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Isolation of Rodent Pancreatic Acinar Cells and Acini by Collagenase Digestion

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Version 1.0, November 13, 2010 [DOI: 10.3998/panc.2010.18]

Isolated pancreatic acini and isolated acinar cells have become the preparation of choice for many studies of acinar cell function including digestive enzyme synthesis and secretion, signal transduction events including measurement of intracellular Ca^{2+}, and ligand binding studies. Isolated or dispersed exocrine pancreatic cells were first described by Amsterdam and Jamieson in 1972 and have been used by multiple investigators since then (1,2,11,33) followed by the description of isolated acini in 1978 which are now the standard preparation (6,24,30,34).

Acini have been prepared using similar technique from pancreas of a variety of species including humans (15,21), pig (20), and duck (32,36) but the focus in this entry is on rodent (rat, mouse and guinea pig) pancreas. Dissociation involves the use of collagenase and proteases to digest the extracellular matrix after which acinar units can be prepared by mild shearing forces. Acini are purified away from ducts, islets and blood vessels based on size and density although some contamination from other cell types remains. Isolated acini which are usually composed of 8-20 cells retain their junctional complexes (tight and adherens junctions) and three dimensional architecture for short term studies of 2-6 hours. They can be cultured in suspension in tissue culture media for 24-48 hours but show marked loss of polarity and some functions over time (8,16,23).

When a Ca^{2+} chelator and/or proteases or hyalurionidase are added during the digestion period smaller acinar units of 2-6 cells mixed with isolated cells can be produced. Fully isolated cells show rapid loss of polarity and regulated secretion although doublets and triplets with a retained lumen and connecting tight junctions are particularly useful for electrophysiological or imaging studies (3,12,31). By contrast, larger clusters of 2-3 acini may give best results for amylase secretion.

In this entry we describe the procedure used by our laboratory and point out a number of variations used by other investigators. Emphasis will be placed on the choice of collagenase which in our experience is the most important ingredient. We also provide a guide for assessing quality of the acinar preparation based on morphology and amylase secretion.

1. Materials

1.1 Isolation of pancreatic acini

1. Animals: Pancreas is typically obtained from young adult animals (rats, mice, or guinea pig). Older animals may have excess fat in the

pancreas which upon emulsification can be toxic to cells. It is best if possible to avoid prolonged anesthesia and use either decapitation or CO_2 asphixiation prior to removal of the pancreas. An acinar prep can be produced from a single mouse although when more acini are required several pancreases can be digested simultaneously in the same flask.

2. Dissecting Tools: Medium large scissors and forceps for opening the abdomen. Small Iris scissors with either straight or curved tips for removal of the pancreas. A small (4 or 4 ½ inch) curved forceps with serrated tips for handling the pancreas.

3. Early studies used Krebs Henseleit Buffer (KHB) a bicarbonate buffer with half strength calcium chloride or Krebs Hepes Buffer (KHB) without bicarbonate but with 10 or 25 mM Hepes (33,34). Some investigators added fumerate, glutamate and pyruvate as additional energy sources (14,24) but we have not found this to be important for amylase secretion. Over the last five years many investigators have shifted to premade Dulbecco's Modified Eagle Medium (DMEM). DMEM is much easier to use unless changing of the ionic composition is required. DMEM contains amino acids and vitamins which must be added to the house made buffer usually as a concentrate of essential amino acids. Glutamine may be present or added separately. Hepes is usually not included. We currently use GIBCO #11995 DMEM. It contains high glucose which is not necessary but doesn't seem to interfere and glutamine and pyruvate. Essential amino acids can also be obtained from Invitrogen (GIBCO #11130) as a x50 concentrate.

4. Gas: 95% O_2 – 5% CO_2 in a tank with regulator for DMEM or other HCO_3 based buffer. 100% O_2 is used for Hepes buffered Ringer. Check the pH of gassed solutions to make sure they are at 7.35-7.40.

5. No. 2 hardened Whatman filter paper

6. Five cc disposable plastic syringes and 27 gauge needles

7. Polystyrene 10 cm Petri dish

8. Clear polycarbonate Erlenmeyer flasks 25, 50, 125 and 250 ml with rubber or neoprene stoppers.

9. Shaking water bath that will maintain temperature at 37^o and shake at 50-150 cpm. We have used Dubnoff metabolic shaking incubators from Precision instruments for many years although we have also used other models. Flask holders that attach to the bottom are ideal although lead doughnuts can be used to hold flasks in place. Some baths such as the Dubnoff type come with metal plates with clamps that hold Erlenmeyer flasks. The clamps for 25 ml and 50 ml flasks are most useful. The baths we use hold two plates each of which holds 18 25 ml flasks so one can run 36 samples. When using blood dilution vials obtain plastic racks for the vials. These may need to be cut down to fit the bathe. Our baths hold 2 racks each of which will hold 21 blood dilution vials.

10. Small polypropylene funnel

11. 150 micron nylon mesh cloth (Sefar Inc, Depew NY) cut into a 4 x 4 inch square. For preparation of guinea pig acini a slightly larger mesh (200-250 microns) is necessary as their acini are larger. For isolated cells or to enrich for smaller acini a 75-100 micron mesh can be used. In the original version of the procedure we used a nylon stocking as a prefilter. This is more important when using a small mesh cloth which would otherwise get clogged. The nylon mesh can be washed and reused many times but be careful to avoid contamination with soap.

12. 16 ml round bottomed polystyrene test tubes

13. Ten ml polypropylene pipettes (Fisher) with a thumbscrew or electric pipettor. The pipettes can have the tip sliced with a razor blade to make a larger orifice. We generally have one pipette with

a 2-2.4 mm orifice and one with a 0.9-1.1 mm orifice.

