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THE EFFECT OF DIBUTYRYL CYCLIC ADENOSINE-3′,5′-MONOPHOSPHATE ON PROTEIN SECRETION FROM THE RAT EXOCRINE PANCREAS *in vitro*

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1 The mechanism by which dibutyryl cyclic adenosine-3′-5′-monophosphate (dibutyryl cyclic AMP) potentiates the secretory effect of carbachol in rat exocrine pancreas was investigated.

2 Dibutyryl cyclic AMP potentiated the secretory effect of carbachol only at carbachol concentrations $\gtrsim 10^{-7}$ mol/l; was independent of carbachol at concentrations $> 10^{-7}$ mol/l and was inversely proportional to extracellular $[Ca^{2+}]$.

3 Carbachol increased and dibutyryl cyclic AMP reduced the rate of ^{45}Ca efflux from the tissue.

4 A-23187 stimulated ^{3}H-protein release in the presence of Ca^{2+} and this effect was potentiated by dibutyryl cyclic AMP; the degree of potentiation was inversely proportional to extracellular $[Ca^{2+}]$. At 10^{-3} mol/l $[Ca^{2+}]$ the potentiation occurred only at ionophore concentrations $\lesssim 10^{-6}$ mol/litre.

5 These results support the hypothesis that dibutyryl cyclic AMP potentiates the effect of secretagogues in rat exocrine pancreas by maintaining an elevated intracellular calcium concentraion. It does so by inhibiting Ca^{2+} efflux. The results also suggest that the limiting factor in carbachol-stimulated secretion, at all concentrations of carbachol, is intracellular $[Ca^{2+}]$.

Introduction

The precise mechanism of hormone-stimulated enzyme secretion from the exocrine pancreas is not yet known but there is little doubt that a critically important element in this process is calcium (Hokin, 1966; Robberecht & Christophe, 1971; Heisler, Fast & Tenenhouse, 1972; Case & Scratcherd, 1972). The most convincing recent evidence is the demonstration by Eimerl, Savion, Heichel & Selinger (1974) and Williams & Lee (1974) that ionophore A-23187, in the presence of calcium, stimulates amylase secretion from the rat exocrine pancreas *in vitro*. The involvement of cyclic nucleotides in this process is still controversial, however the bulk of evidence suggests that cyclic adenosine-3′,5′-monophosphate (cyclic AMP) is not involved in either acetylcholine or pancreozymin-stimulated enzyme secretion (Benz, Eckstein, Matthews & Williams, 1972; Case & Scratcherd, 1972; Heisler *et al.*, 1972). It has been suggested that cyclic GMP mediates the effects of acetylcholine in some tissues (Goldberg, O'Dea & Haddox, 1973) including the exocrine pancreas (Robberecht, Deschodt-Lanckman, De Neef, Borgeat & Christophe, 1974) but the evidence that this nucleotide plays a role in secretion from this tissue is preliminary.

Heisler *et al.* (1972) reported that dibutyryl cyclic AMP was itself a poor secretagogue in the rat exocrine pancreas but that it potentiated the secretory effect of carbachol. This effect was seen at carbachol concentrations that were optimum for secretion and was dependent on extracellular calcium. To explain these observations we proposed that activation of secretion requires an elevation of cytoplasmic ionized calcium. This has been suggested by many investigators for a number of secretory systems (Douglas, 1968; Rasmussen & Tenenhouse, 1968; Rasmussen, 1970; Rasmussen, Goodman & Tenenhouse, 1972) and is now widely accepted. In the case of cholinomimetic secretagogues in the exocrine pancreas the rate limiting factor at optimum concentration of secretagogue is the concentration of cytoplasmic ionized calcium. Dibutyryl cyclic AMP potentiates the activity of these secretagogues by conserving cytoplasmic Ca^{2+}, probably by inhibiting those processes which remove Ca^{2+} from the cytoplasm. The experiments described in this paper were designed to test this hypothesis.

Methods

Female Wistar rats weighing 225 to 250 g were anaesthetized with ether and killed by decapitation.

