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A new, rapid, method for preparation of dispersed pancreatic acini

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A new method for the preparation of pancreatic acini is described. The method is simple and much more rapid than previously described techniques, the time required for preparation of pancreatic acini being 20min from removal of the pancreas. Acini prepared with this method perform in a superior manner when stimulated by either caerulein or secretin. Thus this new technique would be ideal for use in binding and secretion studies.

The study of secretory mechanisms in the exocrine pancreas has been greatly helped by the development of systems *in vitro* using isolated pancreatic acinar cells (Amsterdam & Jamieson, 1972, 1974) and isolated pancreatic acini (Peikin *et al.*, 1978; Williams *et al.*, 1978; Wakasugi *et al.*, 1982). Acini have been found to be more responsive to secretagogues than are isolated acinar cells, probably because of the preservation of cell-to-cell coupling or of apical structures thought to be required for exocytosis (for a review, see Williams, 1984). However, the techniques currently used to prepare pancreatic acini, although relatively simple, require several steps: injection of the pancreas with a collagenase solution, two digestion periods with collagenase and mechanical dissociation through plastic or silicone-treated glass pipettes of decreasing orifice (Peikin *et al.*, 1978; Williams *et al.*, 1978; Wakasugi *et al.*, 1982). Apart from the time required for each of these procedures, one additional point is that the tissue is exposed to a high concentration of collagenase for a longer period of time compared with that needed for isolation of pancreatic islets (Lacy & Kostianovsky, 1967) and liver cells (Seglen, 1976). This, of course, does not represent the ideal milieu for any type of cell. To this end we have developed a simple technique which allows a good yield of viable acini to be prepared in less than 20min from removal of the pancreas, also decreasing by approx. 75% the amount of collagenase used. In this study we have compared this method with that previously employed by us and others (Wakasugi *et al.*, 1982) with respect to preparation time and sensitivity of the tissue to caerulein, a cholecystokinin-like peptide, which is the most potent stimulant of enzyme release (Jensen & Gardner, 1981) acting via mobilization of intracellular calcium (Gardner & Jensen, 1981; Ochs *et al.*, 1983). In addition, we studied the ability of both preparations to respond to secretin, a peptide which causes a severalfold increase in cyclic AMP, but only a small increase in enzyme secretion from the rat pancreas (Collen *et al.*, 1982).

Experimental

Isolated pancreatic acini were prepared from fed male Wistar rats (approx. 200g). Animals were decapitated and the pancreases were rapidly removed and trimmed of visible fat and lymph nodes. The dissociation and incubation medium was a Krebs–Ringer Hepes (KRB–Hepes) buffer, adjusted to pH7.4, containing: 12.5mM-Hepes, 135mM-NaCl, 4.8mM-KCl, 1.0mM-$CaCl_2$, 1.2mM-KH_2PO_4, 1.2mM-$MgSO_4$, 5.0mM-$NaHCO_3$, 5mM-glucose and 0.01mg of aprotinin/ml.

Sources of materials

Hepes, ATP and collagenase (grade suitable for use in isolated fat-cells; lot no. 52F-6812; 160 units/mg dry wt.) were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Collagenase from *Clostridium histolyticum*, of research grade (425 Mandl-Units/mg), was obtained from Serva Feinbiochemica G.m.b.H., Heidelberg, Germany. Aprotinin (Trasylol) was obtained from Bayer A.G., Wuppertal, Germany. Caerulein was generously given by Dr. C. De Paolis, Farmitalia, Milan, Italy. Secretin was purchased from Bachem A.G., Bubendorf, Switzerland.

'New' method

Pancreases from two rats were quickly chopped with scissors into small pieces (approx. 2mm) and then transferred to 10ml conical glass tubes (one

pancreas/tube) to which a volume of KRB–Hepes equal to that of the tissue (usually 1.5–2ml) was added. For digestion, the pancreatic pieces were vigorously shaken by hand in the presence of 0.8–1.0mg of collagenase/ml, to allow complete exposure to the collagenase solution. The length of the digestion period was variable, being 4–5min with Serva and 8–10min with Sigma collagenase, by which time the tissue suspension appeared homogeneous to the eye. Then 5ml of fresh KRB–Hepes with 0.1% human serum albumin (HSA) or bovine serum albumin was added and the tissue was centrifuged at 500rev./min for 30s. The digested tissue was washed twice with repeated centrifugations, resuspended in 20–25ml of fresh KRB–Hepes–HSA, and then filtered on nylon mesh (obtained from Angst and Pfister, Le Lignon, 1219 Geneva, Switzerland). This step consisted of two consecutive filtrations on a 'coarse' mesh (54-GG-315 'Nybolt') of 290μm squares and then a fine mesh (PES 74/42 'Polymon') of 72μm squares. This procedure was greatly facilitated by the use of a Perspex apparatus which held both filters and allowed sequential filtration in one step. The use of this apparatus has been described in detail elsewhere (Offord & Halban, 1978). The dimensions of the apparatus (144mm^2 of filtering surface) are appropriate for the filtration of one digested pancreas, but the yield of tissue can be increased if the same amount of tissue is filtered on two or three apparatuses. Pancreatic acini which passed through the fine filter were then centrifuged at 600rev./min for 1min and resuspended in fresh KRB–Hepes–HSA. This was followed by a 30min preincubation period, during which acini were gassed continuously with 100% O_2 and shaken at approx. 80 cycles/min at 37°C.

