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REGULATION OF AMYLASE RELEASE FROM DISPERSED PANCREATIC ACINAR CELLS

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SUMMARY

1. A study has been made of factors influencing release of amylase from dispersed pancreatic acinar cells.

2. In the basal, unstimulated, condition cells released 2–3 % of the total amylase present in 30 min.

3. The rate of amylase release was stimulated 50–70 % by C-terminal octapeptide of cholecystokinin (CCK-OP, maximally effective concentration, 3×10^{-10} M); carbamylcholine (maximally effective concentration, 10^{-5} M); secretin (maximally effective concentration $> 10^{-6}$ M); vasoactive intestinal peptide (VIP, maximally effective concentration, 10^{-8} M); and adenosine 3′:5′ monophosphate (cyclic AMP) and guanosine 3′:5′ monophosphate (cyclic GMP) as well as their dibutyryl derivatives (maximally effective concentrations, 10^{-3} M).

4. The responses to CCK-OP or carbamylcholine were potentiated by secretin, VIP or dibutyryl cyclic AMP.

5. The responses to secretin or VIP were potentiated by CCK-OP, carbamylcholine, or dibutyryl cyclic GMP.

6. There appear to be two pathways for the regulation of amylase release from pancreatic acinar cells: one pathway can be stimulated by cholecystokinin or cholinergic agonists, and the response to these stimuli is mediated by cyclic GMP; the other pathway can be stimulated by secretin or VIP, and the response to these stimuli is mediated by cyclic AMP.

INTRODUCTION

Although it is well established that cholecystokinin and muscarinic cholinergic agents are potent stimuli for secretion of pancreatic enzymes (see Preshaw, 1974), the mechanisms by which these agents exert their actions are not well understood. Some (Kulka & Sternlicht, 1968; and see

Kimberg, 1974, for review) have suggested that cyclic AMP may act as the intracellular mediator of the enzyme secretory response. Cyclic AMP or its dibutyryl derivative has been reported to stimulate pancreatic protein secretion from mouse (Kulka & Sternlicht, 1968), rabbit (Ridderstap & Bonting, 1969; Knodell, Toskes, Reber & Brooks, 1970), rat (Bauduin, Rochus, Vincent & Dumont, 1971; Heisler, Fast & Tenenhouse, 1972) and guinea-pig (Haymovits & Scheele, 1976). In addition, theophylline has been found to increase pancreatic enzyme output in mouse (Kulka & Sternlicht, 1968), rabbit (Ridderstap & Bonting, 1969; Knodell *et al.* 1970), rat (Heisler *et al.* 1972) and cat (Case & Scratcherd, 1972) and to potentiate the effect of dibutyryl cyclic AMP in rat (Bauduin *et al.* 1971) and of cholecystokinin in rabbit (Ridderstap & Bonting, 1969) and cat (Case & Scratcherd, 1972). Some investigators however, have failed to detect significant effects of exogenous cyclic AMP or of theophylline on pancreatic enzyme secretion (Benz, Eckstein, Matthews & Williams, 1972; Case & Scratcherd, 1972; Haig, 1974; Heisler & Grondin, 1975). Cyclic GMP has also received consideration as a potential mediator of the enzyme secretory response since dibutyryl or 8-bromo cyclic GMP can increase pancreatic enzyme secretion *in vitro* (Haymovits & Scheele, 1976) and cholecystokinin as well as cholinergic agents increase cyclic GMP in the pancreas *in vivo* (Robberecht, Deschodt-Lanckman, DeNeef, Borgeat & Christophe, 1974) and *in vitro* (Albano, Bhoola & Harvey, 1976; Christophe, Frandsen, Conlon, Krishna & Gardner, 1976*b*; Haymovits & Scheele, 1976). Secretin can increase cyclic AMP in the pancreas *in vivo* (Case, Johnson, Scratcherd & Sherratt, 1972; Robberecht *et al.* 1974), in pancreatic fragments (Deschodt-Lanckman, Robberecht, DeNeef Labrie & Christophe, 1975; Robberecht, Deschodt–Lanckman, DeNeef & Christophe, 1975; Deschodt–Lanckman, Robberecht, DeNeef, Lammens & Christophe, 1976). in dispersed pancreatic acinar cells (Robberecht, Conlon & Gardner, 1976; Gardner, Conlon & Adams, 1976*a*; Gardner, Conlon, Fink & Bodanszky, 1976*b*) and has also been found to increase pancreatic enzyme secretion *in vivo* (Wormsley, 1968; Dockray, 1972) and *in vitro* (Deschodt–Lanckman *et al.* 1974, 1976; Robberecht *et al.* 1975). The mechanism through which secretin increased pancreatic enzyme secretion in these previous studies is controversial since the effect may have reflected a direct action of the peptide on acinar cells or a 'washout' of previously secreted enzymes by the fluid whose secretion is increased by secretin. In the present study we have examined the effects of secretin, cholecystokinin and other agents on the release of amylase from dispersed acinar cells prepared from guinea-pig pancreas. Our results indicate that (1) there are two pathways for regulation of enzyme secretion: one is activated by cholecystokinin and cholinergic agents; the

other is activated by secretin and similar peptides, (2) both cyclic AMP and cyclic GMP play significant roles in regulating enzyme secretion and (3) potentiating interactions occur between the two regulatory pathways.