14. Bovine Serum Albumin (BSA): We use Millipore Probumin universal grade (k) pH 7.0, catalogue # 81-003-3. **(Note 1)**

15. Soybean Trypsin Inhibitor (SBTI): Sigma purified Type 1-S (Cat# T9003).

16. Collagenase: Collagenase is an endopeptidase that digests native collagen and is usually prepared from *Clostridium histolyticum* and assayed based on its ability to degrade collagen. Unfortunately, several different units of measurement are used. As prepared, collagenase also contains caseinase, a non specific protease and tryptic activity as well as clostripain, a trypsin like enzyme. Some protease activity is necessary for tissue dissociation as pure recombinant collagenase is not effective. Collagenase is purified by gel filtration which can be done in the lab or as purchased from Worthington. If the collagenase prep is too pure or if isolated cells are desired it may be necessary to add back purified protease (not trypsin). We have in the past used CLSPA purified collagenase from Worthington selected for low clostripain levels and this has been the most common form used. Worthington maintains data sheets for each batch and has a collagenase sampling program. Once a good batch is identified it is useful to purchase a large amount that can be stored frozen for years. We usually use 1000 U per pancreas dissociation although if the purity of the collagenase is over 1200 U/mg it may be necessary to use more to get enough contaminating protease. For purified collagenase it is most effective to dissolve the contents of a single bottle (10,000 U) in 1 ml of saline and store frozen aliquots. Because of difficulties in identifying good lots of Worthington Collagenase we and others have also recently used Collagenase from Crescent Chemicals (Islandia, New York) which is prepared by Serva (4,9,25,26,28). We currently use either Worthington CLSPA purchased as 10,000 U per bottle or Serva NB8 Grade purchased as 0.25 or 1 gram lots and utilize 1mg per digestion. Other collagenase used by investigators includes Collagenase P from Roche (18,37), Sigma Type II (35), IV or V (19), and Worthington CLS 4 (10). Most of these have a higher protease content. Collagenase P is often used to prepare small acinar units for electrophysiology. As will be described later we use cell morphology and amylase dose-response curves to confirm the state of acini prepared with any new batch of collagenase.

17. Simple lab microscope and slides to view drops of acinar suspension

18. Stopwatch or timer

1.2 Measurement of Amylase Release

1. Polystyrene disposable blood dilution vials from Fisher or Evergreen. These have a snap on lid and a flat bottom and are ideal for incubating 1ml acinar aliquots which is necessary for mouse acini where less material is available. Polycarbonate Erlenmeyer flasks are used for larger volume incubations. Twenty five ml flasks are used for 2 ml aliquots of acini.

2. Micropipettes with disposable tips for aliquoting acini and adding secretagogue. We use a Pipettman P-1000 and cut off the tips for aliquoting acini.

3. DMEM medium supplemented with 1 mg/ml of BSA and 0.1 mg/ml of SBT

4. Shaking water bath capable of speeds of 50-200 cpm and temperature control as described for the preparation of acini.

5. Digital timer capable of counting up from 0 to at least 60 min and 0.01 minute resolution. Large numerals are helpful. Timers that measure in seconds can also be used but are not as easy to use quickly.

6. Tabletop microcentrifuge that spins at 2000 g or higher and starts and stops quickly. We use a miniature version that holds 6-8 microcentrifuge tubes and goes on when the top is held down.

7. 1.5 ml snap top microcentrifuge tubes from Evergreen or other supplier in bulk

8. 6 ml (12 x 75 mm) polystyrene test tubes

9. Phadebas amylase assay tablets (Magle Life Sciences, Lund Sweden) and buffer as described in the product insert. **(Note 2)**

10. A spectrophotometer that will read plastic test tubes at 675 nm.

11. Stock solution of CCK-8 (10^{-4}M) or carbachol (10^{-2}M). CCK is normally dissolved in isotonic saline with 1 mg/ml BSA, aliquoted as 50 or 100 ul volume in 0.5ml microcentrifuge tubes and frozen. Carbachol (Carbamylcholine chloride) is dissolved in water to make 1-2 ml and kept in a refrigerator.

2. Methods

2.1 Isolation of Pancreatic Acini

1. Prepare experimental solutions starting by determining the total amount of DMEM that will be used, bubble it with 5% CO_2/95% O_2 using a disposable Pasteur pipette in a graduated cylinder or Erlenmeyer flask (usually 150-200 ml). Bubbling is stopped and SBTI totaling 0.1mg/ml is added to the surface and the vessel sealed with parafilm. After the SBTI dissolves, the container is gently mixed and the solution divided into four components shown below in polycarbonate Erlenmeyer flasks that are sealed with a stopper or parafilm. The amounts listed will work for 1 rat or 1-3 mouse pancreas.

(D)igestion 10ml 25mg BSA 1000U or 1 mg collagenase
(R)inse 10ml 100mg BSA
(C)entriguge 50ml 2000mg BSA
(I)ncubation 100ml 100mg BSA

Dissolve the BSA and collagenase by pouring it on top of the solution and allowing it to absorb and dissolve without shaking or mixing. It can be mixed after it dissolves. We use polycarbonate flasks that are about 2.5-5 times the volume of the liquid to be contained so that the flask can be gassed and hold a reservoir of gas to exchange with the liquid. If neutral BSA is used it is not necessary to check or adjust the pH every time.

2. Sacrifice the rodent (We use decapitation or CO_2 asphyxiation because it is quick and drug free), open the abdomen and displace the intestines to reveal the pancreas. Remove the pancreas by cutting the mesentery with Iris scissors starting with the splenic end, cutting along the stomach and finally along the intestine while putting slight tension on the pancreas by holding it at the splenic end with the serrated forceps. It is desirable to remove the pancreas in one piece without cutting into the gland to facilitate the injection. The pancreas is quickly placed in a Petri dish containing PBS and any obvious large lymph nodes, fat or other tissue cut off. The pancreas is then quickly rolled on Whatman #2 filter paper to remove any adherent blood and fluid and placed in a clean Petri dish with 5 ml digestion solution (D).

