Secretory Response of Cultured Acinar Cells of Rat Pancreas to Cholecystokinin

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To determine the adequate models for studying the functions of pancreatic acinar cells, secretory responses to CCK and to CCK receptor antagonist, L-364, 718 were examined in freshly isolated cells and confluent monolayer cells. The results showed that as CCK concentration increased, releases of amylase and lipase increased dose-dependently reaching a maximum at 10^{-9} M in acinar cells cultured in serum-containing media as well as in serum-free media. Acinar response to CCK was partially inhibited by L-364, 718. L-364, 718 itself had no effect on the releases of both amylase and lipase. Confluent monolayer of acinar cells released relatively low levels of enzymes and exhibited less response to CCK. In conclusion, short-term culture of acinar cells would be suitable to study the regulation of pancreatic enzyme secretion, and serum factors do not influence acinar response to the secretagogues. However, confluency of the acinar cells resulted in the loss of their secretory potential in the aspect of amylase and lipase release.

Key Words: Primary culture, acinar cell, rat pancreas, cholecystokinin

One of the difficulties in studying the regulation of pancreatic secretion is the lack of a pancreatic acinar preparation that is both hormonally responsive and viable in long-term culture. Early attempts in organ culture of the pancreas reported a rapid necrosis of the acinar component while the endocrine and ductal components survived (Hegre *et al.* 1972; Orci *et al.* 1973). Although embryonic pancreatic acinar cells are maintained in organ culture (Pictet *et al.* 1972; Parsa and Marsh, 1976), the survival of this rat pancreatic acinar cells was found to be inversely proportional to its age at the beginning of the culture period (MeEvoy *et al.* 1976). Maintenance of adult

pancreatic acinar cells has been reported by use of a variety of organ explant systems (Malick *et al.* 1981; Resau *et al.* 1983). However, explant cultures contain mixed populations of endocrine, ductal, and acinar pancreatic cells which generally exhibit central necrosis rendering them as unsatisfactory models for studies of the regulation of acinar cell function. Isolated acinar cells were maintained as suspension cultures for several weeks, but no cellular replications were reported (Oliver, 1980). Similarly, isolated acinar cells have also been cultured as colonial aggregates on plastic dishes for several weeks during which there was a decline in protein and DNA synthetic abilities of the cells (Ruoff and Hay, 1979). Cells cultured in serum-containing medium, which are hormonally responsive, do maintain viability or rates of cellular protein synthesis for 24 hrs. However, acinar cells cultured in serum-free media maintained viability for 4 to 5 days and were hormonally responsive, which suggested them for the studies on the regulation of the synthesis of amylase and other pancreatic enzymes *in vitro* (Brannon *et al.*

1985).

For long-term regulatory study on pancreatic acinar cells, the culturing of enriched populations of adult mouse acinar cells as monolayers on collagen was described (Logsdon and Williams, 1986). They eliminated the heterogeneity and the anatomic and physical complexity of the intact pancreas and hormone actions, and the interactions can be studied by precisely controlling hormone levels and ratios in cultured media. This culture system was reported as a useful model for the characterization of *in vitro* requirements for the growth and maintenance of differentiation in the pancreatic acinar cells.

Cholecystokinin (CCK) is the major hormonal regulator of pancreatic enzyme secretion acting via mobilization of intracellular calcium (Gardner and Jensen, 1981). Although its direct effect on pancreatic enzyme synthesis or secretion cannot be inferred from infusion or injection studies because of potential interaction with other hormones or regulators *in vivo*, CCK appears to regulate pancreatic enzymes directly since the CCK receptor antagonist, L-364, 718 blocks its action (Rosewicz *et al.* 1989).

The present study was undertaken to determine the adequate experimental models for studying functions of pancreas acinar cells by comparing the secretory response of acinar cells which were freshly isolated and cultured to be confluent. Secretory responses to CCK and to CCK receptor antagonist, L-364, 718 were observed after a 24 hr-culture of freshly isolated cells and confluent monolayer cells.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (70~100 g) inbred in the Yonsei University Medical Center animal unit were used. The animals were fed *ad libitum* standard chow and maintained on a 12 hr light-dark cycle.

Preparation of acinar cells

Acinar cells were isolated from the pancreata of two rats by collagenase digestion as was described before (Muallem *et al.* 1988). In brief, the pancreas was aseptically removed, injected with sterilized solution A containing (in mM) 140 NaCl; 5 KCl; 1 MgCl$_2$; 1 CaCl$_2$; 10 Hepes (pH 7.4 with NaOH); 10 glucose; 10 pyruvate; 0.1% bovine serum albumin (fraction V: Sigma Chemical Co., St. Louis, MO, USA) and 0.2% soybean trypsin inhibitor (Type 1-S; Sigma Chemical Co., St. Louis, MO, USA) and minced. The minced tissue was suspended in a 5 ml solution containing 750 units of collagenase (Type II; Sigma Chemical Co., St. Louis, MO, USA) in 20 ml of solution A, oxygenated, and incubated for 10min with shaking at 37℃. Then the medium was removed and replaced with a 5 ml of fresh digestion medium and incubated for 10min with shaking. This digestion process was repeated two times. After the digestion, the acinar cells were harvested by centrifugation, washed twice, and resuspended in solution A. The tissue was then dissociated by passage through sterilized plastic pipettes with orifices of decreasing size. Acinar cells were purified by means of filtration through 150 μm nylon mesh and sedimentation through sterilized solution A containing 4% and 1% bovine serum albumin successively. The procedure is reported to enrich acinar cells eightfold over islets as compared with in the intact pancreas (Mossner *et al.* 1984).