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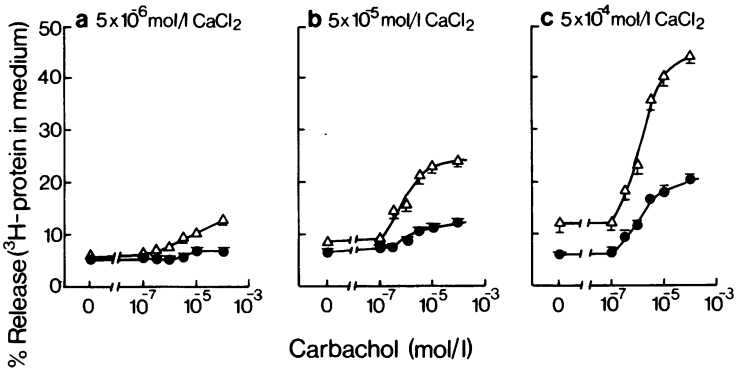

Figure 1 The effect of varying the concentration of carbachol on tritiated protein release from rat pancreas pieces in the presence (△) or absence (●) of 10^{-3} mol/l dibutyryl cyclic AMP. EGTA (5×10^{-5} mol/l) plus (a) 5×10^{-6} mol/l Ca^{2+}, (b) 5×10^{-5} mol/l Ca^{2+} or (c) 5×10^{-4} mol/l Ca^{2+} was present in the Krebs-Ringer bicarbonate buffer as described in the text. Each point is the mean of at least five experimental values; standard errors are indicated by the vertical lines.

The pancreases were quickly removed and placed in ice-cold Krebs-Ringer bicarbonate buffer of the following composition (mM): NaCl 118.5, KCl 4.75, $MgSO_4$ 1.2, KH_2PO_4 1.2, and $NaHCO_3$ 25.0. Calcium ($CaCl_2$) and EGTA (ethylene glycol bis (β-aminoethyl ether)-N, N'-tetraacetic acid) were added as indicated in the results section. The buffer also contained 10 mM glucose and L-amino acids (with the exception of leucine) in concentrations recommended by Eagle (1959).

The tissue was trimmed free of adherent fat and connective tissue in chilled (ice-cold) Krebs-Ringer bicarbonate buffer and minced with scissors into 5 to 15 mg pieces. The proteins of pancreatic zymogen granules were labelled with [^{3}H]-leucine as described by Jamieson & Palade (1967) and the release of radioactive proteins from the pancreas measured as described previously (Heisler *et al.*, 1972).

For investigations of ^{45}Ca efflux, the pancreatic pieces were first incubated for 90 min in Krebs-Ringer bicarbonate buffer containing 0.5 μCi/ml ^{45}Ca and sufficient non-radioactive $CaCl_2$ to make the final calcium concentration 10^{-4} mol/litre. The tissue and medium were filtered through cheesecloth and the tissue washed with Krebs-Ringer bicarbonate buffer. The pancreas pieces were divided into 180 to 200 mg portions; these were transferred to flasks of buffered medium containing 10^{-4} mol/l non-radioactive Ca^{2+}, 7.5×10^{-5} mol/l EGTA, and the stimulating agent(s) to be tested in a final volume of 3 ml. Aliquots (10 μl) of medium were removed at various times and the radioactivity determined in a liquid scintillation counter. At the end of the incubation the tissue was solubilized in NCS and an aliquot counted to determine the tissue content of ^{45}Ca. Results are expressed as the percentage of total tissue ^{45}Ca released into the medium.

Each experiment was done at least three times. Results are given for typical experiments or as the mean of several (at least five) experiments plus or minus the standard error. The significance of differences was determined using Student's test. The ionophore A-23187 was a gift of Dr R.L. Hamill, Lilly Research Laboratories, Lilly & Co., Indianapolis, Indiana, U.S.A.

Results

The effect of varying extracellular calcium concentration on tritiated protein secretion from the pancreas was studied at various carbachol concentrations, using carbachol alone or in combination with dibutyryl cyclic AMP 10^{-3} mol/litre. Based on the results of previous experiments (Heisler *et al.*, 1972) three concentrations of calcium were chosen for these experiments: 5×10^{-6} mol/l Ca^{2+}, at which concentration carbachol alone did not significantly stimulate secretion; 5×10^{-5} mol/l, the lowest Ca^{2+} concentration at which carbachol-dependent secretion could be detected; 5×10^{-4} mol/l, at which concentration ^{3}H-protein release caused by carbachol was near maximum. The results of these experiments, shown in Figure 1, give the per cent of total ^{3}H-protein released into the medium by the tissue as a function of the carbachol concentration.