METHODS

Male Hartley strain guinea-pigs weighing 250–300 g were obtained from the National Institute of Health colony. The animals were allowed water *ad libitum* but were starved for 48 hr before experiment. Dispersed pancreatic acinar cells were prepared by a technique based on that described by Amsterdam & Jamieson (1972, 1974*a*, *b*). At the time of experiment animals were killed by a blow to the head, the pancreas was removed rapidly and dissected free of mesentery and fat. Using a fine hypodermic needle, the pancreas was injected at multiple sites with 5–6 ml. of a buffered saline solution containing collagenase 0·75 mg/ml. (Type I, Sigma Chemical Co., St Louis, Mo.), hyaluronidase 1·5 mg/ml. (Type I, Sigma Chemical Co.), and 0·1 mM calcium. All solutions were equilibrated with 100% O_2 and all incubations were gassed with 100% O_2. The pancreas was incubated for 15 min at 37° C and then drained. The tissue was covered with 8 ml. of the same saline solution without enzymes or calcium but containing 2 mM ethyleneglycol-bis (β-aminoethyl ether) *N*,*N*′-tetraacetic acid (EGTA) and incubated for 10 min at 37° C. The saline solution was removed and the tissue was washed twice with 10 ml. of buffered saline containing 0·1 mM calcium. The tissue was then incubated for 45 min at 37° C in 5 ml. of buffered saline containing 1·25 mg/ml. collagenase, hyaluronidase 2·0 mg/ml., and 0·1 mM calcium. At the end of the second digestion period, tissue and incubation medium were aspirated five times in a Pasteur pipette, and twice in a syringe bearing a 16 gauge needle. The resulting suspension was divided into two parts and each was layered onto 10 ml. of buffered saline containing 4% bovine serum albumin and 1 mM calcium. The suspension was centrifuged at 500 *g* for 5 min. The supernatant was discarded, the cells combined and resuspended in 10 ml. of the saline with 4% albumin and 1 mM calcium. This suspension was centrifuged and washed once more in the same medium. The packed cells, which usually had a volume of approximately 1 ml., were suspended in 25 ml. of buffered saline solution containing 1% albumin and 1 mM calcium.

1 ml. aliquots of cell suspension were pipetted into stoppered polypropylene tubes (internal diameter 15 mm), flushed with O_2, and placed in a shaking water bath at 37° C. After 30 min incubation the tube was removed from the bath, agitated on a vortex mixer, and an aliquot of the suspension centrifuged at 10,000 *g* for 15 sec in the Ultracentrifuge (Beckman Instrument Inc., Palo Alto, Cal.). An aliquot of supernatant was diluted in hypotonic phosphate buffer (0·01 M sodium phosphate pH 7·8 containing 0·1% (w/v) albumin and 0·1% (w/v) sodium dodecylsulphate) and stored at 4° C until assayed for amylase activity. In each series of experiments a sample of the suspension was taken at the start of the incubation, centrifuged and the supernatant collected. In addition, a sample of the suspension was lysed in the hypotonic phosphate buffer, and assayed to estimate the total amylase activity present. Results of amylase assays of supernatant were calculated as the percentage of the total amylase present in the suspension, and the rate of amylase release from the cells was taken as the difference between the activity present in the supernatant at the beginning, and after the 30 min incubation.

Amylase activity was estimated with the Phadebas Amylase Test kit (Pharmacia Laboratories Inc., Piscataway, N. J.) using a twofold dilution of the recommended substrate concentration. The presence of albumin in the hypotonic phosphate buffer used to dilute the supernatant and cell suspension was found to be important in

maintaining enzyme activity. When albumin was not present in the diluent, enzyme activity decreased rapidly, but adding 0·1% albumin to the diluent produced a linear relation between enzyme concentration and product formation over a wide range of enzyme concentrations.

In preliminary experiments there was significant variation in the rate of enzyme release from one experiment to another, and particularly in the magnitude of the increase in the rate of enzyme release caused by adding secretagogues. This variation was decreased to acceptable levels when the stimulation associated with the presence of a secretagogue was expressed as % stimulation = [100 × (stimulated release − basal release)/basal release]. In some experiments two secretagogues were added to the incubation. In these experiments we desired to determine the response to a secretagogue, *A*, in the presence of a second secretagogue, *B*. In this situation the response to *A* was calculated as the difference between the rate of enzyme release with both agents and that of B alone, divided by the rate of enzyme release in the basal, unstimulated condition, i.e. % stimulation by *A* in presence of *B* = 100 × (enzyme released with *A* plus *B* − enzyme released with *B* alone)/basal release. All results shown are means (± 1 S.E. of mean) of at least five separate experiments.

