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

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Summary

A role for microfilaments and microtubules in the secretion of α-amylase is indicated since cytochalasin B and colchicine inhibited the stimulation of α-amylase release by epinephrine (30 or 15 μM) but only cytochalasin B inhibited the stimulation by N^6, $O^{2'}$ dibutyryl adenosine 3',5'monophosphate (1.0 mM). It was necessary to incubate the parotid tissue slices in the presence of cytochalasin B (1 hr.) or colchicine (4 hrs.) before adding the agonist in order to see the inhibitory effects.

Introduction

Lacy proposed that microtubules and microfilaments played an important role in the secretion of insulin (1). In support of this hypothesis Malaisse et al (2) reported that agents, such as colchicine or vinblastine, which interrupt the structural integrity of the microtubular proteins also inhibited the stimulation of insulin release from the β cells by glucose. Similar inhibitory effects of colchicine on the stimulation of thyroid secretion by thyroid stimulating hormone (TSH) and dibutyryl cyclic AMP have been reported by Williams and Wolff (3).

The involvement of microfilaments in the secretory process can be evaluated with cytochalasin B (Cyto. B) an agent which is thought to specifically interfere with the function of the microfilaments (4). Thus Williams and Wolff (5) have shown that

cytochalasin B inhibited the stimulation of thyroid secretion in response to TSH. The in vitro stimulation of growth hormone release by certain agents was also inhibited by cyto. B. In contrast, Orci et al (6) have reported that cyto. B enhanced the release of insulin from β cells in response to glucose. Similar to the latter findings, but with colchicine instead of cyto. B, Temple et al (7) have reported that steroid secretion in an adrenal tumor cell line was enhanced by low concentrations of colchicine to an extent that exceeded the response to the hormone ACTH. In yet another aspect of the effects of these agents on secretion it has been reported that neither cyto. B nor colchicine have an effect on the release of immunoglobulins (8). Thus it would appear that the secretory processes in various tissues exhibit different responses to the agents which interact with the microfilaments or microtubules and these effects must be defined for each tissue. Since the effect of these agents on the secretion of α-amylase from rat parotid tissue has not been defined, the following studies were conducted.

Methods and Materials

Parotid glands from a minimum of two female Sprague-Dawley rats, 8-10 weeks old, were obtained and prepared at 37° according to the procedure of Babad et al (9). Slices were incubated in stoppered, one ounce plastic bottles with a total volume of 2.0 ml of Krebs-Ringer bicarbonate buffer and 95% O_2-5% CO_2 as the gas phase. The length of the incubation periods are described in the legends to the tables. 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

Table 1

The Effect of Cytochalasin B on the Stimulation of α-Amylase Release by Epinephrine and Dibutyryl Cyclic AMP

Additions	Concentration	Per Cent α-Amylase Released: Control	Per Cent α-Amylase Released: Epinephrine 15 μM
None	-----	5.1±0.5	37.1±2.1
Cytochalasin B	13 μM	4.9±0.3	23.9±2.4
	52 μM	5.2±0.3	19.2±0.5
	104 μM	4.6±0.1	18.5±1.1
			Dibutyryl Cyclic AMP 1.0 mM
None	-----	4.4±0.8	33.3±2.7
Cytochalasin B	52 μM	5.0±0.3	18.1±2.3

Cytochalasin in DMSO or DMSO, 10 μl (0.5% v/v final concentration), was added to the appropriate bottles and the tissue slices incubated for 1 hour at 37° before adding the agonists. DMSO alone had no effect on basal or stimulated α-amylase release when tested in concentrations from 0.25% to 1.0% (v/v) (not shown). Tissue slices were separated from the incubation buffer 15 minutes after adding epinephrine and 30 minutes after adding dibutyryl cyclic AMP. The data for the epinephrine and dibutyryl cyclic AMP portions of the table are from two separate experiments.

α-amylase of the tissue homogenates and incubation buffer was assayed by the procedure of Bernfeld (10). All secretion data are listed as the per cent 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.

