EFFECTS OF SECRETIN AND CAERULEIN ON ENZYMES OF CULTURED PANCREATIC ACINAR CELLS

KAREN K. HIRSCHI, SHARON KENNY, JILL D. JUSTICE, AND PATSY M. BRANNON

Department of Nutrition and Food Science and Nutritional Sciences Program,
University of Arizona, 309 Shantz Building, Tucson, Arizona 85721

(Received 3 August 1990; accepted 22 March 1991)

SUMMARY

We examined the effects of secretin (0 to 200 nM) and caerulein (0 to 100 nM) on rat pancreatic acinar cells cultured 0 to 48 h in serum-free medium. The effects of 100 nM secretin with 1 nM caerulein were also studied because secretin may potentiate the effects of caerulein. Cellular and media (secreted) lipase and amylase were analyzed as were cellular DNA and protein content. Cellular lipase and amylase activities significantly decreased (P < 0.0001) over time in all treatment groups, whereas media amylase and lipase significantly increased (P < 0.0001). Neither secretin nor caerulein affected cellular lipase or media amylase. However, secretin significantly increased (P < 0.04) and caerulein tended to increase (P < 0.08) media lipase in a dose-dependent manner. At 12 h, 10 nM secretin maximally increased media lipase (58%), suggesting that cultured acinar cells remain responsive to secretin in vitro. Caerulein, at all concentrations, significantly decreased (P < 0.001) cellular amylase but exhibited a dose-dependent effect only at 24 h when 100 nM caerulein maximally decreased cellular amylase (34%). Secretin (100 nM) did not alter these effects of caerulein. These results support the proposed role of caerulein in the regulation of amylase but not a direct role of secretin in the regulation of lipase.

Key words: caerulein; secretin; amylase; lipase; exocrine pancreas; secretagogue.

INTRODUCTION

The activity, synthesis rate, and mRNA level of lipase adapt to changes in its dietary substrate, triglyceride, increasing 2.4-, 3.1-, and 3.9-fold, respectively, in response to a high fat diet (22). Similarly, amylase activity (9), synthesis rate (15), and mRNA levels (7) increased in response to high carbohydrate diet. The cellular mediators of these pancreatic adaptations remain unknown, but various hormones and nutrients have been proposed as mediators of this dietary adaptation. One hormone, secretin, is proposed to regulate lipase, specifically, because its intravenous infusion for 24 h into rats stimulates lipase synthesis (4-fold) (14). Another gastrointestinal hormone, cholecystokinin, is proposed to regulate amylase and the proteases. Caerulein, a cholecystokinin analog, decreases amylase synthesis (17) without parallel changes in its mRNA levels (21) when infused continuously.

Although these results suggest secretin and caerulein may regulate pancreatic digestive enzymes, interactive effects of these hormones with other hormones or nutrients cannot be eliminated with in vivo infusion studies. To examine the direct effects of each hormone and their possible interactive effects on acinar cells, this present study used an in vitro system—primary cultures of pancreatic acinar cells (2). These cells are maintained in a defined serum-free (SF) medium and are viable for 4 to 5 days. They retain differentiated characteristics, secretagogue-stimulated secretion, and hormonal responsiveness (3) and synthesize amylase de novo (11) for 4 days in culture.

MATERIALS AND METHODS

Materials. The following were purchased from GIBCO Laboratories (Grand Island, NY): Ham's F12 medium, Waymouth's MB 752/1, Hanks' balanced salt solution with no Ca++ or Mg++, heat-inactivated calf serum (HI-CS), 7.5% Na₂HCO₃, 1 M N-2-hydroxyethylpiporazine-N'2-ethane sulfonic acid (HEPES) solution, 200 mM glutamine, 100× antibiotic-antimycotic solution, and 0.5% trypan blue. Collagenase type II and hyaluronidase were obtained from Worthington Biomedical Corp. (Freehold, NJ). The following were purchased from Sigma Chemicals (St. Louis, MO): dexamethasone (DEX), crystallized and lyophilized bovine serum albumin (BSA), EDTA, soybean trypsin inhibitor (STI), gum arabic, synthetic secretin, and caerulein. The following were purchased from indicated sources: sodium tauroglycocholate (ICN Pharmaceuticals, Plainview, NY); epidermal growth factor (EGF; Bethesda Research Labs, Gaithersburg, MD); Wayne Blox rodent diet (Continental Grain Co., Chicago, IL); Nytex filters (Wiretec, Stafford, TX), and 24-well cluster plates (Costar, Van Nuys, CA). Rats were obtained from Harlan (Indianapolis, IN).