3. Suck up as much of the D solution as possible in the Petri dish with a 5 ml plastic syringe, cap with a 27 gauge needle and inject into the pancreas parenchyma until well distended. Repeat multiple times to cover the entire pancreas. This will take 5-10 min after which the pancreas can be cut into 3-4 pieces if desired. Note: in some protocols the pancreas is minced into small pieces under 1 mm in size prior to incubation rather than injecting. We find that injection gives better results as mincing damages many cells. To isolate islets the digestion solution is injected retrograde into the main pancreatic duct before the pancreas is removed. This tends to isolate the islets which after a short digestion can be hand picked but does not dissociate individual acini as well.

4. Transfer the pancreas and any extra D solution to a 25 ml polycarbonate flask, gas with 5% CO_2/95% O_2 (10-15 seconds at a flow rate strong enough to dimple the solution surface), cover with a rubber stopper and incubate at 37^0 shaking 120 cycles per minute for 10 min. Make sure the level of fluid in the bath is as high as the level in the flask to maintain temperature in the flask.

5. Remove the liquid from the flask using a plastic transfer pipette being careful not to suck up the pancreas and then add the remaining 5ml of fresh D solution. Gas, stopper and incubate for an additional 40-45 min under the same condition. With less pure collagenase a shorter incubation time is often used. Gas R and C solutions near the beginning of this incubation period to assure oxygenation and pH regulation when they are used **(Note 3)**.

6. Suck the pancreas up and down with the large orifice 10 ml polypropylene pipette 5-6 times followed by 5-6 times with the narrow orifice 10 ml pipette. This was originally done by mouth which gives finer control but is now done with a thumbscrew (my preference) or motorized pipettor (used by most people) for safety reasons. It is the passage through the orifice that generates the shearing forces. If the pancreas sticks in the pipette blow it out and start over. The resulting mix should be a fine puree without visible large chunks. Too vigorous pipetting can damage the acini.

7. The suspension is then filtered through 150 micron nylon cloth into a 50 ml Erlenmeyer flask through a polypropylene funnel. Wash the filter by pouring through the 10 ml of R solution. There should be a thin residual layer on top of the filter. If you put the filter in PBS or other media and put a drop on a microscope slide, this material will include acini, islets, blood vessels, ducts, and occasional foreign debris. A drop of the 20 ml of filtrate on a microscope slide will reveal predominantly acini and fine debris.

8. Set up four 16 ml tubes in a rack and put about 6 ml of C solution in each. Using a wide orifice (cut to enlarge) soft transfer pipette, layer ¼ of the filtrate on top of the C solution. This requires a gentle approach to keep the solutions as separate layers and to avoid damage to the acini. Let the acini settle by gravity for 5-10 min or centrifuge at 50 x g for 2 min. Remove the supernatant and resuspend each pellet by letting a stream of 5 ml of C solution fall on the pellet by gravity. If it doesn't resuspend, you centrifuged too hard previously. Combine the four tubes into two tubes and allow to settle or centrifuge again.

9. Remove supernatant as above and resuspend each pellet in 5 ml of I solution, combine into one tube and let acini settle out or centrifuge. This will go faster as the solution is less dense. This serves as an extra rinse and to transfer into the new solution. When necessary to completely remove a component of the medium or to remove damaged cells an extra rinse step with I solution can be performed.

10. Remove the supernatant, resuspend acini in 20-30 ml (mouse) or 50 ml (rat) I solution in a 125 or 250 ml polycarbonate flask, gas with 5% CO_2/95% O_2, stopper or seal with parafilm and preincubate in 37^o waterbath for 30 min. Use lead doughnut to secure the flask. If carrying out a drug/inhibitor study, split suspension in two in separate flasks and use one for control and one for treatment. You can also preincubate without shaking (shaking seems to activate some of the MAPKs) and in this case we use 2 hours. Incubating longer than 30-60 min requires repeat gassing. The purpose of the preincubation is to allow any damaged cells to die or recover and for the operator to clean the bench and get read for the incubation phase. Amylase secretion works best with a short preincubation.

11. Let acini settle by gravity (or centrifuge), resuspend acini in 25-75 ml of freshly gassed incubation solution for the experiment. The amount depends on the number of acini and number of samples needed. Amylase release

works fine with dilute acini at a concentration of 0.1 to 0.2 mg protein/ml. For studies of protein phosphorylation or to be followed by immunoprecipitation one may need to use acini at up to 0.5 to 1 mg protein/ml. For most experiments 1 ml aliquots are taken with a pipettor with cut off tips and ensuring that the flask is gently swirled while the acini are taken and that the tip is in the same area of the flask each time. The 1ml aliquots are placed in blood dilution vials. If larger samples are needed, place 2 ml into 25 ml polypropylene flasks. In any case it is important to that the acinar suspension be sucked up and expelled gently to minimize shearing forces and avoid damage.

2.2 Measurement of amylase secretion dose response curve

1. Decide on the experimental protocol. We use either CCK-8 or carbachol as a routine stimulating agent and run a dose-response curve as the shape of the curve as well as basal and maximum levels are important. For CCK use 0, 1, 3, 10, 30, 100, 300, 1000, and 10,000 pM concentrations for a full curve. The 1 and 10,000 pM can be omitted for most purposes. For Carbachol use 0, 0.1, 0.3, 1, 3, 10, and 30 micromolar concentrations. Dilute the secretagogue stock in Incubation medium which contains 1mg/ml BSA and 0.1 mg/ml SBTI and prepare it to a concentration 200 times the desired final. Thus stock for 1 nM CCK will be 200 nM. We make 1-2 ml of each stock in 12 x 75 mm polystyrene tubes which are labeled and placed in an ice bucket. Stocks for lower concentrations are easily made by serial dilution. Each secretagogue concentration is run in duplicate although we often use four flasks, two at the beginning and two at the end for the 0 secretagogue point. Thus a seven point dose response curve will require 16 flasks. Number the sample flasks and set up a sheet that for each flask lists the secretagogue concentration, start and stop times and has room to record the amylase assay value.

2. Label a blood dilution vial for each sample, a microcentrifuge tube, and a test tube to receive the supernatant. Four microcentrifuge tubes labeled T_{oA}, T_{oB}, T_{oC} and T_{oD} will be used for time zero (T_o) samples. This allows the subtraction of amylase in the medium prior to the incubation with secretagogue.