'Old' method

Acini were prepared by the method of Wakasugi *et al.* (1982), except that one chelation step with EDTA was added. Briefly, the procedure involves the injection of pancreatic pieces (approx. 4mm) with dissociation medium containing 4mg of Serva collagenase or 9.4mg of Sigma collagenase per 15ml of KRB–Hepes–HSA buffer. Injected pancreatic pieces were then incubated in this medium for 15min in a 50ml polycarbonate Erlenmeyer flask (shaken at approx. 80 cycles/min) at 37°C. After 15min the medium was replaced by fresh KRB–Hepes–HSA with no Ca^{2+} and no Mg^{2+}, but containing 1mM-EDTA, for 5 min. This was followed by a second digestion period with 6mg of Serva collagenase or 15.6mg of Sigma collagenase per 15ml for 40–60min, depending on the collagenase used. The tissue was continuously gassed during the digestion steps with 100% O_2. Acini were then mechanically dissociated by forceful pipetting through tips of decreasing orifice, filtered through a double layer of medical gauze, layered over KRB–Hepes buffer containing 4% HSA, and centrifuged at 600rev./min for 1min. The pellet was washed three times with standard KRB–Hepes–HSA buffer and then preincubated for 30min as described above.

Yield of tissue and viability of the acini

The yield of tissue was similar with both techniques, but varied with the type of collagenase used, being approx. 0.5g from two pancreases with Sigma collagenase and approx. 0.7g with Serva collagenase. Trypan Blue exclusion was similar in the two preparations (90–95%). Light and electron microscopy revealed well preserved cellular and subcellular structures (not shown).

Secretion studies

At the end of the preincubation period for both methods, acini were allowed to settle under gravity, and the supernatant was removed and replaced by fresh medium. After one further sedimentation and removal of the supernatant, the cells were suspended in the incubation buffer. At the beginning of the incubation (zero time), three 0.5ml samples were taken from the acinar suspension, centrifuged at 10000rev./min for 20s in a Beckman micro-centrifuge, and the supernatant was analysed for amylase released by the method of Bernfeld (1955). This value was then subtracted from all the results obtained at the end of the 30min incubation in the absence or in the presence of the various secretagogues. The pellet was washed twice with cold 0.9% NaCl, sonicated and assayed for amylase content. Total amylase content was obtained by adding the supernatant and the pellet values at zero time. Amylase release in the test vials was measured on triplicate samples and calculated as percentage of total initial content. To compare the sensitivty of the two acinar preparations with the secretory response of the intact organ, we have used the perfused rat pancreas. The pancreas was isolated and perfused as described by Penhos *et al.* (1969). Pancreatic juice was collected by cannulating the major pancreatic duct as described in detail elsewhere (Bruzzone *et al.*, 1984).

Acinar ATP content was measured by the hexokinase method of Lamprecht & Trautschold (1974), with an Aminco fluoro-microphotometer (American Instrument Co., Silver Spring, MD, U.S.A.).

Statistics

Results are expressed as means $\pm$ S.E.M. Statistical analysis was made by analysis of variance and by unpaired Student's *t* test.

Results and discussion

The contents of amylase and ATP were similar for the two methods of acinar preparation: amylase 4622 ± 415 ($n = 6$) and 5241 ± 481 ($n = 5$) units/g wet wt., and ATP 4.5 ± 0.5 ($n = 3$) and 4.5 ± 0.4 ($n = 3$) mM/g wet wt., for 'new' and 'old' methods respectively.

The secretory response to caerulein stimulation is shown in Fig. 1. For acini prepared by the new method, maximal secretion was achieved with 10^{-10}M-caerulein, and for those by the old method with 10^{-9}M-caerulein. The maximal secretion rates were similar in the two preparations. Half-maximal stimulation for acini prepared by the new and old methods was found with approx. 3×10^{-12}M- and approx 10^{-11}M-caerulein respectively. The greater sensitivity of acini prepared by the new method was also shown by the increased amylase secretion at 10^{-12}M-, 10^{-11}M- and 10^{-10}M-caerulein when compared with those by the conventional technique ($F = 5.68$, $P < 0.05$). In fact, at 10^{-12}M-caerulein, a concentration at which we and others (Jensen *et al.*, 1980) find virtually no increase in amylase secretion by acini prepared by the conventional technique, there was a 60% increase in amylase release from acini prepared by the new method. In Table 1 these results are compared with those obtained with the isolated and perfused rat pancreas. Similarly to what was observed in acini prepared with our new technique, in the perfused pancreas maximal stimulation was achieved at 10^{-10}M-caerulein, with lower secretory rates being stimulated by 10^{-11}M and 10^{-9}M-caerulein.

