SHORT COMMUNICATIONS

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The effect of calcium on amylase secretion by rat parotid slices

Exocrine glands demonstrate specific release of digestive enzymes in response to various stimulants[1,2]. In a number of systems, induction of enzyme secretion proceeds in two stages. In the first stage specific hormones, upon reaching their target cells, activate the enzyme adenyl cyclase and as a result, the level of cyclic AMP within the cell is increased[3]. In the second stage, a chain of reactions inside the cell leads to the release of accumulated exportable enzymes[4]. Very little is known about the second stage, apart from the general information that the secretion process is energy-dependent and that it can be induced by addition of cyclic AMP[5,6]. There are also several reports that indicate a requirement for calcium in the secretion process[7-9]. In the rat parotid, however, exclusion of calcium from the incubation medium did not result in an inhibition of amylase secretion[10]. A contradictory finding, although not accompanied by data on amylase secretion, was reported by RASMUSSEN AND TENENHOUSE[11]. Since the parotid gland is one of the systems most suitable for the study of hormone induced secretion, it was decided to investigate the effect of calcium in this system in more detail.

It is difficult to demonstrate a requirement for calcium in the secretion of amylase by parotid slices since the gland contains large amounts of calcium[12]. Furthermore, attempts to deplete the slices of calcium by EDTA results in the activation of endogenous catecholamine which causes amylase secretion[6,13]. To overcome this difficulty the slices were depleted of calcium by ethyleneglycol-bis-(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid (EGTA; a specific chelator for calcium) in the presence of a catecholamine blocking agent. As was shown by SCHRAMM[13] the blocking agent effectively inhibits the secretion of amylase induced indirectly by EDTA. After a period of incubation of slices with EGTA and propranolol which served as the catecholamine blocking agent, the slices were thoroughly washed with calcium-free Krebs–Ringer bicarbonate buffer. Subsequently, induction of amylase secretion was tested in presence and absence of calcium. The butyryl derivatives of cyclic AMP served as inducers of secretion since it has been shown that they are not inhibited by catecholamine blocking agents[13].

The experimental procedures of the rat parotid slice system, used in the present study were those described in detail by BABAD *et al.*[6]. Most of the determinations were carried out in duplicates and all the experiments were repeated at least twice. The overall accuracy of the assay of amylase secretion in parotid slices was $\pm 7\%$ (see ref. 10).

Dibutyryl cyclic AMP was obtained from Boehringer Co., monobutyryl cyclic

Abbreviation: EGTA, ethyleneglycol-bis-(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid.

AMP was prepared by alkaline hydrolysis of dibutyryl cyclic AMP[14]. Propranolol was a product of I.C.I. Ltd. manufactured under the trade name Inderal, and EGTA was obtained from Fluka Co.

It is shown in Table I that after preincubation of slices with EGTA and propranolol addition of calcium enhances amylase secretion induced by dibutyryl cyclic AMP. Furthermore, the value for amylase secretion obtained by re-adding calcium

TABLE I

EFFECT OF CALCIUM DEPLETION ON AMYLASE SECRETION BY RAT PAROTID SLICES

In addition to standard components (*cf.* ref. 6) all incubation media contained 0.1 mM propranolol. Incubation time in the final incubation medium was 60 min. Amylase secreted into the medium is expressed as percent of total amylase in the system (*cf.* ref. 6).

Preincubation		*Final incubation*		
Krebs–Ringer bicarbonate buffer	*Time (min)*	*Inducer, dibutyryl cyclic AMP (1 mM)*	*Krebs–Ringer bicarbonate buffer*	
			Without Ca^{2+}	*With Ca^{2+} (2.5 mM)*
			Amylase secreted (% of total)	
Krebs–Ringer bicarbonate buffer without Ca^{2+} + EGTA 10 mM	60	—	1.0	1.0
		+	23.0	35.0
Krebs–Ringer bicarbonate buffer with Ca^{2+}	60	—	1.7	1.7
		+	1.7	35.0
Krebs–Ringer bicarbonate buffer without Ca^{2+} + EGTA 10 mM	120	—	0.9	1.1
		+	11.6	27.0
Krebs–Ringer bicarbonate buffer with Ca^{2+}	120	—	1.6	1.6
		+	1.7	28.0

TABLE II

EFFECT OF CALCIUM ON AMYLASE SECRETION INDUCED BY VARIOUS AGENTS

In addition to standard components (*cf.* ref. 6) incubation media contained 0.1 mM propranolol except in the final incubation media which contained epinephrine. Incubation in the final incubation media was 60 min. Amylase secreted into the medium is expressed as percent of total amylase in the system (*cf.* ref. 6).

Preincubation		*Final incubation*		
Medium	*Time (min)*	*Inducer*	*Krebs–Ringer bicarbonate buffer*	
			Without Ca^{2+}	*With Ca^{2+} (2.5 mM)*
			Amylase secreted (% of total)	
Krebs–Ringer bicarbonate buffer without Ca^{2+} + EGTA 10 mM	120	—	1.0	3.0
		Monobutyryl cyclic AMP, 1 mM	8.7	25.5
		Cyclic AMP, 1 mM	2.3	2.5
		Epinephrine, 0.1 mM	2.9	7.0

to depleted slices is equivalent to that obtained with slices which had not been depleted of calcium. Amylase secretion which still persists in absence of added calcium is probably due to the incomplete depletion of this cation from the slices. This assumption seems justified in view of the increased dependence on added calcium when preincubation with EGTA was extended from 60 to 120 min (Table I). Relatively long preincubation periods with EGTA are necessary to demonstrate the effect of calcium when glands from rats weighing 250 g or more are used. In the experiments shown in Tables I and II rats weighing 120–160 g were used. Table II shows that after preincubation of slices in presence of EGTA and propranolol, addition of calcium causes a 3-fold increase in the amount of amylase secreted when induced by monobutyryl 3′,5′-cyclic AMP. Epinephrine at 0.1 mM fails to induce amylase secretion efficiently. This is most probably due to the failure to remove the catecholamine blocking agent which accumulated in the tissue during the preincubation period. It is interesting that cyclic AMP which is unable to induce secretion in parotid slices because of the permeability barrier[5] also fails to induce secretion of amylase after preincubation of 2 h in the presence of 10 mM EGTA. This fact might indicate that the permeability properties of the parotid cells, were not drastically changed after calcium depletion.

These experiments clearly demonstrate a requirement for calcium in enzyme secretion by parotid slices. A dependence on calcium could only be demonstrated after prolonged preincubation with EGTA in the presence of a catecholamine blocking agent. Since calcium is required when cyclic AMP derivatives serve as inducers it is obvious that this cation exerts its effect on the secretion process beyond the adenyl cyclase step.

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