THE FUNCTION OF 3'5' CYCLIC AMP IN ENZYME SECRETION[1]

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Studies in this laboratory have established that epinephrin is a potent inducer of enzyme secretion in rat parotid slices (Bdolah et al., 1964; Schramm et al., 1965). The work of Sutherland and others clearly indicates that 3'5' cyclic AMP is a primary intracellular intermediate in the action of epinephrin in several tissues (Rall and Sutherland, 1961). It was therefore of interest to find out whether cyclic AMP mediates enzyme secretion in parotid gland preparations.

When 3'5' cyclic AMP, up to a concentration of 9 mM, was substituted for epinephrin, the slices failed to show significant amylase secretion (Table 1). Many tissues are apparently impermeable to the nucleotide (Rall and Sutherland, 1961) and another experimental approach to the problem was therefore adopted.

It has been shown that theophyllin at relatively high concentrations inhibits the diesterase responsible for the breakdown of cyclic AMP (Butcher and Sutherland, 1962). It therefore seemed possible that the concentration of cyclic AMP in the cell could be raised from a low steady state level by inhibiting its breakdown with theophyllin as an alternative to accelerating its synthesis by epinephrin (cf. Orloff and Handler, 1962). Accordingly it would be expected that theophyllin should cause enzyme

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secretion by the slices if cyclic AMP functions in this process. As shown in Table 1, theophyllin readily induces amylase secretion in the rat parotid slices and the process is inhibited by dinitrophenol as previously reported for epinephrin (Bdolah et al., 1964). Furthermore, addition of theophyllin at low concentration, on top of epinephrin, yields a higher rate of enzyme secretion than that obtained with epinephrin alone.

More direct evidence for the function of cyclic AMP in enzyme secretion was obtained thanks to a generous gift of N^6-2-O- dibutyryl 3'5' cyclic AMP from Dr. Sutherland. This synthetic derivative shows a higher

Table 1

Effect of theophyllin and 3'5' cyclic AMP on enzyme secretion

Experiment	Additions		Amylase secreted % of total
I	None		14
	3'5' cyclic AMP	9 mM	14
	Theophyllin	1 mM	22
	Theophyllin	10 mM	41
	Theophyllin	10 mM + DNP 1 mM	18
	Epinephrin	0.01 mM	44
II	None		4
	Theophyllin	2.2 mM	12
	Epinephrin	0.01 mM	37
	Epinephrin	0.01 mM + Theophyllin 2.2 mM	54

Preparation of slices in Krebs Ringer bicarbonate medium at 37° and other procedures are as described in the accompanying communication (Schramm et al., 1965). Exp. I: Each vessel contained slices equivalent to half a gland (about 2,500 amylase units). Incubation time, 60 min. Preincubation of the slices was omitted in this experiment and the control without additions is therefore relatively high. Exp. II: Each vessel contained slices after preincubation, equivalent to 0.8 glands (about 7,000 amylase units). Incubation time, 45 min.

permeability into cells and furthermore seems to be resistant to hydrolysis by the diesterase (Posternak et al., 1962). Table 2 shows that dibutyryl

cyclic AMP is an efficient inducer of enzyme secretion. The figures indicate that there might still be some permeability barrier for the dibutyryl derivative since the rate of secretion increases with time while the rate obtained with epinephrin is maximal at onset of incubation.

Preliminary experiments indicate that dinitrophenol, which inhibits enzyme secretion caused by epinephrin also inhibits secretion when dibutyryl cyclic AMP serves as the inducer. It is therefore tentatively concluded that energy requirement in the secretion process is not limited to the synthesis of cyclic AMP.

Table 2

Dibutyryl 3'5' cyclic AMP as an inducer of enzyme secretion

Additions		Amylase secreted, % of total	
		15 min incubation	65 min incubation
None		1.5	4.7
Dibutyryl cyclic AMP	1 mM	7.8	48.0
" " "	2 mM	10.0	51.0
Epinephrin	0.01 mM	13.0	43.0

Each vessel contained slices, after preincubation, equivalent to one gland (about 3,500 amylase units).

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