ENZYME SECRETION IN VITRO IN THE ABSENCE OF ALTERATIONS IN OXIDATION[1]

Raymond H. Lindsay, Takao Ueha[2] and Roger W. Hanson

Departments of Pharmacology and Medicine, and College of General Studies, University of Alabama in Birmingham, and Nuclear Medicine Service, Veterans Administration Hospital, Birmingham, Alabama 35233

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SUMMARY: The effects of various concentrations of epinephrine and dibutyryl cyclic 3', 5'-AMP (dcAMP) on enzyme secretion and glucose oxidation by rat parotid in vitro were determined. Epinephrine at 0.33 µg/ml increased α-amylase secretion 43% without significantly altering glucose oxidation. Higher concentrations of epinephrine increased both secretion and glucose oxidation. The cyclic 3',5' -AMP (cAMP) derivative dcAMP stimulated amylase secretion 157% at 200 µg/ml without producing any accompanying increase in glucose oxidation. These results indicate that changes in oxidation are not essential for the cAMP induced phase of enzyme secretion and that the increase in glucose oxidation produced by epinephrine is not mediated by cAMP.

It appears to be well established that the induction of enzyme secretion by rat parotid in vitro is mediated by cAMP (1). According to this concept, chemical inducers such as epinephrine must stimulate adenyl cyclase in the target cell to produce more cAMP. This nucleotide then initiates the sequence of events that results in the discharge of transportable protein from the cell Thus cAMP would have a more specific effect on secretion than epinephrine which must stimulate cAMP formation then, through this nucleotide, enzyme secretion.

It has been demonstrated that epinephrine initiation of enzyme secretion by rat parotid in vitro is accompanied by an increase in oxygen consumption (1) and in substrate oxidation (2). Similar increases in salivary gland

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[2]Present address: Department of Physiology, Osaka Dental University, Osaka, Japan

respiration (cf 3,4) and glucose oxidation (5,6) by inducers of secretion have been observed by others. In addition, Babad et al. (1) have shown that neither epinephrine nor dcAMP stimulates secretion in the presence of uncouplers of oxidative phosphorylation and that epinephrine has no effect in a nitrogen atmosphere. These results strongly suggest that enzyme secretion is energy dependent; consequently, it has been assumed that the increase in oxidative metabolism provides the additional energy required when secretion is induced. However, it has not been established that the increase in tissue oxidation is essential for the export of amylase nor that cAMP mediates the epinephrine effects on metabolism.

Since cAMP is probably the mediator of the epinephrine action on enzyme secretion, the effects of various concentrations of epinephrine and dcAMP on amylase secretion and glucose oxidation by rat parotid in vitro were compared to determine if an increase in tissue oxidation is essential for the dcAMP stimulated phase of secretion and if the increase in tissue oxidation is mediated by cAMP.

MATERIALS AND METHODS

Male Sprague-Dawley rats (200-250 g) were sacrificed by exsanguination while under light ether anesthesia. The parotid glands were quickly removed, freed of fat and extraneous tissue then placed on buffer-moistened filter paper in covered, iced Petri dishes. Parotid glands were minced with fine-tip scissors and 40 ± 2 mg incubated in triplicate in 3.0 ml Krebs-Ringer bicarbonate medium with the appropriate additions. Epinephrine, dcAMP and glucose were all dissolved in Krebs-Ringer bicarbonate before addition to the medium. Radioactive compounds were usually added in 0.05 ml of H_2O and control flasks corrected accordingly. Incubation was carried out in stoppered flasks with a removable center well at 37°C in 95% oxygen - 5% CO_2 at a shaking rate of 120/min.

The activity of α-amylase which was secreted into the medium during 90 minutes of incubation was determined by the method of Myers et al. (7)

and expressed as mg of reducing substance, as glucose, formed per mg of wet tissue wt.

Glucose-6-^{14}C oxidation was determined by measuring $^{14}CO_2$ production. Following incubation, Hyamine (1.0 ml) was injected into the center well and the reaction terminated by the injection of 0.3 ml 4N perchloric acid. The vessels were shaken an additional 30 min for $^{14}CO_2$ collection. The Hyamine was then quantitatively transferred to scintillation vials and counted in a Packard Tri-Carb liquid scintillation spectrometer.