3. Following preincubation of acini, let settle in a 50 ml conical centrifuge tube and gently resuspend in a volume of 10-15 ml more than the total required for the 16 flasks. For mouse acini this is usually 25-35 ml and more for rats. The desired density is 0.1-0.2 mg acinar protein/ml. The acini are held in a 125 ml polycarbonate Erlenmeyer, gassed, covered and left on the desk top.

4. Using a 1ml manual micropipettor with cut off tips, place 1ml aliquots of acini into microcentrifuge tubes T_{oA} and T_{oB} and incubation vials 1 and 2. Immediately cap and centrifuge the To samples for 20 seconds and place in ice bucket. Add 5 ul of concentrated secretagogue to vials 1 and 2, gas, cap, and place in 37^o water bath shaking at 50-60 cpm. Start timer at zero and note time on procedure sheet. This gets repeated for a pair of samples every 1.5 to 2 min with the time noted on the sheet. At the end place two samples in T_{oC} and T_{oD} and centrifuge. Carefully remove the supernatant from the T_o tubes and place in clean tubes. Retain the supernatants and the pellets in the ice bucket. It may help to have more than one ice bucket. Starting the incubation is usually is finished in 15-20 min and for larger experiments the limit is 30 min. The key point in the above procedure is to gently and continuously swirl the large flask of acini and to withdraw aliquots from the middle of the flask to ensure even distribution of acini.

5. Remove each pair of incubation vials 30 min after the start of their incubation with secretagogue and transfer the contents with a cut off pipette tip into the numbered microcentrifuge tube and centrifuge for 10-15 seconds. Remove the supernatant and place in a clean tube on ice.

6. To the T_o pellets add 1 ml of water. Sonicate each sample with a probe type sonicator on ice for several bursts of 3-5 sec, take a 100 µl aliquot and add to 900 µl of ice cold Incubation medium to reduce protease digestion and keep on ice. The samples (T_o's and acinar supernatants) will keep several hours on ice or even overnight in the refrigerator although the latter is for backup assay if necessary

7. Assay amylase in 10 µl of the T_o's, acinar supernatants, and diluted T_o pellets. We have used various forms of starch labeled with a dye but currently use the Phadebas amyase assay substrate. **(Note 1)** Each sample requires 1 ml of reagent in buffer. Prepare a little more than enough for the number of samples by dissolving one pellet per 14 ml buffer that is specified by the product insert in a small beaker with a magnetic stirrer. Pipette out 1 ml aliquots while stirring to prelabeled tubes and then add the 10 ul aliquots at timed interals. Place the rack of tubes in a 37^o shaking waterbath and incubate for 20 min after which the reaction is stopped by adding 300 µl of 0.5 M NaOH. Tubes are centrifuged and supernatant read in a spectrophotometer at 620 nm and the OD recorded. Subtract the appropriate T_o reading from each acinar supernatant and multiply the mean total (T_o pellet) by 10. Because the T_o value will increase over time we use linear extrapolation to estimate the T_o to be subtracted. Per cent release is then obtained by dividing the secreted by the average total **(Note 4)**.

2.2 Assessment of Isolated Acini

In order to obtain the most valid and informative functional information it is critical that isolated acini be structurally and functionally intact. Therefore for individuals learning how to prepare acini and periodically in their use it is important to evaluate their condition. Some of these approaches often are part of an evaluation of function. The following approaches have been used.

1. Microscopic evaluation – Microscopic evaluation is the easiest approach and although qualitative can be recorded digitally and compared to other results. It is best to have a bright field upright microscope in the lab with 10x, 20x and 40x objectives. Phase contrast is not essential and the idea is to be able to take a quick look by putting a drop of acinar suspension on a slide. This will reveal whether debris is present, the size of the acini and whether they look intact with smooth bulging basolateral surfaces. The basal cytoplasm is clear and has a reflective sheen. Enzymatic underdigestion results in large clusters of acini while use of too vigorous shearing forces will lead to broken acini. The most critical structures to look for are blebs which are a sure sign of damage. Persistence in checking each preparation with a microscope is essential for developing an understanding of what constitutes poor, good and excellent acini. Examples of acini are shown in **Fig. 1**. For photographing acini an inverted stage microscope with a digital camera is ideal. If the microscope is equipped for fluorescence it is then possible to visualize GFP as well (8) and to use fluorescent indicators of cell viability (see below). Use a small petri dish with a hole in the bottom with a coverslip glued over it for best resolution. These can be purchased from several suppliers. We normally do not put a coverslip over the drop of acini as they will become squashed and then anoxic in 3-5 min.