At concentrations of carbachol less than 10^{-7} mol/l there was no stimulation of radioactive protein release at any of the calcium concentrations tested. In combination with dibutyryl cyclic AMP 10^{-3} mol/l, carbachol 10^{-7} mol/l or less did not increase ^{3}H-protein release above that caused by the cyclic nucleotide alone; i.e., there was no effect of carbachol plus dibutyryl cyclic AMP until the carbachol con-

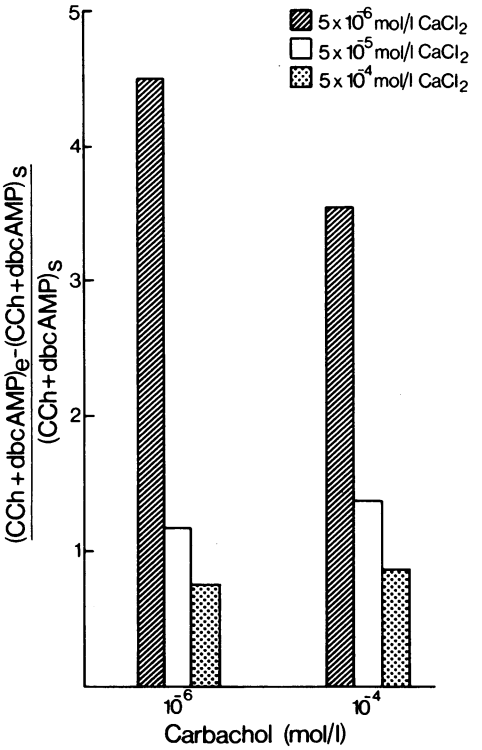

Figure 2 The degree of potentiation of carbachol (CCh)-stimulated ^{3}H-protein release from rat pancreas by dibutyryl cyclic AMP (dbc AMP) at three [Ca^{2+}], 5×10^{-6}, 5×10^{-5} and 5×10^{-4} mol/litre.

centration was greater than 10^{-7} mol/litre. Carbachol (5×10^{-7} mol/l) stimulated ^{3}H-protein release at 5×10^{-5} mol/l Ca^{2+} to 7.3% ($P < 0.05$ compared to non-stimulated release) and at 5×10^{-4} mol/l Ca^{2+} to 9.6% ($P < 0.01$). Tritiated protein release caused by any of the concentrations of carbachol used in the absence of dibutyryl cyclic AMP was not significantly different from non-stimulated release at 5×10^{-6} mol/l Ca^{2+}. Also at 5×10^{-7} mol/l carbachol and at all three concentrations of calcium tested, the stimulatory effect (% total ^{3}H-protein minus % total ^{3}H-protein released in control) of carbachol plus dibutyryl cyclic AMP was greater than the sum of the stimulatory effects of the individual agents. At 5×10^{-5} mol/l Ca^{2+}, for example, the stimulatory effect of 5×10^{-7} mol/l carbachol was 0.8%, that of 10^{-3} mol/l dibutyryl cyclic AMP was 2.0% and the stimulatory effect of carbachol plus dibutyryl cyclic AMP was 8.0%.

Figure 2 illustrates the degree of potentiation of carbachol-dependent ^{3}H-protein secretion caused by dibutyryl cyclic AMP 10^{-3} mol/litre. This value was calculated from the results given in Figure 1 to

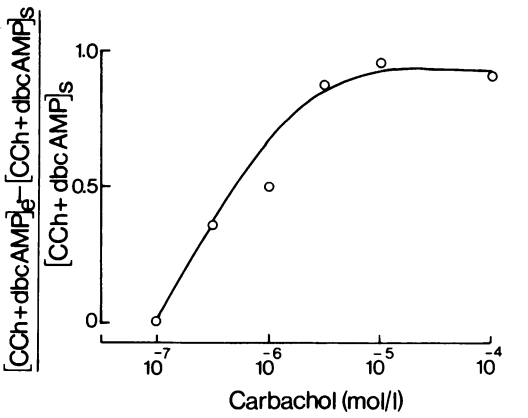

Figure 3 The degree of potentiation of carbachol (CCh)-stimulated ^{3}H-protein release from rat exocrine pancreas by dibutyryl cyclic AMP (dbc AMP, 10^{-3} mol/l) as a function of carbachol concentration, [Ca^{2+}], 10^{-3} mol/litre.

determine whether the degree of potentiation was a function of the concentration of carbachol and/or calcium. The value $(CCh + dbcAMP)_e$, the experimental value for ^{3}H-protein release caused by carbachol plus dibutyryl cyclic AMP, was the per cent of ^{3}H-protein release caused by carbachol plus dibutyryl cyclic AMP minus ^{3}H-protein release with no added stimulus, i.e. the stimulatory effect of carbachol plus dibutyryl cyclic AMP. The theoretical value for secretion caused by carbachol and dibutyryl cyclic AMP (the sum of their individual effect), $(CCh + dbcAMP)_s$, was calculated by adding the stimulatory effect of carbachol alone to that of dibutyryl cyclic AMP alone. The degree of potentiation was calculated from the formula:

$$(CCh + dbc\ AMP)_e - (CCh + dbc\ AMP)_s \,/\, (CCh + dbc\ AMP)_s$$

The degree of potentiation was calculated at 10^{-6} and 10^{-4} mol/l carbachol and at 5×10^{-6}, 5×10^{-5} and 5×10^{-4} mol/l calcium. At 5×10^{-6} mol/l Ca^{2+} the degree of potentiation was 4.5 and 3.5 for 10^{-6} mol/l and 10^{-4} mol/l carbachol respectively; at 5×10^{-5} mol/l Ca^{2+} it was 1.2 and 1.4 for these concentrations of carbachol; at 5×10^{-4} mol/l Ca^{2+} it was 0.5 and 0.9.

