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AMYLASE SECRETION FROM ISOLATED PURE ACINAR CELLS

Madhukar S. Bandisode[1] and Manjit Singh[2]

Diabetes Research[1] and Pancreatic Research[2]
VA Medical Center and Department of Medicine
Medical College of Georgia, Augusta, Georgia 30910

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Summary - Isolation of pure acinar cells of the rat pancreas was achieved employing counterflow sedimentation filtration technique (CSFT). The preparation of purified acinar cells contained an occasional red blood cell (RBC, 200:1) with total absence of endocrine and duct cells. A significant stimulation of amylase secretion from isolated pure acinar cells was produced by octapeptide of cholecystokinin (CCK_8) and insulin produced potentiation of the effect of CCK_8. Synthetic glucagon inhibited basal and CCK_8 stimulated amylase secretion. Non-synthetic purified glucagon stimulated amylase secretion and potentiated the effect of CCK_8. Vasoactive intestinal polypeptide (VIP) did not stimulate amylase secretion but potentiated the effect of CCK_8. No leakage of lactic dehydrogenase (LDH) was detected from the cells in any of the secretion studies. Thus a highly purified preparation of isolated pure acinar cells of rat pancreas could be obtained with excellent morphologic and functional integrity.

The interactions between exocrine and various endocrine cells of the pancreas has been suspected in the past, on the basis of the results of effects of insulin, glucagon and somatostatin (1-7) on amylase secretion. However, a clear definition of the role of endocrine cells in modulation of the function of exocrine cells has not been achieved because of non-availability of pure preparations of the pancreatic cell types. The present report describes a technique of obtaining a preparation of highly purified acinar cells of adult rat pancreas and the results of the effect of the enteric and pancreatic hormones on amylase secretion.

MATERIALS AND METHODS

Starting Sample Suspension: Sequential dissociation of the adult rat pancreas was achieved by incubation of pancreatic lobules with collagenase (C-0130, Sigma) as described earlier (8).

Isolation of Pure Acinar Cells: The counterflow sedimentation technique (CST) employed for isolation of pure beta cells (8) was modified to obtain pure acinar cells. The new technique, counterflow sedimentation filtration technique (CSFT), essentially involved installment of a nylon mesh filter

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(149 micron) between the reservoir and the column of the CST. The gradient of Ficoll (KRB vs 5% Ficoll) was generated as in CST (8). After the fraction of the gradient containing predominantly endocrine cells escaped from the side arm, the remaining gradient from the side arm to the nylon filter containing pure acinar cells was removed by aspiration employing Pasteur pipette, washed twice with KRB-I plus 1% BSA by centrifugation at 550g for 10 minutes, resuspended in KRB-II and employed for further studies.

Morphology

The cell pellets were prepared in 2 ml plastic conical tubes by centrifugation fixed in Bouin's solution, dehydrated and embedded in paraffin. Sections (5 μ) were mounted, rehydrated and employed for differential staining with aldehyde fuchsin stain. Modified Karnovsky fixative was layered on cell pellets and the cells processed for electron microscopy (EM) (9). Antiinsulin, antiglucagon and antisomatostatin antibodies were employed for indirect immunofluorescence staining (10) of the paraffin sections. The sections were examined under Leitz Dialux 20 EB fluorescence microscope with K2 filter.

Studies of Amylase Secretion and LDH Release: After preincubation in KRB-II for one hour, the cells were resuspended in KRB-II and distributed in incubation flasks. Effect of CCK_8, insulin, glucagon and VIP alone or in combination were studied. The amylase and LDH activity in the supernatants of aliquots of cell suspension prior to incubation with the peptides were substracted from values obtained following incubation to determine the enzymes released during the incubation periods. The post-incubated pellets were sonicated, and amylase and LDH activity determined. Amylase was assayed by the method previously described (11).

Preparation of Isolated Acini: Isolated intact acinii were prepared by the method previously described (12).