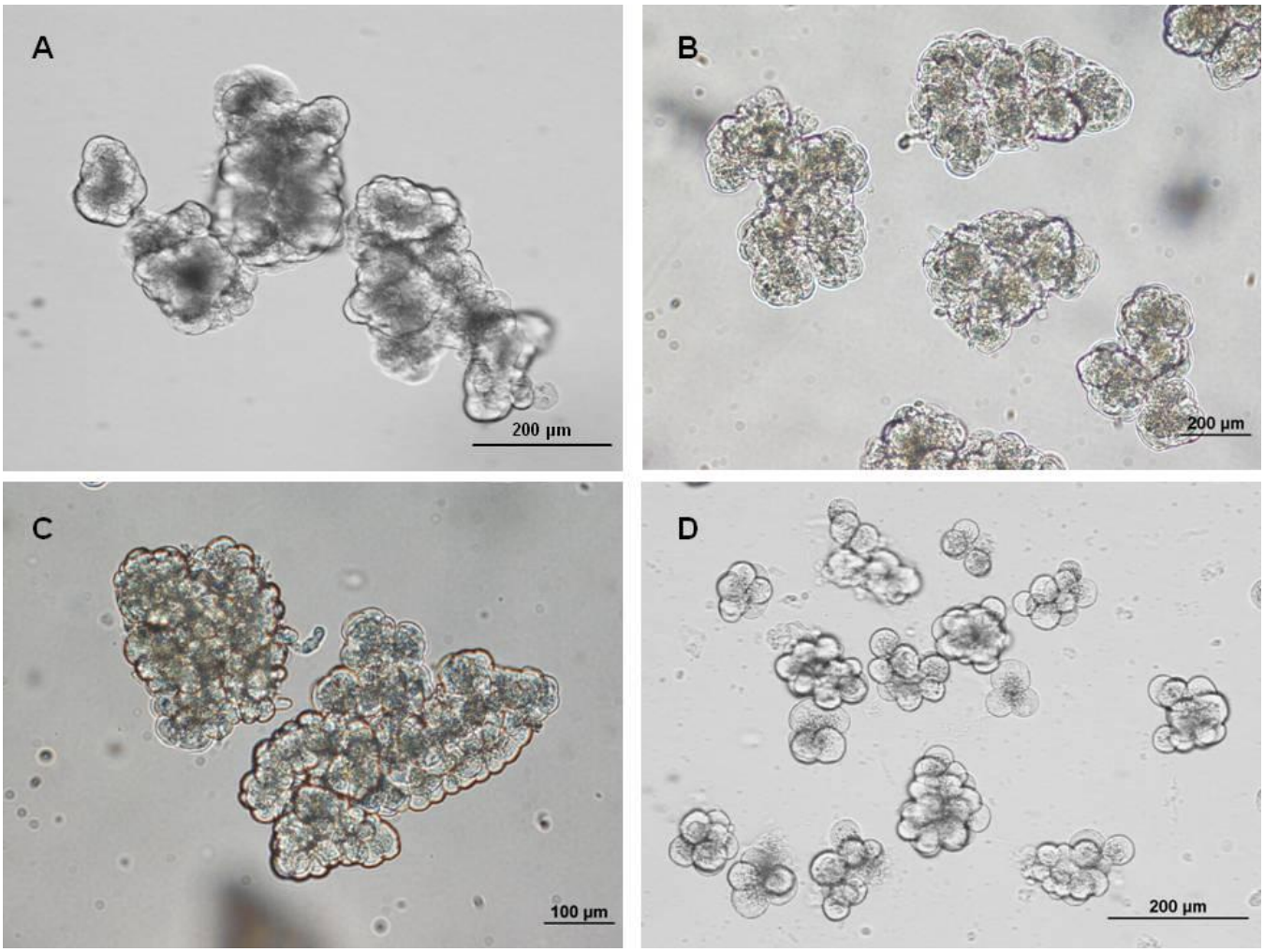

Fig 1. Microscopic appearance of freshly isolated pancreatic acini. A. Mouse acini digested with Crescent Collagenase (lot no longer available) that is pretty good looking but not the greatest. B. Rat acini prepared with Worthingron CLSPA collagenase. A little underdigested and ragged but gave a good amylase release curve (Fig 2). C. Mouse acini, underdigested such that several acini remain in a cluster; gave a reasonable amylase release curve. D. Mouse acini prepared with a 10 min calcium chelation step in between the two collagenase digestions. Note the individual cells tend to bulge out and there are a number of single cells and one beautiful triplet. These acini were used to place in monolayer culture and would not give as good an amylase release curve as the acini in the other panels. The receptors and Calcium signaling however will be in good shape. Images were taken with a Olympus DP71 camera on a Nikon inverted microscope after placing the acinar suspension in a small dish with a cover slip on the bottom.

2. Trypan blue and other vital dyes – Trypan blue is often used to evaluate viability of cultured cells. Intact cells exclude Trypan blue which is a negatively charged complex molecule of MW 883 while damaged cells take up the dye where it is visible upon microscopic evaluation. It can be purchased or dissolved as a 0.4% solution in isotonic NaCl and added at about 1:4 volumes of acinar suspension.

Good viability is considered to be exclusion of dye by over 95% of cells. If one has a fluorescent microscope at hand one can use propidium iodide in a similar manner as it is excluded from live cells and because it concentrates in nuclei the positive cells are easier to count. Commercial live cell staining kits often include a live cell marker such as Calcein-AM and a dead cell marker such as ethidium bromide but are primarily designed for evaluation of isolated cells in suspension with a plate reader. We have not found any of these to be useful for routine evaluation of the state of acini in the middle of an experiment. The signal is often intermediate making determination of dead cells difficult and increases over time on the slide.

Furthermore the three dimensional structure of acini makes it difficult to quantitate positive cells in a suspension. Finally, freshly prepared acini may be slightly leaky and then seal up latter. These methods, however, can be used to evaluate the effect of a damaging agent on stable recovered acini although we have found biochemical measurements such as lactate dehydrogenase (LDH) release to be easier and more reproducible (7, 13, 27).

3. Amylase release dose response curves are in our view the best way to determine the state of acini and to give feedback to new investigators interested in acinar function. Parameters important in evaluation are basal (unstimulated) release as a percent of total content, maximal release and the concentration of secretagogue inducing maximal amylase release. There are clear species differences that need to be taken into account. For rat acini, ideally basal release should be around 2% per 30 min, maximal release 16-20% and peak release at 100 pM CCK8. For mouse acini basal release is higher, usually 3-5% and should not be over 5% and maximal release is 10-16%. Thus fold stimulation is much lower for mouse acini. Peak release is usually at 30 pM CCK8. Guinea pig acini behave more like rat acini and may show even higher stimulated release. In all cases the dose response curve should be biphasic (**Figs 2,3**) with a fall off of amylase release at supramaximal concentrations of CCK.