In Figure 3 the degree of potentiation, as a function of carbachol concentration is illustrated. In these experiments the extracellular Ca^{2+} and the dibutyryl cyclic AMP concentrations were 10^{-3} mol/l and the carbachol concentration varied between 10^{-7} and 10^{-4} mol/litre. As can be seen the degree of potentiation which is zero at 10^{-7} mol/l carbachol, increased to 0.88 as the carbachol concentration

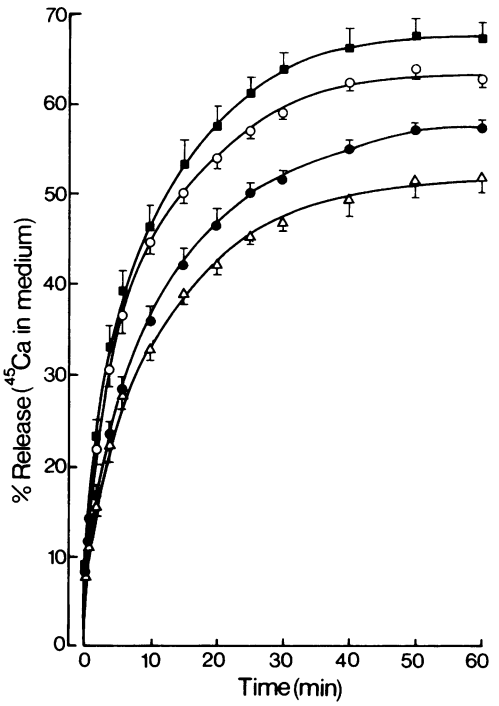

Figure 4 The effect of 10^{-3} mol/l dibutyryl cyclic AMP (△), 10^{-5} mol/l carbachol (■) or the combination of carbachol plus dibutyryl cyclic AMP (○) on the rate of ^{45}Ca efflux from rat pancreas pieces (control = ●). Each point is the mean of at least five separate experimental values; standard errors are indicated by the vertical lines. Details are given in the text.

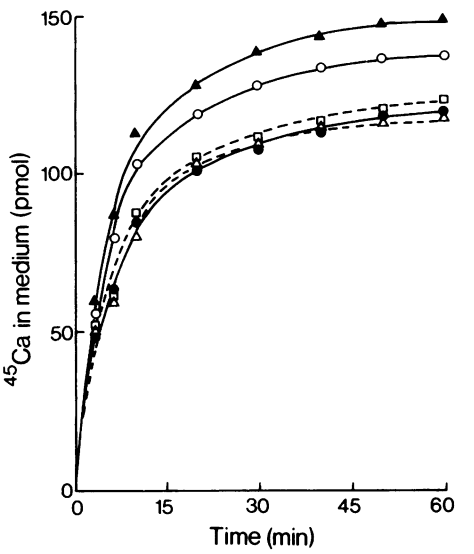

Figure 5 The effect of atropine on carbachol, or carbachol plus dibutyryl cyclic AMP, stimulated ^{45}Ca efflux from rat pancreas pieces. (●) Control; (▲) carbachol 10^{-5} mol/l; (○) carbachol + dibutyryl cyclic AMP 10^{-3} mol/l; (△) carbachol + atropine 10^{-6} mol/l; (□) carbachol + dibutyryl cyclic AMP + atropine.

increased to 5×10^{-5} mol/litre. Further increase in carbachol concentration had no effect on the degree of potentiation.

The rates of uptake and release of ^{45}Ca from pancreatic cells was measured to evaluate the role of calcium transport in protein secretion stimulated by carbachol or dibutyryl cyclic AMP. No difference in the rate of radioactive calcium uptake was observed when carbachol (10^{-5} mol/l), dibutyryl cyclic AMP (10^{-3} mol/l) or a combination of the two agents was added to the medium (unpublished observation).

Efflux of ^{45}Ca from prelabelled tissue was measured as described in the Methods section, and the results of these experiments are shown in Figure 4.