The saline solution used in the cell preparation procedure and the determination of amylase release was based on the incubation saline III described by Krebs (1950). However, it was found difficult to maintain adequate control of pH with a bicarbonate buffered system, and a HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid) buffer was substituted for the bicarbonate/CO_2 system. (Replacing bicarbonate by HEPES did not alter the rate of oxygen consumption, outflux of ^{45}Ca or cellular concentrations of cyclic AMP or cyclic GMP and did not alter the effects of secretagogues on these functions.) The composition of the saline used in the present experiments (mM) was: Na, 140; K, 8; Mg, 1; Cl, 108; HEPES, 25; pyruvate, 5; fumarate, 5; glutamate, 5; phosphate, 2; sulphate, 1. To this mixture was routinely added glucose (14 mM); soybean trypsin inhibitor (100 mg/l.) and the minimum essential amino acid and vitamin supplements described by Eagle (1959). In addition, the saline used in the studies of amylase release also contained 1% (w/v) albumin and 1 mM calcium, and 5 mM theophylline.

Natural porcine secretin, and natural porcine VIP were gifts from Professor Viktor Mutt, Gastrointestinal Hormone Research Unit, Karolinska Institutet, Stockholm, Sweden and CCK-OP was a gift from Dr Miguel A. Ondetti, Squibb Institute for Medical Research, Princeton, N.J. Three preparations of synthetic secretin were used. One was a gift from Drs V. Hruby & D. Wright (Department of Chemistry, University of Arizona, Pheonix, Arizona), one was a gift from Dr E. Wunsch (the Max Planck Institute fur Eiwiess- und Lederforschung, Munich, Germany) and one was purchased from Schwarz/Mann (Orangeburg, New York). Carbamylcholine chloride (Carbachol), N^6, O^2-dibutyryl adenosine 3′:5′ monophosphate (dibutyryl cyclic AMP) and N^2, O^2-dibutyryl guanosine 3′:5′ monophosphate (dibutyryl cyclic GMP) were purchased from Sigma Chemical Co., St Louis, Mo., U.S.A.

RESULTS

Effects of secretagogues on amylase release. Release of amylase from dispersed pancreatic acinar cells occurred at a constant rate for at least 3 hr (Fig. 1) and was significantly increased by CCK-OP, VIP, secretin and carbachol (Figs. 1 and 2). The basal rate of enzyme release was not altered by 1 mM atropine. With increasing concentrations of CCK-OP, VIP or carbachol the rate of amylase release increased, became maximal and

then decreased (Fig. 2). Because our supplies of secretin were limited it was not possible to test this peptide at concentrations above 10^{-6} M and our results suggest that 10^{-6} M was probably a submaximal concentration (Fig. 2). Results obtained with synthetic secretin were not significantly different than those obtained with natural secretin. The relative potencies (and concentrations giving maximal rates of amylase release) of the agents tested were CCK-OP (3×10^{-10} M) > VIP (10^{-8} M) > secretin (above

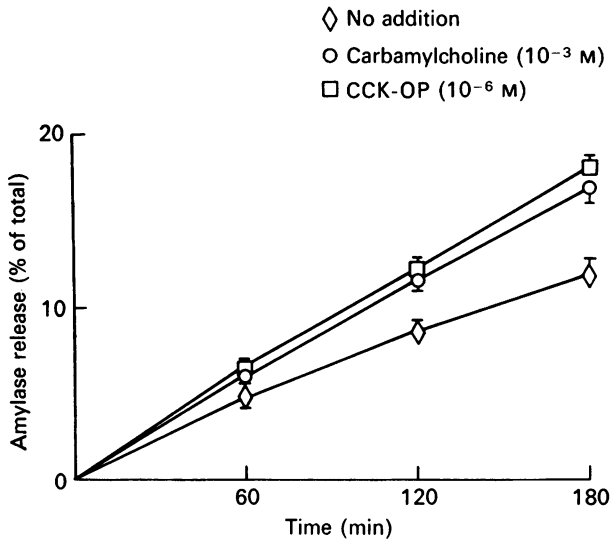

Fig. 1. Release of amylase from dispersed pancreatic acinar cells as a function of time. 1 ml. aliquots of the cell suspension were placed in stoppered tubes, flushed with O_2, and incubated at 37° C. At intervals a sample of the suspension was centrifuged, and the amylase activity in the supernatant was determined. This activity was calculated as a percentage of the total amylase present in the sample and corrected for amylase present in the supernatant at the start of the incubation.

10^{-6} M) > carbachol (10^{-4} M) (Fig. 2). The maximal rate of amylase release caused by CCK-OP was the same as that caused by carbachol and was significantly greater than that caused by VIP.

