ADENOSINE 3',5' CYCLIC PHOSPHOROTHIOATE: AN EFFICIENT INDUCER OF AMYLASE SECRETION IN RAT PAROTID SLICES

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1. Introduction

Since its synthesis by Posternak and Sutherland [1] dbcAMP has found wide use as a cAMP derivative which can penetrate into intact cells and initiate biological processes mediated by the cyclic nucleotide. One of the first cellular processes in which the action of dbcAMP was demonstrated was the secretion of amylase by rat parotid slices [2]. The findings contributed strong proof that cyclic AMP is an intermediate in the induction of enzyme secretion by epinephrine in the parotid acinar cell [3]. However, free butyric acid which may be formed from the dbcAMP has been reported to cause various cellular effects which are completely unrelated to the function of the cyclic nucleotide [4–6]. It would therefore be desirable to have alternative cyclic AMP derivatives which are active on intact cells. Previous work has shown that cAMPS activates protein kinase almost as effectively as cAMP and that cAMPS is relatively resistant to hydrolysis by the phosphodiesterase [7]. Since the sulphur atom might also contribute to the permeability of cAMPS through the cell membrane, cAMPS was tested for its ability to initiate amylase secretion in rat parotid slices. The rat parotid system seemed particularly suited for this purpose since it is well characterized with respect to the actions of neurohormones and cAMP [8,9] and since free cAMP is without effect even at a concentration of 9 mM [2].

2. Materials and methods

cAMPS was synthesized as previously described [7]. Solutions of the cyclic nucleotides in H_2O were prepared fresh before each experiment. Rat parotid glands were taken from overnight fasted rats. The slice system and the assay of amylase secretion were as previously described [10]. Slices equivalent to one gland (about 5000 amylase units) were incubated in a 25 ml erlenmeyer containing 2.5 ml of Krebs–Ringer bicarbonate medium without Ca^{2+}. The system was continuously incubated with shaking at 37°C under a gas phase of 95% O_2/5% CO_2. To block endogenous catecholamines [11] 2 μM propranolol was added to systems receiving cyclic nucleotides and to controls without inducer of secretion.

3. Results and discussion

It is shown in fig.1 that cAMPS, dbcAMP and isoproterenol are about equally effective in causing sustained and extensive amylase secretion in rat

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Abbreviations: cAMP, adenosine 3',5'-monophosphate; dbcAMP, N^6,C^2 dibutyryl cAMP; cAMPS, adenosine 3',5'-cyclic phosphorothioate.

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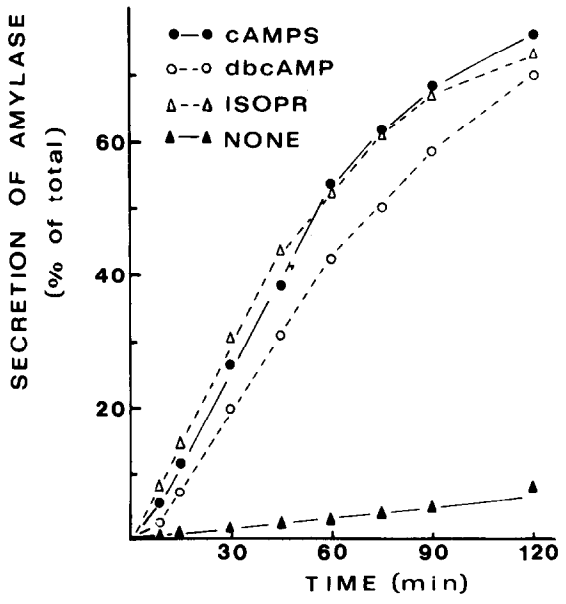

Fig.1. Comparison of isoproterenol, 2 μM; cAMPS, 1 mM; and dbcAMP, 1 mM as inducers of amylase secretion.

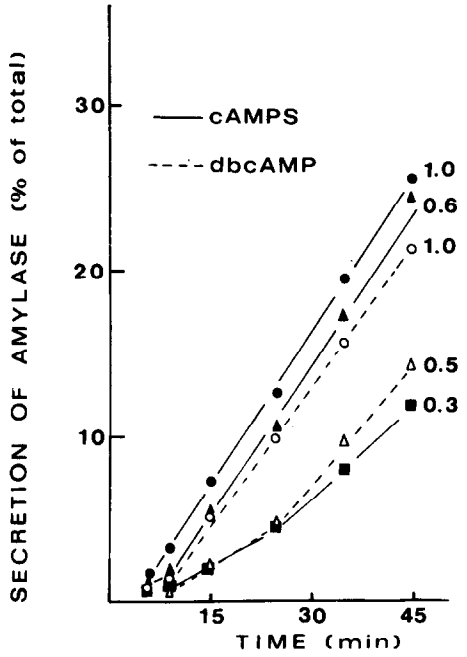

Fig.2. Induction of amylase secretion by cAMPS and by dbcAMP at various concentrations. The numbers in the figure indicate the mM concentration.

parotid slices. The concentration of isoproterenol used produces a maximal rate of amylase discharge. Fig.2 compares the rates of secretion obtained with different concentrations of cAMPS and dbcAMP. cAMPS seems to be somewhat more effective than dbcAMP.

Experiments were also conducted to test whether secretion declines when the inducer of secretion is removed from the medium by washing the slices. Table 1 shows that all systems which had contained

Table 1
Decline of amylase discharge after removal of inducers of secretion by washing the slices

Inducers of secretion	Rate of amylase secretion (% of total per min)		
	In presence of inducer	After removal of inducer	
	5–25 min period	0–10 min period	10–20 min period
Isoproterenol	0.6	0.5	0.2
cAMPS	0.8	0.6	0.3
dbcAMP	0.5	0.8	0.5
None	0.08	0.08	0.08

Concentrations of inducers were: isoproterenol, 2 μM; cAMPS, 1 mM; dbcAMP, 1 mM. The first 5 min of incubation did not produce a constant rate of secretion. Therefore the rate is given for the 5–25 min period. After 25 min incubation the slices were rapidly rinsed with fresh medium and then reincubated in medium without added inducers of secretion.

an inducer continue to secrete at a high rate during the period 0–10 min after washing the slices. Apparently enough inducer was retained in the tissue during the washing procedure. Even during the period 10–20 min after washing the slices, the system which had been incubated with dbcAMP fails to show a significant decline in amylase secretion. In contrast, the system initially incubated with cAMPS shows a considerable decrease in the rate of secretion after washing and incubation in fresh medium. cAMPS was in this sense similar to isoproterenol. It is therefore assumed that cAMPS can be removed from the cells more easily than dbcAMP.

4. Conclusion

The findings suggest that cAMPS is an effective agent for the study of cAMP function in intact cells.

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