Carbachol alone or a combination of carbachol plus dibutyryl cyclic AMP caused a significant increase in ^{45}Ca efflux ($P < 0.05$ compared to control values) as early as 2 min after the addition of the stimulus. At 2 min ^{45}Ca efflux caused by carbachol was 23.0% and efflux caused by carbachol plus dibutyryl cyclic AMP was 21.8%; control efflux was 16.8%. Dibutyryl cyclic AMP alone decreased the amount of ^{45}Ca released into the medium. This effect of dibutyryl cyclic AMP became significant ($P < 0.05$) after 25 min of stimulation, when efflux caused by dibutyryl cyclic AMP was 45.4% and control efflux was 50.0%. Although radioactive calcium efflux caused by carbachol plus dibutyryl cyclic AMP was always less than that with carbachol alone, this difference was not statistically significant if each time point was regarded as a separate entity. However the probability that twelve points for ^{45}Ca efflux caused by carbachol plus dibutyryl cyclic AMP would be less than thirteen points for ^{45}Ca efflux with carbachol alone due entirely to chance, was less than 0.002 using Binomial Theorem. The combination of carbachol plus dibutyryl cyclic AMP, therefore, was having a significantly different effect from carbachol alone on the efflux of ^{45}Ca. Radioactive calcium efflux reached a plateau after 40 min for both control and stimulated efflux. Efflux at 40 min was 55.1% of total ^{45}Ca for the control, 49.5% for dibutyryl cyclic AMP, 66.0% for carbachol and 62.4% for carbachol plus dibutyryl cyclic AMP. Figure 5 shows that the effects of carbachol and carbachol plus dibutyryl cyclic AMP on ^{45}Ca efflux are abolished by atropine (10^{-6} mol/litre).

The ionophore A-23187 is believed to insert itself into cell membranes and act as a freely mobile carrier to equilibrate the concentration of divalent cations,

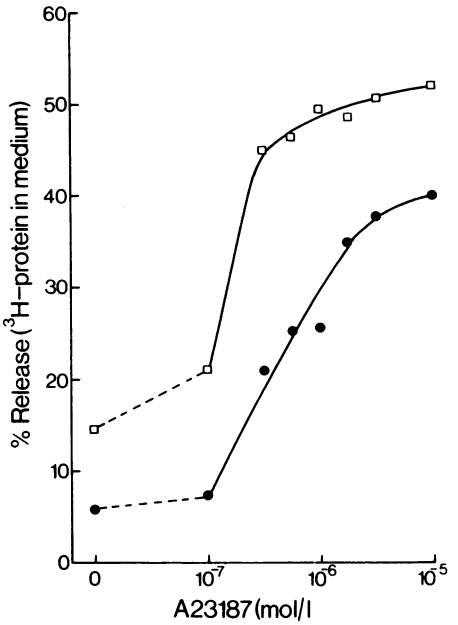

Figure 6 The effect of A-23187 on the release of ^{3}H-labelled protein from rat exocrine pancreas in the absence (●) and presence (□) of dibutyryl cyclic AMP (10^{-3} mol/litre).

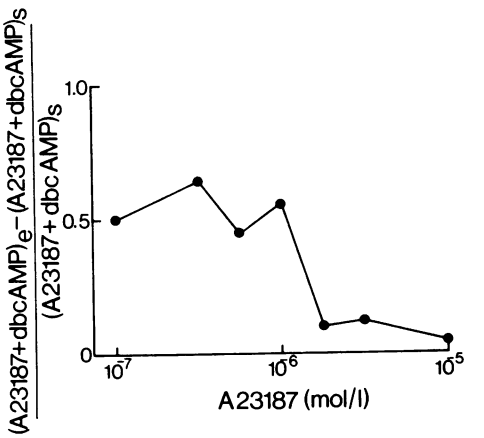

Figure 7 The degree of potentiation of A-23187-stimulated ^{3}H-protein release from rat pancreas by dibutyryl cyclic AMP (dbcAMP) as a function of A-23187 concentration in presence of [Ca^{2+}] 10^{-3} mol/litre.

particularly Ca^{2+}, across these membranes (Reed & Lardy, 1972). The effects of A-23187 on protein secretion from the exocrine pancreas was studied; results are shown in Figure 6. EGTA (5×10^{-5} mol/l) was present during both the 60 min chase and the 60 min incubation with the stimulus.