Interactions between secretagogues. With secretin or VIP the magnitude of the maximal response to CCK-OP was significantly greater than that observed with CCK-OP alone indicating that secretin and VIP potentiated the stimulation of amylase release caused by CCK-OP (Fig. 3). The magnitude of the potentiation, like the response to CCK-OP alone, depended on the concentration of CCK-OP. As the octapeptide concentration increased, the potentiation caused by secretin or VIP increased, became maximal at 3×10^{-10} M-CCK-OP and then decreased to the extent

that with 10^{-8} M-CCK-OP there was no detectable effect of secretin or VIP (Fig. 3). Carbachol did not potentiate the action of CCK-OP and the magnitude of the responses to maximal or supramaximal concentrations of CCK-OP plus carbachol were identical to those obtained with CCK-OP alone. Since in Fig. 3 the results with CCK-OP plus carbachol have had the effect of carbachol acting alone subtracted, the curve for the effect of CCK-OP in the presence of carbachol is below the curve for the

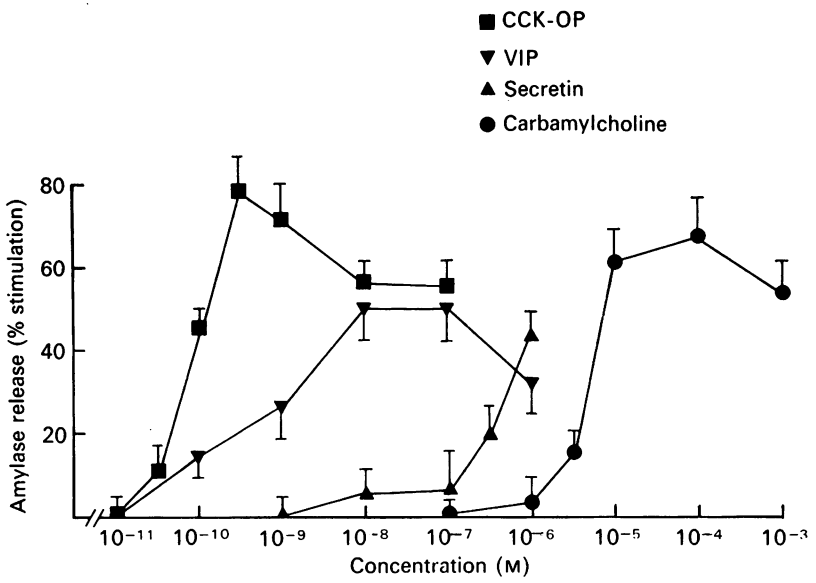

Fig. 2. Effects of CCK-OP, VIP, secretin and carbachol on amylase release from dispersed pancreatic acinar cells. 1 ml. aliquots of the cell suspension, containing the indicated concentrations of secretagogues, were incubated for 30 min at 37° C, and the increase in the activity of amylase in the supernatant was determined. In each series of experiments a sample of the suspension was incubated in the absence of added secretagogue, and this sample was used to estimate the basal, unstimulated rate of amylase release. The observed stimulation by a secretagogue was expressed as a percentage above the basal rate of amylase release in each series of experiments.

effect of CCK-OP acting alone and the difference between these two curves is attributable to the effect of the concentration of carbachol used in these experiments.

Secretin and VIP also potentiated the stimulation of amylase release caused by carbachol (Fig. 4). The magnitude of the potentiation like the effect of carbachol alone, depended on the concentration of carbachol in that as the carbachol concentration increased, the magnitude of the potentiation caused by secretin or VIP increased, became maximal and

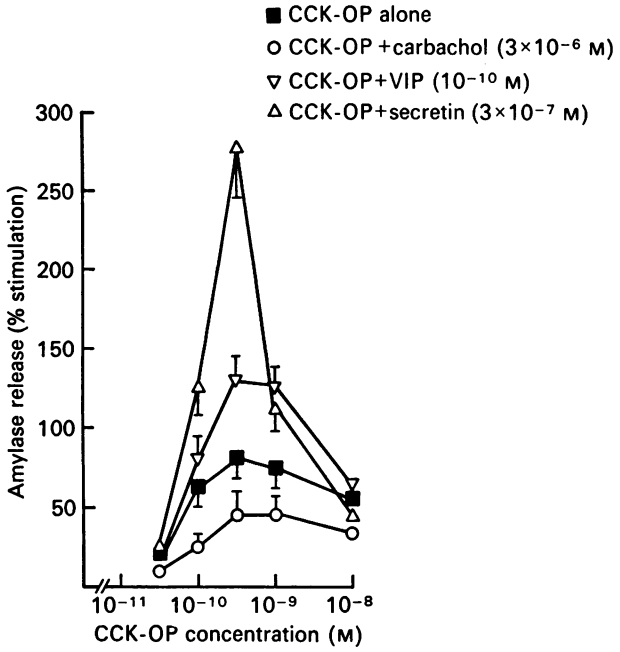

Fig. 3. Effects of secretin, VIP and carbachol on stimulation of amylase release by CCK-OP. These experiments were performed as described in the legend to Fig. 2 and effect of CCK-OP was calculated as the algebraic difference between the rate of amylase release observed with and without CCK-OP divided by the basal rate of amylase release in the absence of secretagogues.