RESULTS

The dissociated cells ($3.1 \pm 0.21 \times 10^6$, M ± SE; n=10) when subjected to CSFT, separated into three clear zones. Zone 1, which was allowed to overflow, contained predominantly endocrine cells (90 - 94%) and a few acinar and duct cells (6 - 10%). Zone 2 contained predominantly acinar cells and occasional red blood cells (200:1). Zone 3 contained mostly aggregates of acinar and duct cells and red blood cells trapped under the nylon mesh filter. Exclusion of nylon mesh filter increased the number of red blood cells (100 acinar to ∿ 10 red blood cells) and duct cells (100 acinar to ∿ 10 duct cells) in the cells obtained from Zone 2. The acinar cells obtained from Zone 2 were round with clear, sharp refractile cell membranes as observed by light microscopy. They contained well-distinguished, large zymogen granules. Presence of unstained acinar cells and absence of endocrine cells was observed by differential staining technique.

The isolated pure acinar cells were structurally intact by EM, (Figure 1), and appeared round with uniform plasmalemma, dense large zymogen granules,

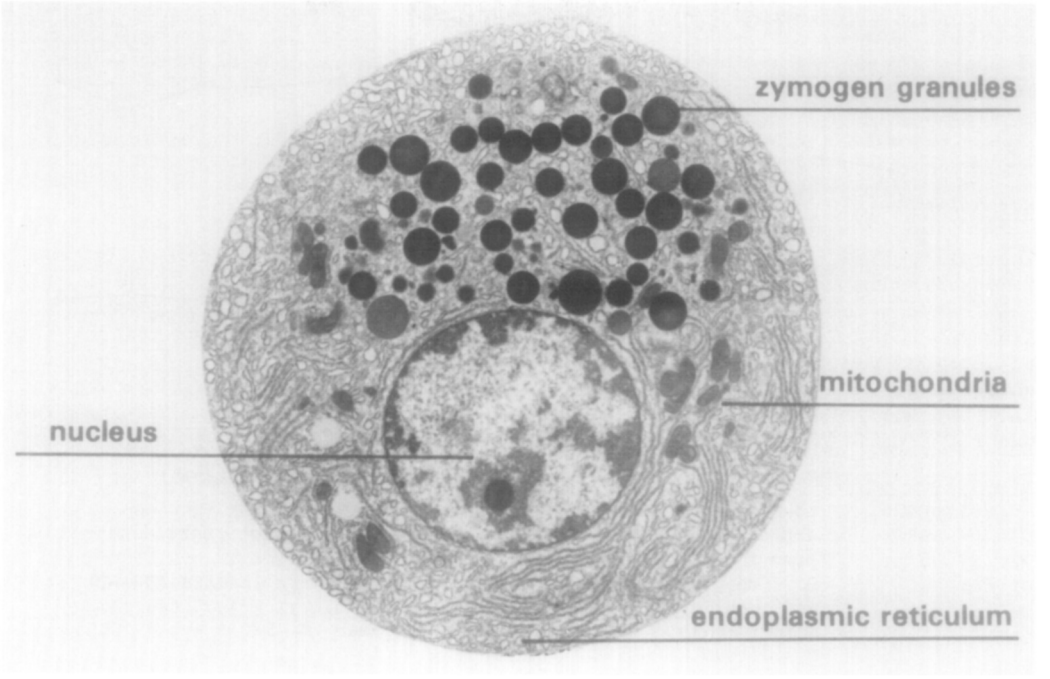


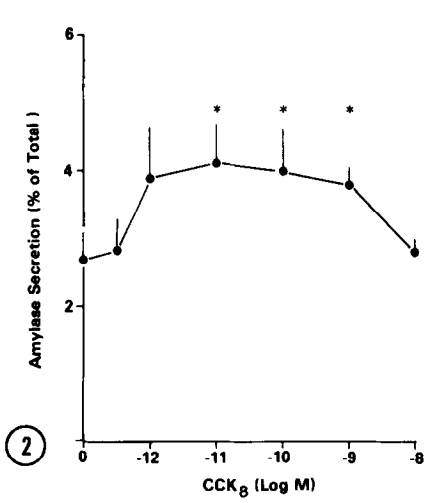
Figure 1 **Electron micrograph of isolated acinar cell.**

intact golgi complexes, intact nuclii, normal mitochondria, and well-preserved rough endoplasmic reticulum. No endocrine or duct cells were evident in the preparations by EM. The endocrine cells were not detected by immunofluorescence staining technique.

Viability of the isolated purified acinar cells was greater than 96% by trypan blue exclusion technique. The absence of release of LDH in the incubation in secretion studies indicated that the structural integrity of cells was well preserved.