As the ionophore is insoluble in water, it was dissolved in 100% ethanol and diluted 1:100 with incubation buffer. Ethanol (1%) did not affect control release of ^{3}H-protein nor radioactive protein release caused by 10^{-3} mol/l dibutyryl cyclic AMP alone. Protein release caused by A-23187 or A-23187 plus dibutyryl cyclic AMP was maximal at a calcium concentration of 10^{-3} mol/l (see Figure 8). At this concentration of calcium, the stimulatory effect (% total ^{3}H-protein released minus % total ^{3}H-protein released in control) of 5×10^{-7} mol/l A-23187 was 8% and that of 5×10^{-6} mol/l A-23187 was 21%. The stimulatory effect of dibutyryl cyclic AMP plus 5×10^{-7} mol/l A-23187 was 25% at 10^{-3} mol/l Ca^{2+}; that of dibutyryl cyclic AMP plus 5×10^{-6} mol/l A-23187 was 35%. The stimulatory effect of dibutyryl cyclic AMP alone at 10^{-3} mol/l Ca^{2+} was 8%. Thus the experimental value for the stimulatory effect of dibutyryl cyclic AMP plus either concentration of A-23187 was approximately 1.5 times the sum of the individual stimulatory effects of the two agents, i.e. dibutyryl cyclic AMP potentiated the secretory effect of A-23187. The degree of potentiation as a function of A-23187 concentration was calculated and the results illustrated in Figure 7. In contrast to that seen when carbachol was the secretagogue (Figure 3) the degree of potentiation of A-23187 dependent secretion by dibutyryl cyclic AMP was maximal and constant (approximately 0.5) at low secretagogue concentrations (10^{-7} to 10^{-6} mol/l) and decreased rapidly to a minimal value of 0.045 as the concentration of A-23187 was increased from 10^{-6} to 10^{-5} mol/litre.

The effect of A-23187 on ^{3}H-protein release from pancreas pieces as a function of Ca^{2+} concentration is illustrated in Figure 8. When extracellular Ca^{2+} concentration was less than 10^{-4} mol/l A-23187 (5×10^{-7} or 5×10^{-6} mol/l) had no effect on ^{3}H-protein release. As the extracellular Ca^{2+} concentration increased A-23187-dependent release of ^{3}H-protein increased to a maximum at 10^{-3} mol/l Ca^{2+}. This concentration of Ca^{2+} was optimum for both concentrations of A-23187 tested (5×10^{-7} and 5×10^{-6} mol/litre). The potentiation of A-23187-dependent protein release by dibutyryl cyclic AMP was also Ca^{2+}-dependent and optimum at 10^{-3} mol/l Ca^{2+}. Using the data in Figure 8 the degree of potentiation was calculated to determine whether it was a function of the concentration of A-23187 and/or calcium. The results are illustrated in Figure 9. In contrast to carbachol (see Figure 2) the degree of potentiation of A-23187-dependent protein release decreased when the concentration of the ionophore was increased; this occurred at 5×10^{-4} or 10^{-3} mol/l Ca^{2+}. The degree of potentiation was also inversely related to the Ca^{2+}

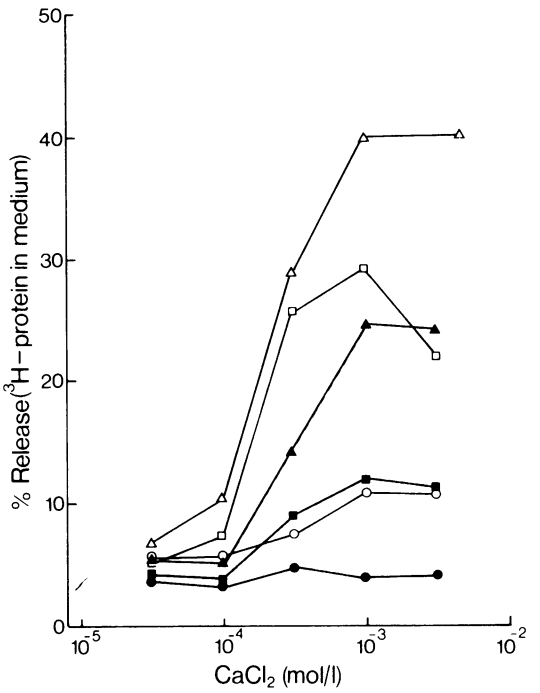

Figure 8 The effect of A-23187 5×10^{-7} or 5×10^{-6} mol/l, in the presence or absence of dibutyryl cyclic AMP 10^{-3} mol/l, on ^{3}H-labelled protein release from rat pancreas pieces as a function of the concentration of extracellular calcium. EGTA (5×10^{-5} mol/l) and the desired concentration of calcium were present in the Krebs-Ringer bicarbonate buffer as indicated. The data are from one representative experiment: (●) 1% ethanol; (○) 1% ethanol + dibutyryl cyclic AMP 10^{-3} mol/l; (■) A-23187, 5×10^{-7} mol/l; (□) A-23187, 5×10^{-7} mol/l + dibutyryl cyclic AMP; (▲) A-23187, 5×10^{-6} mol/l; (△) A-23187, 5×10^{-6} mol/l + dibutyryl cyclic AMP.