Epinephrine bitartrate and colchicine were obtained from the Sigma Chemical Company. Cytochalasin B was purchased from Gallard Schlesinger Company and N^6, $O^{2'}$ Dibutyryl adenosine 3',5' mono-

phosphate (DBC) was from Schwarz-Mann Biochemicals. Colcemid was a gift from the Ciba-Geigy Corporation. Sprague-Dawley rats were obtained from the Charles River breeding farms. Dimethyl sulfoxide (DMSO) was a product of Matheson, Coleman and Bell.

Results and Discussion

Table 1 shows that cyto. B at three different concentrations produced a significant inhibition of the release of α-amylase in response to epinephrine. These results are similar to the effects of cyto. B on TSH induced thyroid secretion (5). However the thyroid was more sensitive to cyto. B since treatment with 3 µg of cyto. B/ml resulted in complete inhibition of TSH induced secretion (5) and in the present study 50 µg of cyto. B/ml (104 µM) inhibited the effects of epinephrine by only 50 per cent. Similar to the effect of epinephrine, the stimulatory action of dibutyryl cyclic AMP was also inhibited (Table 1). Since cyclic AMP is thought to mediate the actions of epinephrine on the parotid gland (11) this would suggest, but not prove, that cyto. B is exerting its effect as a result of an action on the microfilaments and not at the membrane level by inhibiting the binding of epinephrine or activation of adenyl cyclase.

Table 2 demonstrates that colchicine and colcemid inhibited the action of epinephrine on α-amylase release. This is in contrast to a recent paper by Temple *et al* (7) which reported that vinblastine but not colchicine inhibited the stimulation of α-amylase release by epinephrine. A major difference between our protocols could account for this discrepancy since we incubated the parotid tissue slices for 4 hours in the presence of colcemid or colchicine before adding epinephrine as opposed to the two hour period used in the work of Temple *et al* (7). In other unpublished experiments it was observed that 1.0 mM colcemid gave a greater

Table 2

The Effect of Colchicine and Colcemid on the Stimulation of α-Amylase Release by Epinephrine and Dibutyryl Cyclic AMP

Additions	Concentration	Per Cent α-Amylase Released: Control	Per Cent α-Amylase Released: Epinephrine 15 μM
None	-----	7.8±0.5	36.0±1.0
Colchicine	10 μM	11.0±1.6	29.2±1.8
	100 μM	13.5±1.8	19.6±2.5
Colcemid	100 μM	14.2±1.6	29.7±0.1
			Dibutyryl Cyclic AMP 1.0 mM
None	-----	13.3±1.1	30.3±0.8
Colchicine	100 μM	14.4±0.2	28.6±0.4

Tissue slices were incubated in the presence or absence of colchicine and colcemid for 4 hours before adding epinephrine of dibutyryl cyclic AMP. Colchicine was dissolved in Krebs-Ringer bicarbonate buffer and colcemid in dimethyl sulfoxide. After the 4-hour period the indicated flasks were incubated with epinephrine for 15 minutes or dibutyryl cyclic AMP for 30 minutes. The data for the epinephrine and dibutyryl cyclic AMP portions of the table are from separate experiments.

inhibition of the stimulation of α-amylase release by epinephrine than did 0.1 mM colcemid as shown in table 2.

It was pointed out by Temple et al (7) that colchicine was taken up to a significant extent during the 2 hour pre-incubation period. Thus it would appear that the colchicine was taken up during the first 2 hours but had not yet disrupted the structure of the microtubules. Prolonged exposure to colchicine has been used in other studies in order to observe increased effects on secretion (2,3).

It can also be seen from table 2 that 100 μM colchicine had no effect on the stimulation of α-amylase release by dibutyryl cyclic AMP; while under the same conditions it did inhibit the action of epinephrine. Results of unpublished experiments have shown, under the same conditions used in table 2, that 100 μM vincristine also inhibited the action of epinephrine on α-amylase release but not that of dibutyryl cyclic AMP. Experiments are in progress to determine if either colchicine or vincristine interfere with the action of epinephrine on the intracellular levels of cyclic AMP or on adenyl cyclase directly.

It can be concluded on the basis of the information presented in these studies that agents such as cytochalasin B and colchicine which disrupt microfilaments and microtubules, respectively, also appear to interfere with the secretion of α-amylase from parotid tissue.

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