RESULTS AND DISCUSSION

The results obtained with epinephrine and dcAMP, which apparently enters cells more readily than cAMP and is resistant to degradation by phosphodiesterase, are presented in Table 1. Low concentrations of epinephrine (0.33 µg/ml) increased α-amylase secretion 43% without any significant change in glucose metabolism and at 0.66 µg epinephrine, amylase secretion increased almost 100% while glucose oxidation increased only 28.6%. At higher concentrations, epinephrine produced a marked stimulation of both glucose oxidation and enzyme secretion confirming previous observations that increases in α-amylase secretion were accompanied by increases in oxidative processes (1,2). The maximum stimulation of amylase occurred at a concentration between 0.67 and 1.0 µg/ml whereas glucose oxidation continued to increase at levels above 1.0 µg/ml with the percent increase doubling from 1.0 to 8.0 µg/ml. The plateau reached with respect to secretion may represent complete discharge of the enzyme from zymogen granules, after which no further increment in enzyme secretion occurs.

It is apparent from Table 1 that dcAMP is a potent inducer of enzyme secretion producing an increase of 157% at 200 µg/ml. Although secretion of amylase was evident with dcAMP at 50 µg/ml and increased in essentially a linear fashion with increasing concentrations, the oxidation of glucose was unaffected by even the highest concentration tried. Preliminary experiments indicate that similar effects were produced by theophylline and that

Table 1. The effects of various concentrations of epinephrine and dcAMP on glucose oxidation and α-amylase secretion[1]

Additions (μg/ml)	Glucose-6-^{14}C Oxidized		α-Amylase Secreted[2]	
	dpm $^{14}CO_2$/100 mg tiss	% Chg	units	% Chg
Epinephrine				
0	11,979[3]		65	
0.33	13,050	8.9	93	43
0.67	15,410	28.6	126	94
1.0	20,336	69.7	145	123
3.3	26,490	121.2	145	123
8.0	31,126	159.8	151	132.3
dcAMP				
0	5,707[4]		49	
10	5,475	-4.1	56	14.3
25	5,839	+2.3	57	16.3
50	5,380	-5.7	69	40.8
100	5,567	-2.5	84	71.4
200	5,513	-3.4	126	157.1

1 The results shown are averages of three experiments, each done in triplicate.

2 Units of α-amylase activity indicate mg of reducing substance, as glucose, per mg of tissue.

3 0.5 μCi glucose/4.0 μmoles/3 ml.

4 0.25 μCi glucose/4.0 μmoles/3 ml.

amino acid oxidation was also unaffected by dcAMP.

In the sequence of events leading to discharge of exportable protein, epinephrine must stimulate adenyl cyclase and the production of cAMP. This mediator then induces secretion. The results obtained with dcAMP demonstrate that the phase of enzyme secretion stimulated by this nucleotide did not require an increase in tissue oxidation and, since glucose oxidation was not altered, that the increase in oxidation produced by epinephrine was not mediated by cAMP.

Since the effect of dcAMP was abolished by 2,4-DNP (1) and by nitrogen (8), it must be concluded that cAMP induction of secretion is energy dependent. However, results from the present investigation, using glucose oxidation as an indicator of tissue metabolism, indicate that sufficient energy was produced by the parotid cells at their normal, unstimulated rate of metabolism to provide all the energy required for cAMP to induce enzyme secretion.

The action of epinephrine may require an increase in energy production to support an increase in the formation of cAMP. Stimulation of cAMP synthesis apparently can occur to some extent, as seen with 0.33 μg of epinephrine/ml, in the absence of a measurable change in metabolism. However higher concentrations of epinephrine stimulated both glucose oxidation and enzyme secretion suggesting that substantial production of cAMP may create a demand for energy that can be met only by an increase in oxidation. On the other hand, the increase in glucose oxidation may be a side effect of epinephrine which is unrelated to its action on secretion.

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