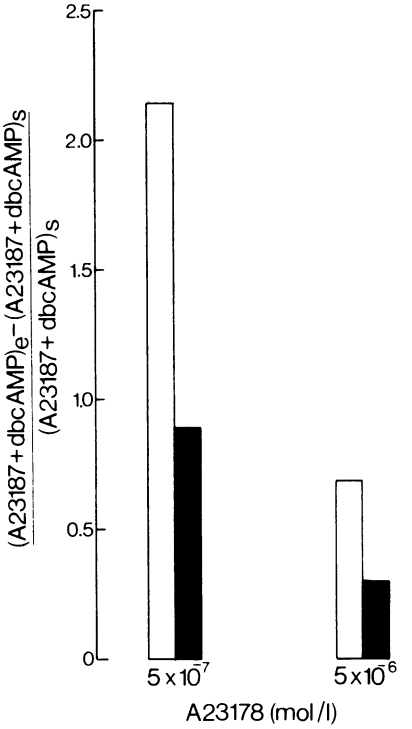

Figure 9 The degree of potentiation of A-23187-stimulated ^{3}H-protein release from rat pancreas by dibutyryl cyclic AMP (dbcAMP) at two [Ca^{2+}], 5×10^{-4} mol/l (open columns) and 10^{-3} mol/l (closed columns).

concentration, i.e. as the Ca^{2+} concentration was increased the degree of potentiation decreased.

Discussion

The studies described in this paper were designed to elucidate the mechanism by which dibutyryl cyclic AMP potentiates the secretory effect of carbachol and specifically to test the hypothesis that dibutyryl cyclic AMP acts by maintaining intracellular calcium concentration at a level which is optimum for secretion. When protein secretion was stimulated by carbachol the absolute amount of radioactive protein released depended on the concentration of extracellular calcium as well as on the concentrations of carbachol and dibutyryl cyclic AMP. The degree of potentiation of the carbachol effect by dibutyryl cyclic AMP however, was independent of the concentration of carbachol at carbachol concentrations greater than 10^{-7} mol/l; that is, once the carbachol concentration was sufficient to stimulate secretion on its own the degree of potentiation depended only on the concentration of extracellular calcium and was inversely related to it. This relationship is predicted from the proposal that the limiting factor in carbachol stimulated secretion at all concentrations of carbachol is the cytoplasmic [Ca^{2+}] and that dibutyryl cyclic AMP acts by conserving and/or mobilizing intracellular Ca^{2+}.

Further evidence that dibutyryl cyclic AMP was acting by conserving intracellular Ca^{2+} was obtained from the investigation of ^{45}Ca flux between the tissue and incubation medium. Increased calcium influx into the acinar cell does not appear to be a major component of enzyme secretion initiated by cholinomimetic drugs (Matthews, Petersen & Williams,

1973), a conclusion which is supported by our results.

In our studies it was found that enzyme secretion from rat exocrine pancreas caused by carbachol or carbachol plus dibutyryl cyclic AMP was associated with an increased ^{45}Ca efflux. Similar results have been reported by others (Case & Clausen, 1973; Heisler & Grondin, 1973; Matthews *et al.*, 1973). Although ^{45}Ca efflux and enzyme secretion were stimulated in a similar dose-dependent fashion by carbachol, radioactive calcium efflux was not directly related to protein secretion; the greatest amount of protein release occurred with carbachol plus dibutyryl cyclic AMP but the largest efflux of ^{45}Ca was caused by carbachol alone. This suggests that the zymogen granules are not the major or sole source of this Ca^{2+} and thus implicates some other intracellular pool. These experiments also suggest that cholinomimetic drugs are able to mobilize Ca^{2+} from some intracellular pool(s).

Dibutyryl cyclic AMP plus carbachol decreased the rate of ^{45}Ca efflux to less than that caused by carbachol alone, and dibutyryl cyclic AMP alone decreased the rate of ^{45}Ca efflux to less than control values. Dibutyryl cyclic AMP is thus able to conserve intracellular Ca^{2+}, and this action is independent of activation of the secretory process by carbachol.