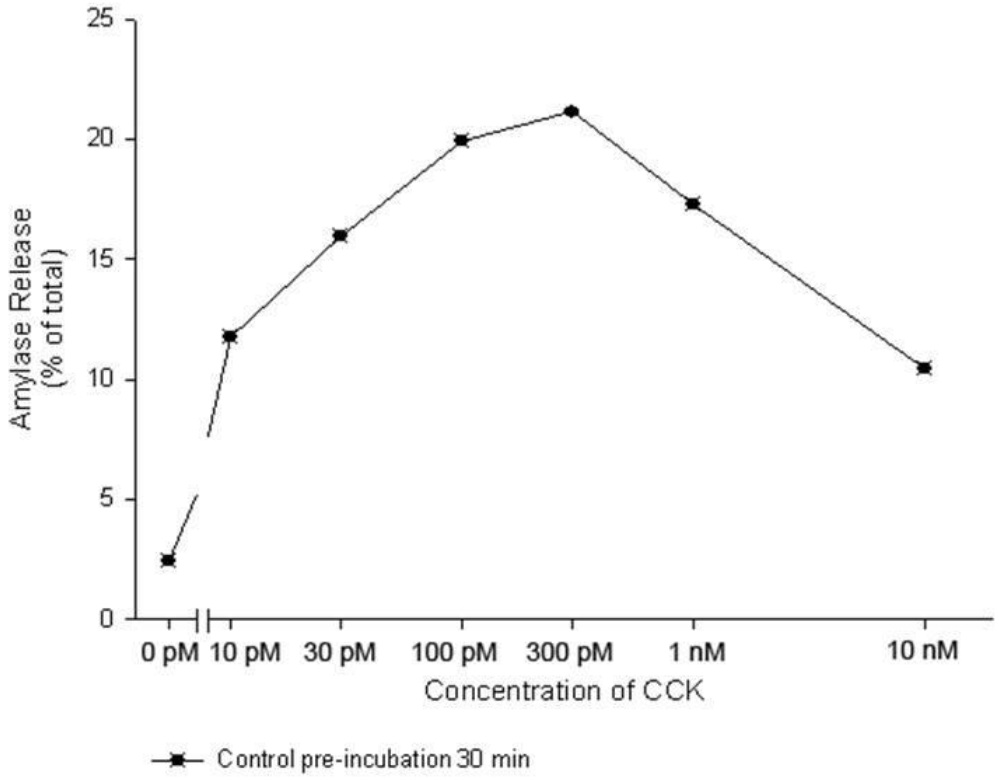

Fig 2. Single experiment amylase release curve in response to CCK for rat acini shown in **Fig 1B**. The basal release of slightly over 2%/30 min and the 10 fold stimulation are excellent. Likewise the prep shows good sensitivity to CCK with half maximal stimulation at 10 pM. However, the curve is broader than ideal and peak release is at 300 pM rather than 100 pM.

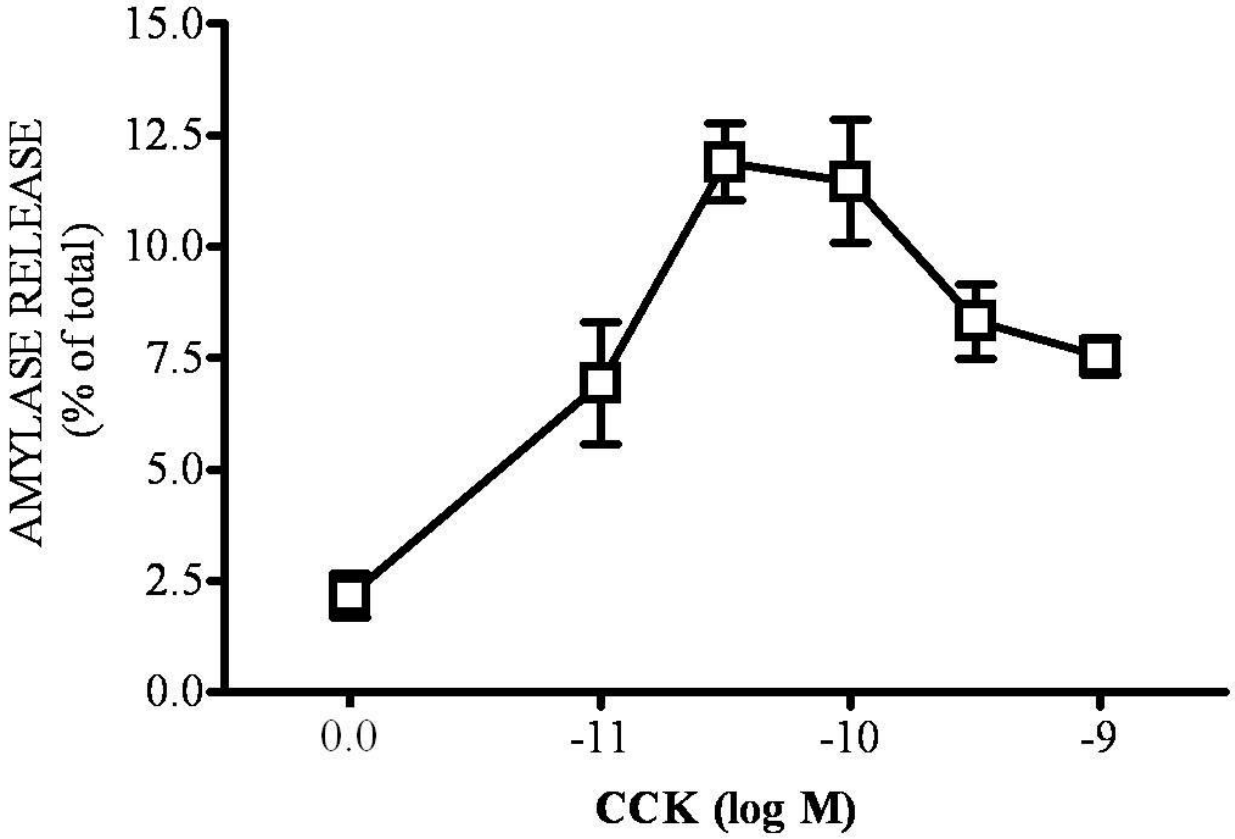

Fig 3. Single experimental amylase release curve from ICR mouse acini in response to CCK.
This is a typical good curve for mouse acini with basal release of about 2.5%/30min, a 5 fold stimulation and peak release at 30 pM CCK_8

4. More sophisticated morphological evaluation focuses on maintainance of cell polarity and a normal actin cytoskeleton. This requires brief fixation in 4% formaldehyde, pelleting and freezing followed by evaluation of frozen sections by wide field or confocal microscopy. We cut sections at 5 μm and stain with DAPI and fluorescent tagged phalloidin which binds to filamentous actin. Alternatively, fixed acini can be stuck down on a glass slide and stained with fluorescent phalloidin followed by z-series confocal microscopy (29). If desired one can also immunostain to reveal zymogen granules or evaluate by differential interference contrast (DIC). In well preserved acini, ZG cluster around the lumen in the apical pole of the cell and a prominent subapical band of filamentous actin is observed.