In isolated pure acinar cells, CCK_8 stimulated amylase secretion in concentrations of 10^{-11}M (52%), 10^{-10}M (49%), and 10^{-9}M (33%) (Figure 2).

Insulin had no effect on amylase secretion, (Figure 3), but potentiated the effect of CCK_8(10^{-9}M) in concentrations ranging from 10^{-10}M to 10^{-7}M. The magnitude of this potentiation ranged from 18 to 27% more than CCK_8 alone. ($p < .05$). Synthetic glucagon 10^{-5}M and 10^{-6}M did not alter amylase secretion (Figure 4). However, in concentrations of 10^{-7}M, 10^{-8}M and 10^{-9}M it inhibited



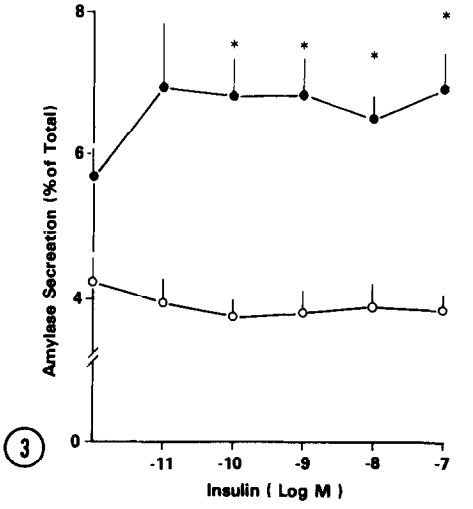


Figure 2 Dose-response curve of amylase secretion with CCK_8 (●—●) employing isolated pure acinar cells. Each point represents Mean ± SE. * - $p < 0.05$ N = 4 to 7.

Figure 3 Dose-response curves of amylase secretion with insulin (○—○) and insulin plus CCK_8 (10^{-9}M, ●—●) employing isolated pure acinar cells. Each point represents Mean ± SE, * - $p < 0.05$. N = 4 to 9.

the amylase secretion (21% - NS, 29% - $p < 0.05$, 21% - $p < 0.05$) respectively. Reduction in amylase secretion was also evident with CCK_8 10^{-9}M and synthetic glucagon 10^{-8}M and 10^{-9}M. Non-synthetic purified glucagon (NSP-glucagon) 10^{-5}M stimulated amylase secretion and combination of NSP-glucagon 10^{-5}M with CCK_8 10^{-9}M potentiated the effect of CCK_8 (Table 1).

VIP 10^{-10}M to 10^{-6}M did not increase amylase secretion but the combination of VIP 10^{-6}M with CCK_8 10^{-9} potentiated the effect of CCK_8.

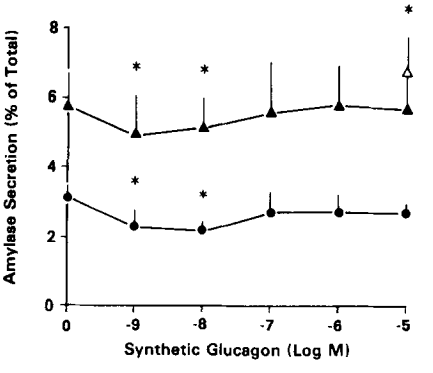


Figure 4 Dose-response curves of amylase secretion with synthetic glucagon (▲—▲) and synthetic glucagon plus CCK_8 (10^{-9}M, ●—●) from isolated pure acinar cells. N = 4 to 8. Δ represents response to non-synthetic purified glucagon. Each point represents M ± SE, * - $p < 0.05$.

TABLE I

Effects of Non-Synthetic Purified Glucagon (10^{-5}M) and VIP (10^{-6}M) Alone or in Combination with CCK_8 (10^{-9}M) on Amylase Secretion from Isolated Pure Acinar Cells

Experimental Condition	Amylase Secretion (% of Total)	P Value
Control	4.01 ± 0.26	
CCK_8	5.77 ± 0.66	$p < 0.05$
Glucagon (NSP)	8.10 ± 1.13	$p < 0.05$
CCK_8 + Glucagon (NSP)	10.61 ± 2.06	$p < 0.05$
VIP	5.14 ± 0.48	N.S.
CCK_8	5.53 ± 0.47	$p < 0.05$
CCK_8 + VIP	8.90 ± 1.19	$p < 0.01$

Results of amylase secretion are expressed as % of total in the sample. Values are mean ± SEM of 4 - 12 experiments.

