between stimulated amylase release and the peak $[Ca^{2+}]_i$ as measured with quin2 in mouse pancreatic acini for submaximal and maximal concentrations of carbachol, and POWERS *et al.* (1985) showed the same for cholecystokinin octapeptide. However, BRUZZONE *et al.* (1986) indicated that both caerulein and carbachol could also stimulate enzyme release without affecting $[Ca^{2+}]_i$, for example, when caerulein was applied at a concentration of 10^{-11} M. There are several possible explanations for the differences between these published results. A) Since quin2 is a high-affinity Ca^{2+} chelater, there is the possibility of buffering and masking of any small $[Ca^{2+}]_i$ transients elicited by low concentrations of the secretagogues. B) The quin2 loading itself may influence the secretory function of the acini. For example, it has been reported that in quin2-loaded acini the maximal amount of stimulated amylase release was decreased (OCHS *et al.*, 1985) and that the maximal stimulating dose of secretagogue was shifted to the right (POWERS *et al.*, 1985), compared with that required in control acini. As measurement of $[Ca^{2+}]_i$ and secretory function in quin2-loaded acini may not be accurate, this was why we attempted to use a newly synthesized Ca^{2+} indicator, fura-2. Fura-2 displays a much higher fluorescence intensity than quin2 (approximately 30 times on a molecular basis) (GRYNKIEWICZ *et al.*, 1985), a property which means that the amount which must be given to adverse intracellular loading is considerably reduced and consequently $[Ca^{2+}]_i$ transients should be less buffered. In our study,

1 μM fura-2 loading for 30 min did not alter the acinar cell's viability. The resting $[Ca^{2+}]_i$ (110 ± 2 nM) was the same as that of the previous studies using quin2 (OCHS *et al.*, 1985; PANDOL *et al.*, 1985; POWERS *et al.*, 1985; BRUZZONE *et al.*, 1986), but we did find a significant rise in $[Ca^{2+}]_i$ in response to 10^{-11} M caerulein. BRUZZONE *et al.* (1986) claimed that, although fura-2-loaded acini were used, 10^{-11} M caerulein induced a significant release of amylase without causing any change in $[Ca^{2+}]_i$. It was suggested that the method of cell preparation might make the difference.

The present findings using fura-2 indicate that carbachol, caerulein, bombesin, and neuromedin B and C each stimulate a rapid increase in $[Ca^{2+}]_i$, followed by return to a sustained plateau phase near the resting level within 2 min. On the other hand, secretagogue-stimulated enzyme secretion consists of an initial burst release for several minutes, followed by a significant sustained release. When the time course of secretagogue-induced amylase release and $[Ca^{2+}]_i$ change are compared, it is noticeable that the sustained release of amylase occurs at near resting $[Ca^{2+}]_i$. Our findings indicate that the peak value of $[Ca^{2+}]_i$ correlates with the total enzyme secretion as well as with the initial release, during submaximal and maximal stimulation with any secretagogue which increases $[Ca^{2+}]_i$. When "Ca^{2+}-mobilizing" secretagogues were used, a certain rise in $[Ca^{2+}]_i$ induced a proportionate amylase release, and there was a dose relationship between the peak $[Ca^{2+}]_i$ and amylase release. It is suggested that the initial rise in $[Ca^{2+}]_i$ participates in not only initial enzyme secretion but also sustained phase of enzyme secretion, at least on condition that the extracellular Ca^{2+} exists sufficiently, although the change in $[Ca^{2+}]_i$ cannot explain the whole mechanism. Sustained secretion may be regulated by the intracellular Ca^{2+} mobilization-linked or associated mechanism.

In the presence of supramaximal concentrations of bombesin, and neuromedin B and C, both amylase release and peak $[Ca^{2+}]_i$ were comparable to those achieved with maximal concentrations. By contrast, amylease release declined in the presence of supramaximal concentrations of carbachol ($> 10^{-4}$ M) or caerulein ($> 10^{-9}$ M), while the peak $[Ca^{2+}]_i$ achieved levels comparable to those produced by maximal stimulation by carbachol and caerulein. From careful comparison of the time courses of the stimulated amylase release in the perifusion system in response to 10^{-5} and 10^{-3} M carbachol, it was clear that in response to 10^{-3} M carbachol both the first and second phases of amylase release were inhibited. By contrast, in the responses to 10^{-9} and 10^{-7} M neuromedin C, the patterns of amylase release were nearly the same. For this "secretagogue-induced unresponsiveness", i.e., the lack of relationship between enzyme secretion and $[Ca^{2+}]_i$ in the presence of supramaximal concentrations of the secretagogue, several possible explanations have been proposed: that it is associated with a different character of cell surface receptor for secretagogue (PANDOL *et al.*, 1982), an alteration in cell morphology or physiology (BURNHAM and WILLIAMS, 1982), or a result of intracellular regulatory mechanism. Our data indicate that at least it is not associated with excessively high $[Ca^{2+}]_i$ or lowered $[Ca^{2+}]_i$. If secretagogue-induced unresponsiveness may be the result of some other intracellular event that occurs in the presence of high concentrations of

agonists such as carbachol or caerulein, such a regulating or modulating mechanism of acinar secretory function is supposed to include protein kinase C, prostaglandins, and the adenylate cyclase system. It seems that this regulating mechanism is somewhat different for bombesin, and neuromedin B and C than for carbachol and caerulein.

In summary, the findings of this study demonstrate that an initial rapid increase in $[Ca^{2+}]_i$ correlates closely with enzyme secretion in the rat exocrine pancreas, although taking account only of the $[Ca^{2+}]_i$ during sustained secretion cannot elucidate the whole mechanism. Other factors, which are linked to or associated with Ca^{2+}-mobilizing mechanism, may be indispensable in regulating enzyme secretion in pancreatic acinar cells. At supramaximal stimulations, it is suggested that the intracellular regulating mechanisms are different for carbachol and caerulein, and for neuropeptides belonging to the bombesin family. However, it may be that the mobilization of cellular Ca^{2+} is one of the most important processes in stimulus-secretion coupling in pancreatic acinar cells, even if the initial rapid increase in $[Ca^{2+}]_i$ is simply one of the sequential steps of the intracellular messenger system rather than the only and absolute "second messenger."

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