

EFFECT OF CYTOCHALASIN B AND COLCHICINE ON α -AMYLASE RELEASE FROM RAT PAROTID TISSUE SLICES

Dependence of the Effect on N⁶, O^{2'}-Dibutyryl Adenosine 3', 5'-Cyclic Monophosphate Concentration

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INTRODUCTION

We have recently reported (3) that cytochalasin B, which disrupts microfilaments, and colchicine, which disrupts microtubules, interfered with the release of α -amylase from rat parotid tissue slices. Our observations on the effect of cytochalasin B on α -amylase release have been confirmed by Rifkin et al. (14). Inhibitory actions of cytochalasin B or colchicine in several other systems have also been reported (5, 11, 18, 19, 22).

In contrast to the inhibitory action of either cytochalasin B or colchicine, these agents have also been shown to exert a stimulatory action in some systems. For example, Orzi et al. (12) have shown that cytochalasin B enhanced the effect of glucose on insulin release from isolated pancreatic islets over the release observed with glucose alone. Similarly, Temple et al. (18) have observed that colchicine enhanced steroid production by an adrenal tumor cell line.

Although the varied effects of cytochalasin B and colchicine might be a function of the system under study, they might also be a function of the compound, or the concentration of the compound used to alter the rate of a given process. In this paper we report the results from a study of this latter possibility.

MATERIALS AND METHODS

Parotid glands from a minimum of two female Sprague-Dawley rats, 8-10 wk old, were obtained and prepared at 37°C according to the procedure of Babad et al. (1). Slices were incubated in stoppered, 1-oz plastic bottles with a total volume of 2.0 ml of

Krebs-Ringer bicarbonate buffer and 95% O₂-5% CO₂ as the gas phase. The length of the incubation periods is described in the legend to Table I. Cytochalasin B was dissolved in dimethylsulfoxide (DMSO). When we used light-sensitive compounds the studies were carried out in plastic bottles covered with foil. At the end of the incubation periods the tissue slices were separated from the buffer by filtering through nylon gauze fitted over the tops of the plastic bottles. Tissue slices were homogenized in 0.02 M potassium phosphate-6.7 mM NaCl, pH 6.9. The α -amylase of the tissue homogenates and incubation buffer was assayed by the procedure of Bernfeld (2). All secretion data are listed as the percent of the total α -amylase released into the incubation buffer. All data are expressed as the average with the indicated range from individual paired experiments which have been repeated at least three times.

Colchicine was obtained from the Sigma Chemical Co., St. Louis, Mo. Cytochalasin B was purchased from Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, N. Y. and N⁶,O^{2'}-dibutyryl adenosine 3',5'-cyclic monophosphate (db-c-AMP) was obtained from Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y. Colcemid was a gift from the Geigy Pharmaceuticals, Div. Ciba-Geigy Corporation, Ardsley, N. Y. Sprague-Dawley rats were obtained from the Charles River Breeding Laboratories, Inc., Wilmington, Mass. DMSO was a product of MC&B Manufacturing Chemists, Norwood, Ohio.

RESULTS

Fig. 1 shows that cytochalasin B (52 μ M) exerted an effect on the release of α -amylase that was dependent on the concentration of db-c-AMP. At

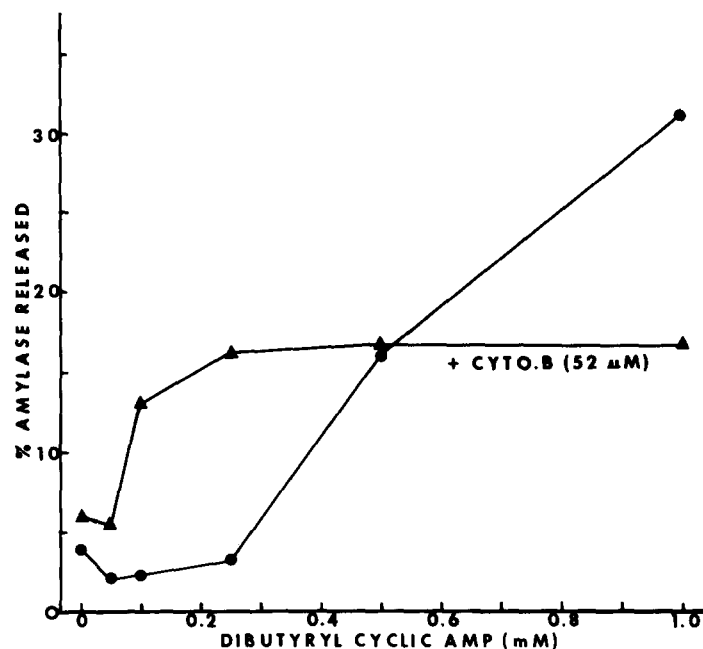


FIGURE 1 The effect of cytochalasin B on the stimulation of α -amylase release by various concentrations of db-c-AMP. Tissue slices from three rats were incubated for 1 h in the presence (triangles) or absence (circles) of cytochalasin B (cyto. B), after which time db-c-AMP was added for an additional 90 min.