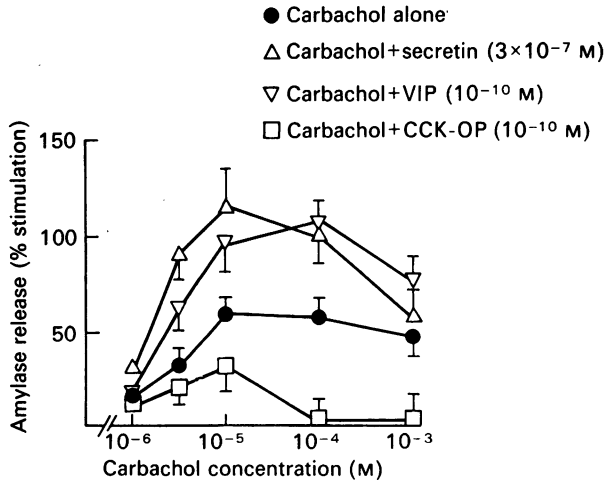

Fig. 4. Effects of secretin, VIP and CCK-OP on stimulation of amylase release by carbachol. The conditions of incubation and expression of results are generally similar to those of the experiments shown in Fig. 3.

then decreased. CCK-OP did not potentiate the effect of carbachol and since the results in Fig. 4 have been 'corrected' for the effect of CCK-OP acting alone, the curve for the effect of carbachol in the presence of CCK-OP is below the curve for the effect of carbachol acting alone.

With CCK-OP or carbachol the magnitude of the response to secretin was significantly greater than that observed with secretin alone indicating that CCK-OP and carbachol potentiated the stimulation of amylase

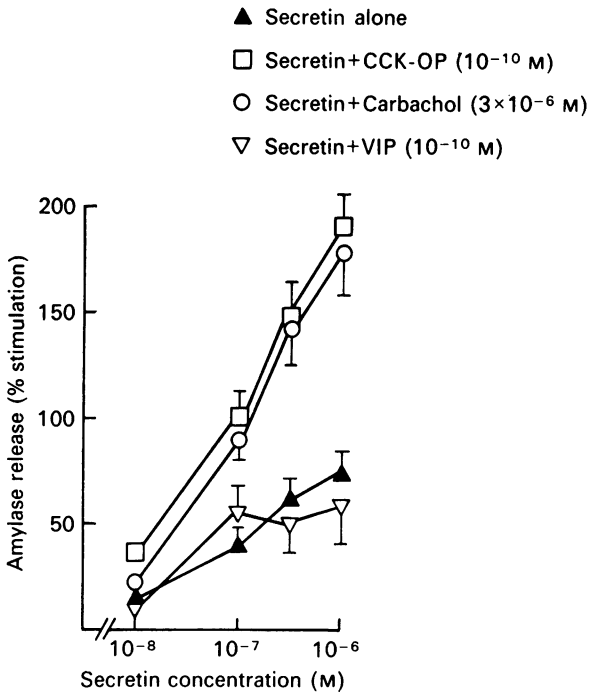

Fig. 5. Effects of VIP, carbachol and CCK-OP on stimulation of amylase release by secretin. The conditions of incubation and expression of results are generally similar to those of the experiments shown in Fig. 3.

release caused by secretin (Fig. 5). The magnitude of the potentiation, like the response to secretin alone, showed a progressive increase with increasing concentrations of secretin. We were unable to demonstrate a maximal response to secretin in our system and the effects of secretin in the presence of VIP were not significantly different from those of secretin alone indicating additive effects of VIP and secretin.

CCK-OP and carbachol also potentiated the stimulation of amylase release caused by VIP (Fig. 6). The magnitude of the potentiation, like the effect of VIP alone, depended on the concentration of VIP in that

as the VIP concentration increased, the magnitude of the potentiation caused by CCK-OP or carbachol increased, became maximal and then decreased. Secretin did not potentiate the action of VIP and the responses to maximal or supermaximal concentrations of VIP plus secretin were identical to those obtained with VIP alone. Since in Fig. 6 the results with VIP plus secretin have been 'corrected' for the effect of secretin acting alone, the curve for the effect of VIP in the presence of secretin falls below the curve for the effect of VIP acting alone and the difference between the two curves is attributable to the effect of the concentration of secretin used in these experiments.

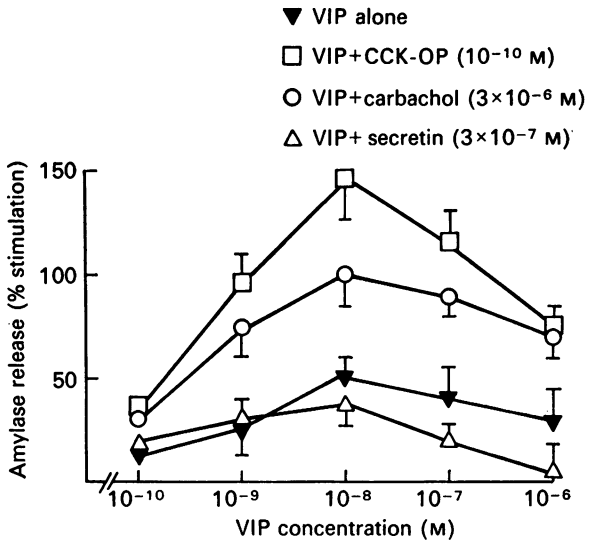

Fig. 6. Effects of secretin, carbachol and CCK-OP on stimulation of amylase release by VIP. The conditions of incubation and expression of results are generally similar to those of the experiments shown in Fig. 3.

