FACTORS CONTROLLING THE PROCESS OF ENZYME SECRETION BY THE RAT PAROTIS SLICE*

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A previous study of the rat parotis gland indicated that it contains abundant amounts of α-amylase, largely aggregated in the intracellular zymogen granules (Schramm and Danon, 1961). The present communication describes the effect of stimulants, metabolic inhibitors and medium components on amylase secretion by the parotis slice.

When epinephrine (10^{-5} M) was added to slices incubated aerobically in Krebs 'medium I' (Krebs, 1950) 30-40% of the amylase was secreted in 60 minutes. In the control without epinephrine only 10-16% of the enzyme appeared in the extracellular medium (see Table I). Other drugs such as pilocarpine, acetylcholine and carbamylcholine caused similar stimulation of amylase secretion (cf. Hokin, 1951; Hokin and Sherwin, 1957; Eggman and Hokin, 1960). Electrical pulses (McIlwain, 1952) passing through the incubation medium were also effective. Enzyme secreted under the effect of such stimulants had a specific activity comparable to that of the zymogen granules and about twice as

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high as that of a whole homogenate of the slice. This finding indicated that the system indeed demonstrates specific secretion and not autolysis.

Table I shows that the enhanced secretion in presence of epinephrine was dependent on oxygen and was completely suppressed by cyanide or DNP but not by iodoacetate. A requirement for ATP generated by oxidative phosphorylation is thus indicated. It was also found that the slice secreted DNAse. The per cent of total DNAse found in the medium was almost the same as that of amylase, both with respect to stimulation by epinephrine and suppression by metabolic inhibitors. Since we were able to show* that the intracellular distribution of DNAse in the parotis is similar to that of amylase, it was not surprising to find that both enzymes are secreted to the same extent.

A study of the requirements of the secretion process for various components of the medium revealed that the salt mixture of Krebs 'medium I', without organic substrates, supported undiminished secretion. Apparently the slice contains sufficient endogenous substrate under the experimental conditions adopted (specified under Table I). Variations in the composition of the inorganic salt medium indicated that potassium ion concentration plays a dominant role in the secretion process. Table II shows that if Na^+ is replaced by K^+ in the Krebs Ringer bicarbonate medium (Krebs and Henseleit, 1932), dependence of secretion on epinephrine is abolished. The finding that epinephrine is not required when K^+ concentration is high (100-150 mM) is also demonstrated using a KCl +$KHCO_3$ medium. Under such conditions of high K^+ concentration

* Unpublished results.

TABLE I

EFFECT OF OXYGEN AND METABOLIC INHIBITORS ON AMYLASE SECRETION BY EPINEPHRINE STIMULATED PAROTIS SLICES

	Amylase units in slices (a)	Amylase units in medium (b)	Per cent secreted b X 100 / a+b	Specific activity units/mg protein in slices	Specific activity units/mg protein in medium
With epinephrine					
Aerobic	2400	1200	33	370	660
Aerobic + iodoacetate	3200	1600	33	390	680
" + KCN	3600	700	16	460	490
" + DNP	3800	500	12	450	500
Anaerobic	2900	600	17	440	640
Without epinephrine					
Aerobic	4100	800	16	470	560

Isolation of glands and assays for amylase and protein were as previously described (Schramm and Danon, 1961). Eight glands from rats previously starved for 24 hours were each cut into 12 slices with a scalpel. All slices were pooled, washed and placed in a 100 ml erlenmeyer containing 25 ml Krebs 'medium I'. Pre-incubation was carried out at 37° for 15 minutes with shaking (200 strokes/min.). The gas phase was 95% O_2, 5% CO_2. The medium was discarded. 16 slices were then put in each 25 ml erlenmeyer containing 4 ml fresh medium including the additions indicated in the Table. Epinephrine was 10^{-5} M and inhibitors were 10^{-3} M. Incubation was carried out under conditions described above. N_2 replaced O_2 for anaerobic experiments. After 60 minutes incubation the medium and a homogenate prepared from the slices were assayed for amylase and protein. To avoid damage to the slices these were not weighed before placement in the experimental vessel. As a result, the total amount of amylase in each vessel varied. The per cent value of amylase secreted fluctuated in duplicate experiments in the range of ∓2%.

secretion was still inhibited by DNP. Table II also demonstrates that if K^+ is omitted from the Krebs Ringer bicarbonate medium, maximal secretion is not achieved even in presence of epinephrine. This finding is also corroborated by an experiment not shown in the Table. In a medium containing 140 mM NaCl + 10 mM $NaHCO_3$, in presence of epinephrine,

TABLE II

EFFECT OF POTASSION ION CONCENTRATION ON AMYLASE SECRETION BY PAROTIS SLICES

Medium*	Epinephrine 10^{-5} M	Amylase secreted % of total	Specific activity of amylase units/mg protein in slices	in medium
K.R.B.	+	34	380	840
K.R.B.	-	13	460	670
K.R.B., Na^+ replaced by K^+	+	35	370	990
K.R.B., Na^+ replaced by K^+	-	32	360	900
K.R.B., K^+ replaced by Na^+	+	24	360	680
$NaCl + NaHCO_3$	+	19	400	660
$KCl + KHCO_3$	+	28	380	810
$KCl + KHCO_3$	-	30	330	770

* K.R.B., Krebs Ringer bicarbonate (contains 5 mM K^+). In the medium containing only NaCl and $NaHCO_3$ concentrations were 144 mM and 10 mM respectively. The same concentrations were adopted for the $KCl + KHCO_3$ medium. The experimental procedure was as outlined under Table I. Total amylase content per vessel was on the average 3500 units.

19% of the amylase was secreted in 60 minutes. Under the same conditions but with 4 mM K^+ secretion was as high as 29%.

The present results may suggest that penetration of K^+ into the cell triggers the secretion process. At high K^+ concentration penetration is achieved by diffusion while at low K^+ concentration epinephrine facilitates K^+ transport. Work on other tissues indicates that K^+ enhances respiration and phosphate turnover (cf. Yoshida and Quastel, 1962). Thus, stimulation of enzyme secretion by K^+, its dependence on oxygen and its inhibition by DNP appear to

implicate high energy phosphate as a determining factor of the secretion process.

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