Although the source of the increased intracellular calcium has not been firmly established for any secretory system, Borle (1973) showed that in many cells a likely source is the mitochondria. Because of the number of mitochondria and the rapid rate of movement of Ca^{2+} across the mitochondrial membrane, this organelle can quickly increase or decrease the concentration of cytoplasmic ionized Ca^{2+} in amounts which are believed necessary for physiological processes. Borle (1974) has also suggested that cyclic nucleotides may directly control uptake and release of Ca^{2+} by mitochondria in some cells, thereby controlling the Ca^{2+} concentration in the cytoplasm. In our experiments dibutyryl cyclic AMP cannot be only mobilizing Ca^{2+} from intracellular stores since this would result in an increased rate of ^{45}Ca efflux from the cell, whereas a decrease in ^{45}Ca efflux is actually observed. The primary action of dibutyryl cyclic AMP, therefore, must be to maintain cytoplasmic ionized calcium by inhibiting efflux of Ca^{2+} from the cytoplasm to the extracellular space and perhaps inhibiting 'efflux' from the cytoplasm into storage organelles. This suggests that the inability of dibutyryl cyclic AMP itself to stimulate secretion is due to the fact that this nucleotide does not promote Ca^{2+} mobilization from intracellular stores but can only maintain an elevated cytoplasmic Ca^{2+} concentration caused by some other mechanism.

This conclusion leads to the prediction that in any situation where the limiting factor in secretion is the cytoplasmic calcium concentration and where the secretagogue does not act by inhibiting those processes which remove calcium from the cytoplasm, dibutyryl cyclic AMP should potentiate the effect of the secretagogue. This prediction was tested with the ionophore A-23187 as the secretagogue. Reed & Lardy (1972) showed that this compound inserted itself into the mitochondrial membrane and acted as a carrier of divalent cations in the direction of the concentration gradient. Eimerl *et al.* (1974) have shown that this ionophore causes the release of amylase from exocrine pancreas and that this effect is dependent on extracellular $[Ca^{2+}]$. If the action of the ionophore is specific then one must conclude that simply increasing the intracellular concentration of calcium is sufficient to initiate the process of amylase secretion. The concentration of intracellular calcium is determined by the difference between the rate of movement of calcium into the cell and the rate of movement of calcium out of the cell. In the presence of A-23187 calcium influx is essentially a function of $[\text{A-23187}] \times [Ca^{2+}]_o$, and calcium efflux is the sum of $[\text{A-23187}] \times [Ca^{2+}]_i$ plus the contribution of the physiological calcium efflux processes. When the concentrations of ionophore and calcium are small the contribution of the physiological mechanisms of calcium extrusion ('Calcium pump') are maximized and therefore any agent which inhibits this pump would contribute maximally to maintaining a greater intracellular calcium concentration. The contribution of this 'pump' is minimized as the ionophore concentration increases and as the extracellular concentration of calcium is increased. It should be noted that the Ca^{2+} 'pump' referred to here may be at the plasma membrane and/or at the membrane of any intracellular organelle. The only assumption is that it pumps Ca^{2+} out of the cytoplasm. One would therefore predict that if dibutyryl cyclic AMP potentiates the action of secretagogues by inhibiting this Ca^{2+} 'pump' it would maximally potentiate the effect of A-23187 at low ionophore and/or low extracellular $[Ca^{2+}]$ concentration and that the degree of potentiation would vary inversely as the concentration of ionophore and Ca^{2+}. This in fact is what was found: the potentiating effect of dibutyryl cyclic AMP is maximal at low ionophore and low extracellular Ca^{2+} concentrations and almost totally disappears at a concentration of ionophore of 5×10^{-6} mol/l and extracellular $[Ca^{2+}]$ of 10^{-3} mol/litre. These results are convincing support for the argument that in the rat exocrine pancreas an increase in cellular calcium is sufficient to initiate the secretion process and that dibutyryl cyclic AMP acts by inhibiting calcium efflux from the cytoplasm thus potentiating the action of secretagogues.

One striking difference between the interaction of dibutyryl cyclic AMP and ionophore is that at the optimum extracellular calcium concentration dibutyryl cyclic AMP continues to potentiate the effect of carbachol even at carbachol concentrations which are $10 \times$ optimum whereas the dibutyryl cyclic

AMP potentiation of the A-23187 effect is almost nil at optimum [A-23187]. This suggests that under the influence of carbachol the maximum rate of accumulation of calcium into the cytoplasm is never sufficient to achieve a calcium concentration optimal for protein secretion, i.e. the 'cytoplasmic' calcium concentration is always the limiting factor in secretion when carbachol is the secretagogue.

It is tempting to postulate that cyclic AMP, which accumulates in various tissues in response to hormone stimulation, functions to maintain cytoplasmic calcium concentrations by a mechanism similar to that proposed for the dibutyryl derivative. However, because of the increasing number of reports of the divergent effects of endogenous cyclic AMP and exogenously administered analogues of that nucleotide such a conclusion is premature.

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