Effects of exogenous cyclic nucleotides on amylase release. In preliminary experiments the rate of enzyme release from dispersed pancreatic acinar cells was significantly increased by cyclic AMP, cyclic GMP, as well as by their dibutyryl derivatives. However, the effects of the nucleotides were variable in that the magnitude of the responses varied substantially from one experiment to another and in some instances no response was observed. We found that the reproducibility of effects of exogenous cyclic nucleotides was improved considerably by increasing the period of exposure to EDTA from 10 to 20 min. Although this modification increased the basal rate of amylase release, the cells retained their sensitivity to peptides as well as to carbachol and responded consistently to cyclic

nucleotides. We assume that the basis for this improvement was increased permeability of the cells to exogenous cyclic nucleotides. The magnitudes of the maximal responses to dibutyryl derivatives of cyclic AMP and cyclic GMP were usually greater than those to native cyclic nucleotides. Dibutyryl cyclic AMP (0·1 mM) and dibutyryl cyclic GMP (0·1 mM) each caused a small, but significant increase in the rate of amylase release from dispersed pancreatic acinar cells (Fig. 7). When

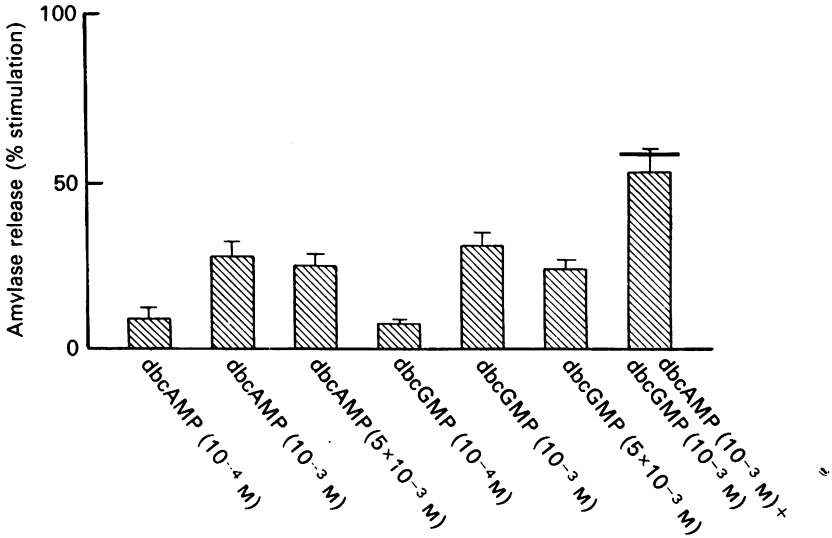

Fig. 7. Effects of dibutyryl cyclic GMP and dibutyryl cyclic AMP on amylase release. The conditions of incubation and expression of results are generally similar to those of the experiments shown in Fig. 3. The solid bar above the column at the right of the Figure indicates the sum of the effect of 10^{-3} M dibutyryl cyclic AMP and 10^{-3} M dibutyryl cyclic GMP acting separately.

present at 1 mM each cyclic nucleotide caused a 20–30 % increase in the rate of amylase release and this effect was not altered by increasing the nucleotide concentration to 5 mM. The increase in amylase release caused by both cyclic nucleotides together was significantly greater than the maximal effect of either alone but was not significantly greater than the sum of their independent actions indicating that the effects of the two nucleotides were additive (Fig. 7).

The increase in amylase release caused by secretin (10^{-6} M) or VIP (10^{-8} M) was not altered by dibutyryl cyclic AMP (Fig. 8). In contrast, the effect of CCK-OP (3×10^{-10} M) or carbachol (10^{-4} M) plus dibutyryl cyclic AMP was significantly greater than the sum of the effect of the secretagogue and the nucleotide alone (Fig. 8). In an analagous series of

experiments the increase in amylase release caused by CCK-OP (3×10^{-10} M) or carbachol (10^{-4} M) was not altered by dibutyryl cyclic GMP (Fig. 9). On the other hand, the effect of secretin (10^{-6} M) or VIP (10^{-8} M) plus dibutyryl cyclic GMP was significantly greater than the sum of the effect of the peptide and the nucleotide alone (Fig. 9).