3. Notes

1. Most albumin is purified by isoelectric precipitation at pH 5.5. This will result in an acidic solution particularly at a high concentration when this albumin is dissolved. Use of neutral albumin avoids having to adjust the pH latter. We have not found any better results with fatty acid depleted BSA but feel a high purity albumin is worth the expense.

2. An alternate assay system is to use 4-Nitrophenyl α-D-maltohexaoside (Sigma #73681) as a substrate in a end point or kinetic assay in which the product is measured at 405 nm and can be modified for 96 well plates and a plate reader (17). This and related substrates can also be used in an autoanalyzer.

3. An alternative method to dissociate acini was published in which rat pancreas was minced and then vigorously hand shaken for 4-10 min (5) Although this "new" method was claimed to give better results it was mainly due to their "old" method results being substandard. In their favor the preparation time was reduced and less collagenase was used.

4. To obtain absolute values for amylase concentration in plasma, the Phadebas assay kit requires one dye pellet per plasma sample and the results are read against a standard curve that comes with the dye. To keep the cost reasonable and because we are calculating relative amylase release we dilute the dye. Another technical issue is that to get accurate percent release all the amylase in the pellet must be solubilized. Some investigators have used detergent for this (0.1% Triton X-100 in 150mM NaCl) but we found that sonicating in water works similarly although the sample must be immediately diluted in incubation media to inhibit trypsin activity. Because total amylase is in the denominator when calculating amylase release an error here will make the entire release curve appear high or low but the shape and fold release will be normal. For this reason some investigators report release as per cent of control release but if this is done the mean basal release should also be reported.

4. References

1. Amsterdam A, Jamieson JD. Structural and functional characterization of isolated pancreatic acinar cells. Proc. Natl Acad Sci USA 69:3028-3032, 1972. PMID: 4342974
2. Amsterdam A, Jamieson JD. Studies on dispersed pancreatic exocrine cells. I. Dissociation technique and morphological characteristics of separated cells. J Cell Biol. 63:1037-1056, 1974. PMID: 4436379
3. Ashby MC, Camello-Almaraz C, Gerasimenko OV, Petersen OH, Tepikin AV. Long distance communication between muscarinic receptors and Ca^{2+} release channels revealed by carbachol uncaging in cell-attached patch pipette. J Biol Chem. 278:20860-20864. PMID: 12657637
4. Bi Y, Williams JA. A role for Rho and Rac in secretagogue-induced amylase release by pancreatic acini. Am J Physiol Cell Physiol. 289:C22-C32, 2005. PMID: 15743890
5. Bruzzone R, Halban PA, Gjinovci A, Trimble ER. A new, rapid, method for preparation of dispersed pancreatic acini. Biochem J. 226:621-624, 1985. PMID: 2581556
6. Bunham D, McChesney DJ, Thurston KC, Williams JA. Interaction of cholecystokinin and vasoactive intestinal polypeptide on function of mouse pancreatic acini in vitro. J. Physiology 349:475-482, 1984. PMID: 6204039
7. Chandler DE, Williams JA. Intracellular uptake and amylase and lactate dehydrogenase releasing actions of the divalent cation ionophore A23187 in dissociated pancreatic acinar cells. J Membrane Biol. 32:201-230, 1977. PMID: 405495
8. Chen X, Edwards JAS, Logsdon CD, Ernst SA, Williams JA. Dominant negative Rab3D inhibits amylase release from mouse pancreatic acini. J Biol Chem 277:18002-18009, 2002. PMID: 11875077
9. DiMagno M, Lee S-H, Owyang C, Zhou S. Inhibition of acinar apoptosis occurs during acute pancreatitis in the human homologue ΔF508 cystic fibrosis mouse. Am J Physiol Gastrointest Liver Physiol 299:G400-G412, 2010. PMID: 20522641
10. Frick TW, Fernàndez-del-Castillo C, Bimmler D, Warshaw AL. Elevated calcium and activation of trypsinogen in rat pancreatic acini. Gut. 41:339-343, 1997. PMID: 9378389
11. Gardner JD, Jackson MJ. Regulation of amylase release from dispersed pancreatic acinar cells. J Physiol. 270:439-454, 1977. PMID: 198531
12. Giovannucci DR, Bruce JIE, Straub SV, Arreola J, Sneyd J, Shuttleworth TJ, Yule DI. Cytosolic Ca^{2+} and Ca^{2+}-activated Cl^- current dynamics: insights from two functionally distinct mouse exocrine cells. J Physiol. 540:469-484, 2002. PMID: 11956337