1.0 mM db-c-AMP, cytochalasin B reduced the amount of α -amylase released by one-half. However, cytochalasin B was without effect at 0.5 mM db-c-AMP even though this concentration of db-c-AMP alone stimulated α -amylase release. At lower concentrations of db-c-AMP, which had little or no effect on α -amylase release, cytochalasin B enhanced α -amylase release. Thus, depending on the concentration of db-c-AMP, cytochalasin B inhibited, was without apparent effect, or potentiated the release of α -amylase.

We also obtained results similar to those above with agents that disrupt the structural integrity of the microtubule system. From Fig. 2 it can be seen that colchicine potentiated α -amylase release at 0.1 and 0.5 mM db-c-AMP, but was without effect at higher db-c-AMP concentrations in contrast to the results presented in Fig. 1 for cytochalasin B.

Since the effects of cytochalasin B could represent some type of irreversible cytotoxic effect, we conducted studies on the reversibility of the effect of cytochalasin B as shown in Table I. Although both the synergistic and inhibitory effects of cytochalasin B were reversible, this does not establish that these effects were the result of an interaction

of cytochalasin B with the microfilaments. It does, however, rule out enhanced release of α -amylase as a result of a permanent cytotoxic effect. In addition, if cytochalasin B enhanced α -amylase release at low db-c-AMP concentrations by a cytotoxic mechanism, cytochalasin B alone should elevate basal α -amylase release by a corresponding amount; but it does not.

Kletzien et al. have shown that cytochalasin B is a potent antagonist of sugar transport (9). In an attempt to gain insight into this latter possibility, we have conducted studies in the absence of glucose and other oxidizable substrates and have obtained the same effect of cytochalasin B given above (not shown).

It is known that the release of α -amylase is strictly dependent upon a continuing energy supply (1). Although no information on the effect of cytochalasin B on ATP levels in the parotid is available, Warner and Perdue (20) reported that cytochalasin B did not alter ATP levels in chick fibroblasts.

DISCUSSION

This is the first report of an effect of either cytochalasin B or colchicine that is a function of the

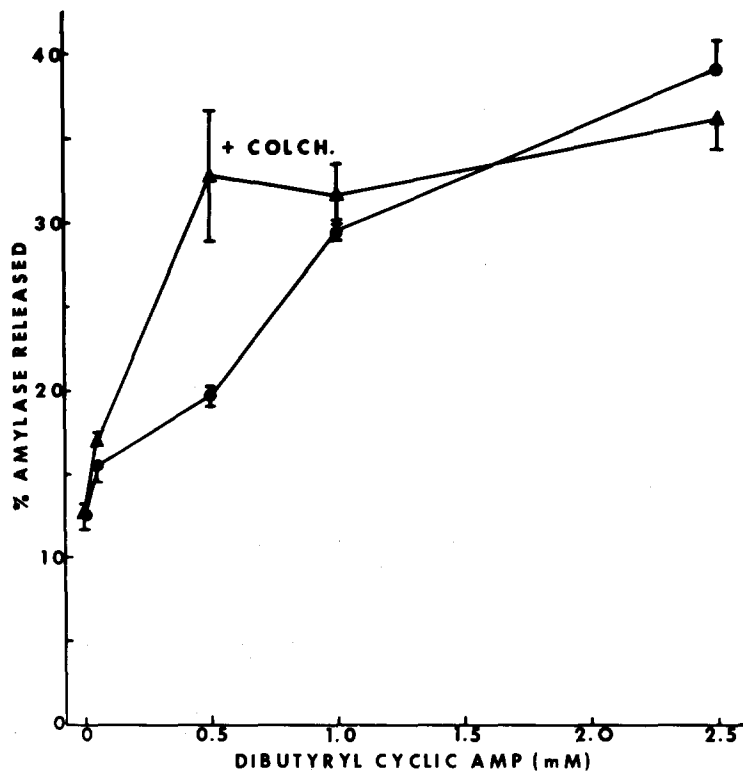


FIGURE 2 The effect of colchicine on the stimulation of α -amylase release by various concentrations of db-c-AMP. Tissue slices from three rats were incubated for 4 h in the presence (triangles) or absence (circles) of colchicine (colch.), after which time db-c-AMP was added for an additional 90 min.