Secretin stimulated amylase release from acini prepared by both techniques. The fold stimulation seen with acini prepared by the 'old' method was similar to that published by Bissonette *et al.* (1984). For acini prepared by the 'new' technique, the incremental secretion (above basal) at maximal stimulation with secretin was approximately double that for the 'old' technique of preparation (Fig. 2; $F = 15.3$, $P < 0.001$).

In conclusion, we have described a new technique which produces as many intact acini as with previously used techniques. The preparation time was considerably decreased, together with a diminished exposure to collagenase, which allowed us to use approx. 75% less collagenase in the new method. In addition, acini proved to be either more sensitive or more responsive to the secretagogues

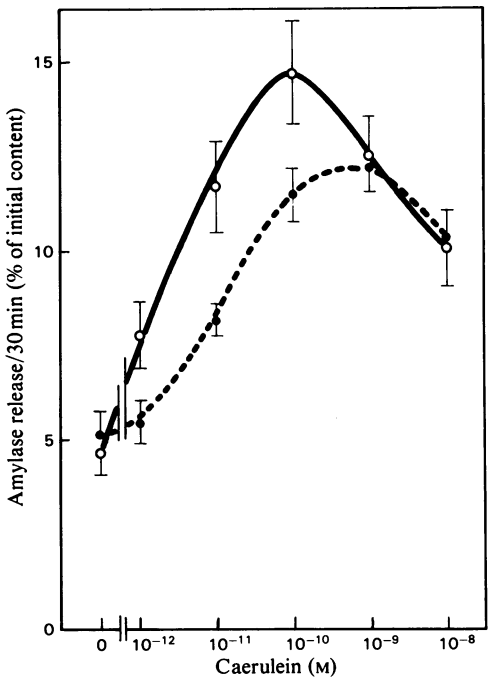

Fig. 1. *Dose-dependence of caerulein-induced amylase release from rat pancreatic acini*

For details, see the Experimental section. Results are shown as means ± S.E.M.: ○, 'new' method ($n = 5$); ●, 'old' method ($n = 4$).

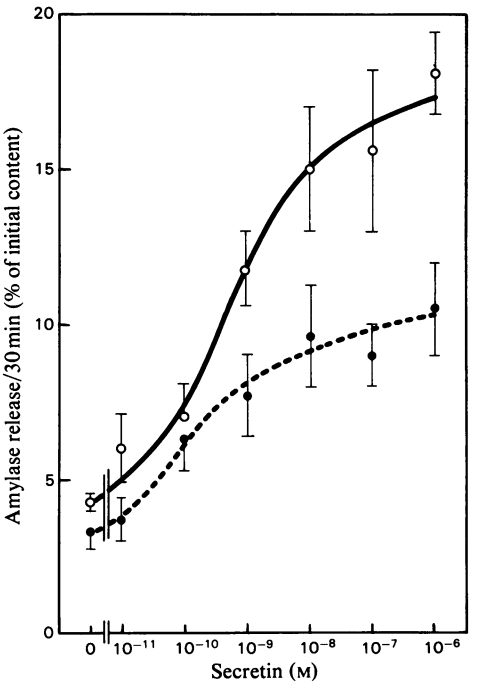

Fig. 2. *Dose-dependence of secretin-induced amylase release from rat pancreatic acini*

For details, see the Experimental section. Results are shown as means ± S.E.M. for six separate experiments for each group: ○, 'new' method; ●, 'old' method.

Table 1. *Comparison of caerulein-induced amylase release from the isolated perfused rat pancreas and from dispersed pancreatic acini*

All results (means ± S.E.M.) are expressed as percentages of maximal stimulation. The numbers of experiments are indicated in parentheses.

	Stimulation (% of maximum)			
Caerulein (M) ...	10^{-12}	10^{-11}	10^{-10}	10^{-9}
Perfused pancreas	21 ± 1 (4)	83 ± 5 (4)	100 (4)	65 ± 13 (4)
Acini, 'new' method	55 ± 5 (5)	77 ± 1 (5)	100 (5)	86 ± 4 (5)
Acini, 'old' method	45 ± 5 (4)	67 ± 3 (4)	94 ± 4 (4)	100 (4)

tested. Therefore, in addition to its advantages of simplicity and rapidity, this method is clearly a very satisfactory technique for use in binding and secretion studies.

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