Animals and diet. Male, weanling Sprague-Dawley rats weighing 40 to 100 g were housed individually, maintained on a 12 h light-dark cycle and fed ad libitum a commercial nonpurified diet that contained 24% crude protein, 4% crude fat, and 4.5% crude fiber.
\textbf{Fig. 1.} Effect of secretin on cellular and media lipase activity in cultured acinar cells. Cells were isolated from rats fed commercial unpurified diet and cultured in SF medium for 6, 12, or 24 h with various concentrations of secretin. Each point represents the mean of triplicate or quadruplicate samples from three experiments. The average freshly isolated cellular lipase activity was 20.93 ± 8.55 U/mg protein (mean ± SD). A significant decrease occurred over time ($P < 0.001$) in cellular lipase activity ($6 > 12 > 24$ h) and a significant increase ($P < 0.0001$) in media lipase activity ($6 < 12 < 24$ h), but no interactive effect of secretin with culture time. Secretin increased media lipase ($P < 0.04$), but did not affect cellular lipase activity. \#Values at each time point not sharing a superscript differed significantly by 1-way ANOVA ($P < 0.04$).

\textbf{Acinar cell culture.} Two rats per cell preparation were decapitated and the pancreata were removed aseptically and pooled. Pancreatic acinar cells were isolated and cultured according to the method of Brannon et al. (2,3). Briefly, pancreata were minced into 1- to 2-mm pieces and incubated with 20 ml HBSS containing 1 mM EDTA for 15 min at 37°C while shaking 120 cycles per min (cpm). The chelated mixture was centrifuged for 2 min at 500 $\times$ g. The resulting supernatant was discarded; the pellet was rinsed with 20 ml Ham’s F12 medium and centrifuged for 2 min at 500 $\times$ g; the supernatant was again discarded. The tissue pellet was digested with 20 ml of 170 U/ml collagenase type II and 462 U/ml hyaluronidase with 1% HI-CS in Ham’s F12 medium at 37°C for 20 min at 120 cpm in a shaking water bath. After centrifugation at 500 $\times$ g for 2 min, the supernatant was discarded; the pellet was rinsed with 20 ml of HBSS. The chelation and digestion were repeated in sequence as described. After the second digestion and centrifugation, the pellet was rinsed with Ham’s F12 medium with 5% HI-CS, centrifuged for 2 min at 500 $\times$ g and resuspended in 10 ml Ham’s F12:5% HI-CS. The suspension was filtered through 500- and 25-$\mu$m Nyrex filters, layered onto 40 ml 5% HI-CS:6% Ficoll and centrifuged for 10 min at 200 $\times$ g. The acinar pellet was rinsed with 20 ml Ham’s F12:5% HI-CS then with 20 ml Waymouth’s medium 752:1 with 25 mM HEPES buffer, 2.4 mM glutamine, 0.01% STI and 1× antibiotic-antimycotic. The pellet was then resuspended in 10 ml Waymouth’s medium. Cell number and viability were determined by trypan blue dye exclusion using a cell suspension containing 0.10% trypan blue dye. Then cells were plated in 24-well petri clusters at a density of 1 × 10^6 cells per well in 2.0 ml Waymouth’s SF medium with 0 to 200 nM secretin, 0 to 100 nM caerulein, or 1.0 nM caerulein plus 100 nM secretin. Cells were incubated at 37°C with 5% humidified CO$_2$ for up to 48 h. SF medium contained 10 mg/ml BSA, 1 × 10^{-9} M DEX, and 42 pM EGF. In

\textbf{Fig. 2.} Effect of secretin on cellular and media amylase activity in cultured acinar cells. Cells were isolated from rats fed commercial unpurified diet and cultured in SF medium for 6, 12, or 24 h with various concentrations of secretin. Each point represents the mean of triplicate or quadruplicate samples from three experiments. The average freshly isolated cellular amylase activity was 84.27 ± 18.05 U/mg protein (mean ± SD). A significant decrease occurred over time ($P < 0.0001$) in cellular amylase activity ($6 > 12 > 24$ h), and a significant increase ($P < 0.0001$) in media amylase activity ($6 < 12 < 24$ h), but no interactive effect of secretin with culture time. At any given time, there was no significant effect of secretin.