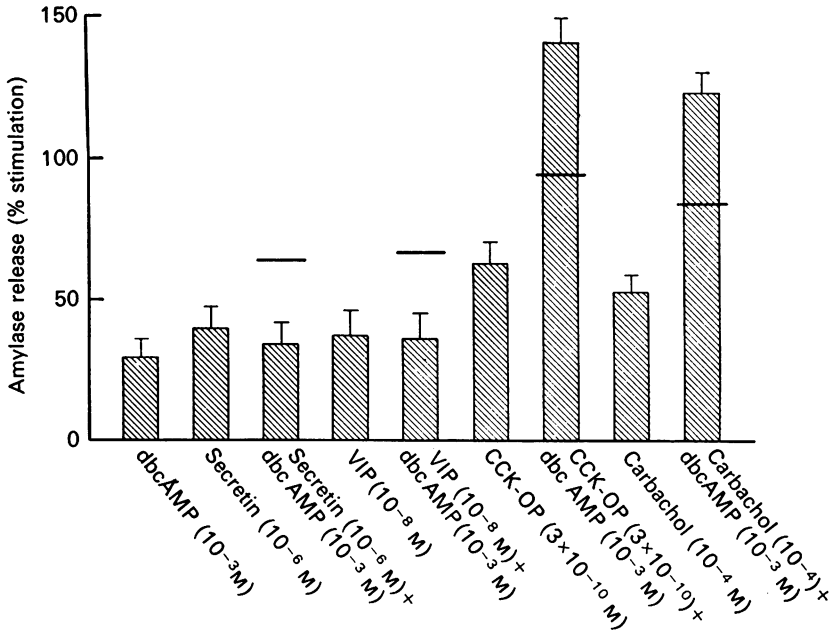

Fig. 8. Interactions between dibutyryl cyclic AMP and secretin, VIP, CCK-OP and carbachol. The conditions of incubation and expression of results are generally similar to those of the experiments shown in Fig. 3. The solid bars shown on some columns indicate the sums of the effect of 10^{-3} M dibutyryl cyclic AMP and a second secretagogue acting separately.

DISCUSSION

Others have reported that secretin can increase pancreatic enzyme output *in vivo* (Wormsley, 1968; Dockray, 1972) as well as *in vitro* (Deschodt–Lanckman *et al.* 1975); however, since secretin also stimulates pancreatic fluid secretion these results could have resulted from a 'washout' of previously secreted enzymes by secretin-stimulated flow of fluid through the duct system (Case & Scratcherd, 1972). Our present results indicate a direct action of secretin on acinar cells and cannot be attributed to a 'washout' phenomenon. VIP is a naturally occurring octacosapeptide which is similar in structure and spectrum of biologic activities to secretin (Said & Mutt, 1972; Bodanszky, 1974). In the present studies stimulation

of amylase output from pancreatic acinar cells caused by VIP was similar to that caused by secretin in that the effect of these two peptides were potentiated by CCK-OP, carbachol and dibutyryl cyclic GMP but not by dibutyryl cyclic AMP. VIP, however, was significantly more potent than secretin in stimulating amylase release.

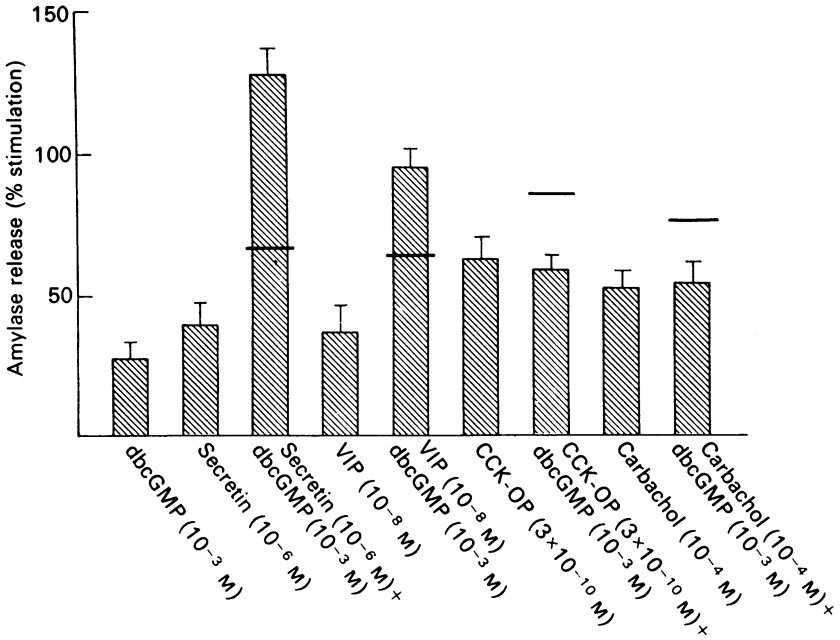

Fig. 9. Interactions between dibutyryl cyclic GMP and secretin, VIP, CCK-OP and carbachol. The conditions of incubation and expression of results are generally similar to those of the experiments shown in Fig. 3. The solid bars shown on some columns indicate the sums of the effect of 10^{-3} M dibutyryl cyclic GMP and a second secretagogue acting separately.