13. Gukovskaya AS, Gukovsky I, Zaninovic V, Song M, Sandoval D, Gukovsky S, Pandol SJ. Pancreatic acinar cells produce, release, and respond to tumor necrosis factor-alpha. Role in regulating cell death and pancreatitis. J Clin Invest. 100:1853-1862, 1997. PMID: 9312187
14. Hoffman KM, Tapia JA, Berna MJ, Thill M, Braunschweig T, Mantey SA, Moody TW, Jensen RT. Gastrointestinal hormones cause rapid c-Met receptor down-regulation by a novel mechanism involving clatherine-mediated endocytosis and a lysosome-dependent mechanism. J Biol Chem. 281:37705-37719, 2006. PMID: 17035232
15. Ji B, Bi Y, Simeone D, Mortensen RM, Logsdon CD. Human pancreatic acinar cells lack functional responses to cholecystokinin and gastrin. Gastroenterology 121:1380-1390, 2001. PMID: 11729117
16. Logsdon CD, Williams JA. Pancreatic acini in short-term culture: regulation by EGF, carbachol, insulin, and corticosterone. Am J Physiol. 244:G675-G682, 1983. PMID: 6190410
17. Lorentz K. Evaluation of alpha-amylase assays with 4-nitrophenyl-alpha-oligosaccharides as substrates. J Clin Chem Clin Biochem. 21:463-471, 1983. PMID: 6604787
18. Means AL, Meszoely IM, Suzuki K, Miyamoto Y, Rustgi AK, Coffey Jr RJ, Wright CVE, Stoffers DA, Leach SD. Pancreatic epithelial plasticity mediated by acinar cell transdifferentiation and generation of nestin-positive intermediates. Development 132:3767-3776, 2005. PMID: 16020518
19. Mooren FC, Turi S, Günzel D, Schlue W-R, Domschke W, Singh J, Lerch MM. Calcium-magnesium interactions in pancreatic acinar cells. FASEB J. 15:659-672, 2001. PMID: 11259384
20. Morisset J, Levenez F, Corring T, Benrezzak O, Pelletier G, Calvo E. Pig pancreatic acinar cells possess predominantly the CCK-B receptor subtype. Am J Physiol. 271:E397-E402, 1996. PMID: 8770035
21. Murphy JA, Criddle DN, Sherwood M, Chvanov M, Mukherjee R, McLaughlin E, Booth D, Gerasimenko JV, Raraty MG, Ghaneh P, Neoptolemos JP, Gerasimenko OV, Tepikin AV, Green GM, Reeve Jr. JR, Peterson OH, Sutton R. Direct activation of cytosolic Ca2+ signaling and enzyme secretion by cholecystokinin in human pancreatic acinar cells. Gastroenterology 135:632-641, 2008. PMID: 18555802
22. Park HS, Betzenhauser MG, Won JH, Chen J, Yule DI. The type 2 inositol (1,4,5)-triphosphate ($InsP_3$) receptor determines the sensitivity of $InsP_3$-induced Ca^{2+} release to ATP in pancreatic acinar cells. J Biol Chem. 283:26081-26088, 2008. PMID: 18658132
23. Park MK, Lee M, Petersen OH. Morphological and functional changes of dissociated single pancreatic acinar cells; testing the suitability of the single cell as a model for exocytosis and calcium signaling. Cell Calcium 35:367-379, 2004. PMID: 15036953
24. Peikin SR, Rottman AJ, Batzri S, Gardner JD. Kinetics of amylase release by dispersed acini prepared from guinea pig pancreas. Am J Physiol. 235:E743-E749, 1978. PMID: 736135
25. Sabbatini ME, Chen X, Ernst SA, Williams JA. Rap1 activation plays a regulatory role in pancreatic amylase secretion. J Biol Chem. 283:23884-23894, 2008. PMID: 18577515
26. Sabbatini ME, Bi Y, Ji B, Ernst SA, Williams JA. CCK activates RhoA and Rac1 differentially through Galpha13 and Galphaq in mouse pancreatic acini. Am J Physiol Cell Physiol. 298:C592-C601, 2010. PMID: 19940064
27. Saluja AK, Bhagat L, Lee HS, Bhatia M, Frossard JL, Steer ML. Secretagogue-induced digestive enzyme activation and cell injury in rat pancreatic acini. Am J Physiol Gastrointest Liver Physiol. 39:G835-G842, 1999. PMID: 10198325
28. Sans MD, Xie Q, Williams JA. Regulation of translation elongation and phosphorylation of eEF2 in rat pancreatic acini. Biochem Biophys Res Commun. 319:144-151, 2004. PMID: 15158453
29. Schäfer C, Ross SE, Bragado MJ, Groblewski GE, Ernst SA, Williams JA. A role for the p38 mitogen-activated protein kinase/Hsp 27 pathway in cholecystokinin-induced changes in the actin cytoskeleton in rat pancreatic acini. J Biol Chem. 273:24173-24180, 1998. PMID: 9727040
30. Schultz GS, Sarras Jr P, Gunther GR, Hull BE, Alicea HA, Gorelick FS, Jamieson JD. Guinea pig pancreatic acini prepared with purified collagenase. Exp Cell Res. 130:49-62, 1980. PMID: 6256185
31. Toescu EC, Lawrie AM, Petersen OH, Gallacher DV. Spatial and temporal distribution of agonist-evoked cytoplasmic Ca^{2+} signals in exocrine acinar cells analysed by digital image microscopy. EMBO J. 11:1623-1629, 1992. PMID: 1563357

32. Wang BJ, Liang HY, Cui ZJ. Duck pancreatic acinar cell as a unique model for independent cholinergic stimulation-secretion coupling. Cell Mol Neurobiol. 29:747-56, 2009. PMID: 19370412
33. Williams JA, Cary P, Moffat B. Effects of ions on amylase release by dissociated pancreatic acinar cells. Am J Physiol. 231:1562-1567, 1976. PMID: 793424
34. Williams JA, Korc M, Dormer RL. Action of secretagogues on a new preparation of functionally intact, isolated pancreatic acini. Am J Physiol: Endocrinol Metab Gastrointes Physiol. 235:E517-E524, 1978. PMID: 215042
35. Won JH, Cottrell WJ, Foster TH, Yule DI. Ca^{2+} release dynamics in parotid and pancreatic exocrine acinar cells evoked by spatially limited flash photolysis. Am J Physiol Gastrointest Liver Physiol 293:G1166-G1177, 2007. PMID: 17901163
36. Xiao R, Cui ZJ. Mutual dependence of VIP/PACAP and CCK receptor signaling for a physiological role in duck exocrine pancreatic secretion. Am J Physiol Regul Integr Comp Physiol. 286:R189-R198, 2004. PMID: 12947031
37. Zhao H, Muallem S. Na^+, K^+, and Cl^- transport in resting pancreatic acinar cells. J Gen Physiol. 106:1225-1242, 1995. PMID: 8786358