concentration of the effector compound used to alter the rate of the process under investigation. It has been noted previously by others (13) that db-c-AMP can reverse the changes in cell morphology induced by either Colcemid or cytochalasin. Kirkland and Burton (8) have also noted that cyclic AMP appeared to stabilize the microtubules to dissociation induced by low temperatures. Butcher and Perdue (4) have previously reported that cytochalasin B did not inhibit the induction of tyrosine aminotransferase by db-c-AMP while cytochalasin B did inhibit the induction of tyrosine aminotransferase in the same system by either insulin or cortisol. Thus, the present findings along with the others cited above give indirect evidence for some type of interaction between cyclic AMP and the microtubular and microfilament networks. Such an interaction might involve protein phosphorylation by a cyclic AMP-dependent protein kinase in a manner analogous to activation of glycogen phosphorylase kinase (10). This hypothesis gains support from the recent observations of Goodman et al. (7) who noted that tubulin iso-

lated from bovine brain could serve as a substrate for protein kinase. In this regard it is of interest that Schramm et al. (16) have reported that secretory granules incubated with cyclic AMP and a protein kinase formed a significantly greater number of pseudopodia than the control secretory granules. It is possible that the formation of pseudopodia represents a mechanism involving phosphorylation of a protein of either the microfilament or microtubule protein. This would imply that the microtubule and microfilament systems were present in secretory granules as well as the cytoplasm of the parotid acinar cell.

An additional factor that might influence the interaction of cyclic AMP with the microtubular and microfilament systems is calcium. It is known that calcium is required for the normal functioning of a number of secretory systems (15) including secretion of α -amylase from the rat parotid gland (17). A direct effect of calcium on microtubule function is also suggested by the observation that calcium inhibits the *in vitro* assembly of microtubule subunits (21). It has also been observed that calcium

TABLE I
Reversal of the Effect of Cytochalasin B on the Stimulation of α -Amylase Release by db-c-AMP

First additions*	Second additions	Percent α -amylase released
		%
Cytochalasin B 52 μ M	None	4.8 \pm 1.7 \ddagger
“	db-c-AMP 0.25 mM	9.6 \pm 1.3
“	1.0 mM	29.8 \pm 2.1
“	Cyto. B 52 μ M	6.2 \pm 0.3
“	+ db-c-AMP 0.25 mM	15.3 \pm 2.9
“	1.0 mM	17.5 \pm 0.1
None	None	5.4 \pm 0.9
“	db-c-AMP 0.25 mM	8.4 \pm 0.5
“	1.0 mM	33.1 \pm 1.6
“	Cyto. B 52 μ M	5.9 \pm 1.2
“	+ db-c-AMP 0.25 mM	16.8 \pm 0.9
“	1.0 mM	17.3 \pm 1.0

* Tissue slices from four rats were divided into two equal portions, each incubated separately either in the presence or in the absence of 52 μ M cytochalasin B for 1 h. After this time the two lots of tissue slices were washed twice with 10 ml of Krebs-Ringer bicarbonate buffer and then added to plastic bottles for the second incubation period. Cytochalasin B was added for either the first or second time as indicated, and after 1 h the appropriate concentrations of db-c-AMP were added. After an additional 30-min period the percent of α -amylase released was determined.

\ddagger Average of duplicate incubations from one experiment with the indicated range.

concentration exerts an effect on the intracellular macromolecular binding of colchicine (6).

In order to account for the present findings it is possible that the low concentrations of db-c-AMP are sufficient to promote reassembly of the microfilament or microtubule subunits. The cytoplasmic activity caused by subunit reassembly might cause the movement of the secretory granules to the surface of the cells where they fuse with the plasma membrane and spill their contents into the lumen. This assumes that a rate-determining step in α -amylase release is transport of secretory granules to the cell surface.

Thus a stabilizing effect of db-c-AMP on microfilament or microtubule structure could account for the enhancement of α -amylase release at low levels of db-c-AMP. As the concentration of db-c-AMP is increased other factors, such as a possible effect on intracellular calcium distribution, might prevent the positive effects of db-c-AMP on microfilament or microtubule subunit reassembly, resulting in inhibition of α -amylase release. Consequently, the inhibitory effect of cytochalasin B on the stimulation of α -amylase by high concentrations of db-c-AMP (1.0 mM) in Fig. 1 may represent a disequilibrium in favor of subunit disaggre-

gation whereas the effect observed at low concentrations represents a disequilibrium in favor of subunit assembly. The point of no inhibition of α -amylase (Fig. 1) then would represent an equilibrium between these two opposing rates.

SUMMARY

The effect of cytochalasin B (52 μ M) and colchicine (100 μ M) on the stimulation of α -amylase release by db-c-AMP from rat parotid tissue slices was a function of db-c-AMP concentration. Cytochalasin B inhibited α -amylase, but colchicine was not inhibitory. At submaximal concentrations of db-c-AMP, neither cytochalasin B nor colchicine inhibited α -amylase release. In the presence of concentrations of db-c-AMP that alone had little or no effect on α -amylase release, cytochalasin B and colchicine enhanced the release of α -amylase above that observed with db-c-AMP alone.

This investigation was supported by the American Cancer Society grant IN 45-L and Biological Sciences Support Grant.

Received for publication 6 August 1973, and in revised form 16 October 1973.

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