In isolated acini, amylase secretion increased 105%, 494%, 710%, 350%, 290% and 250% above control values with CCK_8 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6}M respectively. It was increased 480%, 405%, and 25% with synthetic glucagon 10^{-5}, 10^{-6} and 10^{-7}M. However, significant decrease in amylase secretion ($p < 0.05$) was observed with synthetic glucagon 10^{-9} and 10^{-8}M. Amylase secretion increased 80% above control values with NSP glucagon 10^{-5}M (Figure 5).

DISCUSSION

Digestive enzyme secretion from pancreatic acinar cells is known to be regulated by enteric hormones (CCK_8 and secretin) and cholinergic neural mechanisms. The role of adjacent endocrine cells or their hormones (insulin, glucagon, somatostatin, gastrin and pancreatic polypeptide) and the duct cells in pancreatic enzyme secretion has alluded clear definition. In order to define clearly the type and nature of endocrine-duct-exocrine cell interactions, development of isolated pure cell preparations is necessary.

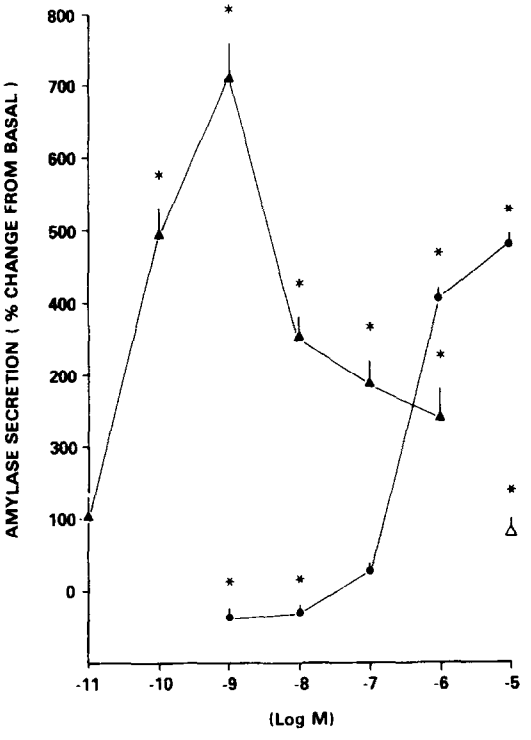


Figure 5 Dose-response curves of amylase secretion with CCK_8 (▲—▲) and synthetic glucagon (●—●) from intact acini. N = 6 to 8. Δ represents response to non-synthetic purified glucagon. Each point represents M ± SE, * - $p < 0.05$.

The technique of counterflow-sedimentation (CST) was initially developed to obtain pure beta cells of the pancreas (8, 15). Excellent purity of acinar cells (99%) was achieved with total exclusion of endocrine and duct cells. Sufficient number of cells could be obtained for studies of enzyme secretion.

The magnitude of response to secretagogues from the isolated purified acinar cells compared favorably with those reported previously from our laboratory (13) and other (12, 14).

The results of previous studies in diabetic animals and humans were suggestive of a regulatory role of insulin in acinar cell function (1-4). It is not clear, however, if the results were (a) direct effects of insulin on the acinar cells, (b) indirect effects secondary to alterations in functions of other pancreatic cells, or (c) indirect effects on acinar cells secondary to improved metabolic control. The results of studies of pure

acinar cells clearly indicate that insulin potentiates the effect of CCK_8. The observations that synthetic glucagon inhibited and NSP-glucagon stimulated amylase secretion raise the possibility of contamination of NSP-glucagon by other stimulatory factor/s and that glucagon appears to be primarily an inhibitor of acinar cells.

In summary, isolated pure acinar cells were morphologically intact and functionally active. The preparation will serve as an excellent model to study endocrine-duct-exocrine cell interactions. CCK_8 stimulated amylase secretion and insulin and VIP potentiated the effects of CCK_8. Synthetic glucagon inhibited both control and CCK_8 stimulated amylase secretion.

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