Previous studies (Christophe, Conlon & Gardner, 1976*a*; Christophe *et al.* 1976*b*; Gardner *et al.* 1976*a*, *b*) indicate that in pancreatic acinar cells (1) secretin and VIP increase cyclic AMP while cholecystokinin (as well as synthetic fragments and analogs) and cholinergic agents increase cyclic GMP, (2) VIP and secretin do not alter cellular concentrations of cyclic GMP, and (3) cholecystokinin and cholinergic agents do not alter cellular concentrations of cyclic AMP or the increase in cyclic AMP caused by secretin or VIP. In view of these previous observations our present findings are consistent with the hypothesis that in pancreatic acinar cells the effects of CCK-OP and carbachol on enzyme release are mediated by cyclic GMP while the effects of secretin and VIP on enzyme release are mediated by cyclic AMP.

In pancreatic acinar cells cholecystokinin and cholinergic agents increase cellular cyclic GMP as a consequence of their ability to mobilize and cause release of membrane-bound, exchangeable calcium (Gardner, Conlon, Klaeveman, Adams & Ondetti, 1975; Shelby, Gross, Lichty & Gardner, 1976). The dose–response curves for stimulation of amylase release by CCK-OP and carbachol obtained in the present studies are similar to the dose response curves for stimulation of calcium release and cellular cyclic GMP by CCK-OP and carbachol obtained previously (Gardner *et al.* 1975; Christophe *et al.* 1976*b*; Shelby *et al.* 1976). Our findings that exogenous dibutyryl cyclic GMP increases amylase release under conditions in which the nucleotide does not alter calcium outflux (Christophe *et al.* 1976*b*) indicate that cyclic GMP is the proximate mediator of cholecystokinin- or carbachol-stimulated enzyme release, and that stimulation of calcium outflux occupies an earlier position in the excitation-secretion coupling sequence.

The dose–response curves for stimulation of amylase release by secretin and VIP obtained in the present studies differ from the dose–response curves for the effects of these two peptides on cyclic AMP in pancreatic acinar cells obtained previously (Robberecht *et al.* 1976; Christophe *et al.* 1976*a*). These earlier studies indicate that pancreatic acinar cells possess two classes of receptors with different affinities for secretin and VIP. One class has a high affinity for secretin and a low affinity for VIP; the other class has a low affinity for secretin and a high affinity for VIP. The present results are consistent with the possibility that stimulation of amylase release by secretin and VIP is mediated by the receptor having a high affinity for VIP and a low affinity for secretin. The role of the other class of receptors in acinar cell function remains to be determined.

Previous studies of pancreatic secretion *in vivo* (Brown *et al.* 1967; Hendrickson & Worning, 1967; Way & Grossman, 1970; Grossman, 1971; Meyer *et al.* 1971; Folsch & Wormsley, 1973) and *in vitro* (Deschodt–Lanckman *et al.* 1975) have shown potentiating interactions between secretin and cholecystokinin in that the effect of the two peptides in combination was greater than the sum of the effect of each agent alone. In the present studies potentiation was observed with the combination of any agent which increases cellular cyclic AMP (VIP or secretin) plus any agent which increases cyclic GMP (CCK or carbachol) but not with the combination of two agents each of which increases the same cyclic nucleotide (i.e. VIP plus secretin or CCK-OP plus carbachol). Potentiation was also observed with the combination of any agent (e.g. CCK-OP) which increases cellular concentration of one cyclic nucleotide (e.g. cyclic GMP) and the dibutyryl derivative of the other cyclic nucleotide (e.g. dibutyryl cyclic AMP) but not with the dibutyryl derivative of the same cyclic

nucleotide (e.g. dibutyryl cyclic GMP). The stimulation of amylase secretion obtained with dibutyryl cyclic AMP plus dibutyryl cyclic GMP was equal to the sum of the effect of each nucleotide alone. This lack of potentiation may be attributable to a difference between endogenous and exogenous cyclic nucleotides, to a difference between native and dibutyryl derivatives of cyclic nucleotides or to the nucleotide concentrations used for these experiments. Evidence which suggests that our failure to detect potentiation with the two cyclic nucleotides may be attributable to our having tested them at maximally effective concentrations (1 mM) is our finding that with two peptides which show potentiation, the magnitude of the potentiation can be reduced by sufficiently increasing the concentration of one peptide (for example see Fig. 3). Thus, it may be that potentiation only occurs with submaximal concentrations of cyclic nucleotides.

Although the present results do not elucidate the biochemical basis for potentiation of amylase release from pancreatic acinar cells they do suggest that there are two parallel pathways for stimulation of enzyme release. One pathway can be activated by secretin or VIP and is mediated by cyclic AMP. The other pathway can be activated by cholecystokinin or mucarinic cholinergic agents and is mediated by cyclic GMP. The pattern of potentiation suggests that these two pathways can interact at some step distal to the generation of cyclic nucleotides and this interaction results in multiplication of the effect of each nucleotide on enzyme release.

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