Cell culture

Acinar cells were finally washed and resuspended in serum-containing culture media, then 2-ml aliquots (900 μg, ~1×10^6 cells) seeded onto a 6-well plate precoated with soluble collagen (Falcon Plastics, Lincoln Park, NJ, USA). Cell viability higher than 95% in the final cell suspension was verified by the trypan blue dye exclusion method. Cells were maintained in Waymouth's MB 752/1 medium (GIBCO, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.2 mg/ml soybean trypsin inhibitor, 5 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml selenium, 1 mM putrescine, 10 μg/ml dexamethasone, and 50 ng/ml epidermal growth factor (Sigma Chemical Co., St. Louis, MO, USA). Culture was maintained at 37℃ in a 5% CO$_2$/95% O$_2$ water-

saturated atmosphere. For comparative study, the cells were cultured in the serum-free media in which serum, insulin, transferrin, selenium, and putrescine were removed from the serum-containing media.

Acinar response to cholecystokinin (CCK) and its antagonist L-364, 718

For study of secretory response of acinar cells to CCK, 10^{-12} M - 10^{-7} M CCK-8 (Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂; Sigma Chemical Co., St. Louis, MO, USA) was added to freshly isolated acinar cells and cultured either in serum-containing media or in serum-free media for 24 hr which was determined as the critical response period of the cells to the secretagogues (Arias and Bendayan, 1991). To compare the time response of acinar cells cultured in serum-containing media, the releases of both amylase and lipase were determined at 2 hr- and 24 hr-culture in which the cells were incubated with or without 10^{-9} M CCK. For 24 hr-culture studies on basal and CCK stimulated cells, a specific CCK receptor antagonist, L-364, 718 (10^{-6} M), a generous gift from Dr. V.J. Lotti (Merck Sharp and Dohme Research Laboratories, West Point, PA, USA), was added to the culture medium 15 min prior to CCK (10^{-9} M). For the study on confluent monolayer cells, the culture medium (serum-containing media) was renewed 24 hr after plating and every other day until the cells were confluent. When the cells reached their confluency, 7 days after the seeding, the medium was changed with fresh serum-containing media and CCK with or without L-364, 718 (10^{-6} M) was then added as the same manner as described above. At their confluency, the cells were morphologically intact having zymogen granules without any fibroblastic overgrowth under the light microscope which was also reported by the morphological study of mouse pancreatic acinar cells in the monolayer culture (Rosewicz et al. 1989).

Biochemical evaluation

Amylase content of culture media was determined by the method of Bernfeld (1955). Lipase content of the media was estimated by an autotitrimetric method with olive oil as the substrate in the presence of excess colipase (Sabb et al. 1986).

Statistical analysis

All values represent mean ±s.e.m. from 6 separate experiments. Differences among groups were determined by one-way ANOVA with Newman-Keuls test (Zar, 1984). Values were considered significantly different if $P < 0.05$.

RESULTS

Releases of amylase and lipase from acinar cells

Pancreatic acinar cells were cultured in serum-containing media for 2 hr and 24 hr and the releases and intracellular contents of amylase and lipase were determined. Basal releases of amylase and lipase were 34.0±2.5 IU/ml and 7.0±0.3 U/ml at 2 hr and 132.0±3.5 IU/ml and 9.1±0.1 U/ml at 24 hr, respectively (Fig. 1). 10^{-9} M CCK stimulated the releases of amylase and lipase. The releases of amylase and lipase of CCK-stimulated acinar cells were 105.0±4.0 IU/ml and 10.0±0.4 U/ml at 2 hr and 221.0±7.0 IU/ml and 20.0±0.5 U/ml at 24 hr culture period. At 0 hr, the releases of amylase and lipase of untreated control cells were 18.0±2.0 IU/ml and 2.0±0.2 U/ml, respectively, which were not significantly different
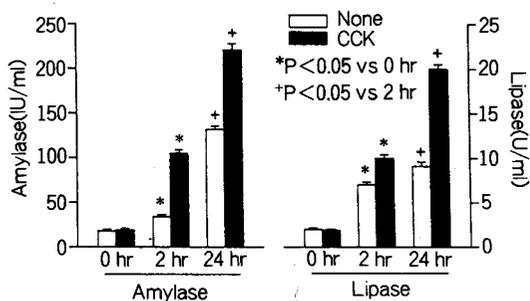
Fig. 1. Releases of amylase and lipase from acinar cells. Acinar cells were incubated with or without CCK (10^{-9} M) in serum-containing media for 2 hr and 24 hr. Values are mean ± s.e.m. from 6 separate experiments. *: $P < 0.05$ vs 0 hr-culture, +: $P < 0.05$ vs 2 hr-culture

from amylase release (19.0±2.0 IU/ml) and lipase release (1.9±0.1 U/ml) of the cells before CCK stimulation.

Dose-response of acinar cells to CCK in amylase release

To compare the secretory response of acinar cells cultured either in serum-containing media, in serum-free media, or those of the confluent monolayer cells, 10^{-12} M - 10^{-7} M of CCK was added to the culture media and amylase release were measured after a 24 hr-culture (Fig. 2A). Basal amylase releases of acinar cells cultured in serum-containing media and serum-free media were 132.0±3.5 IU/ml and 32.0±2.5 IU/ml while basal amylase release of confluent monolayer cells was 1.05±0.02 IU/ml. The amylase release was increased by increasing concentrations of CCK dose-dependently and reached maximum at 10^{-9} M. Acinar cells showed this biphasic response to CCK regardless of their culture condition. Amylase releases of CCK (10^{-9} M) - stimulated acinar cells cultured in serum-containing media, serum-free media, and confluent monolayer cells were 221.0±7.0 IU/ml (167% of control which is basal cumulative release of amylase from untreated cells), 60.0±2.5 IU/ml (187% of control), and 1.46±0.04 IU/ml (139% of control), respectively.
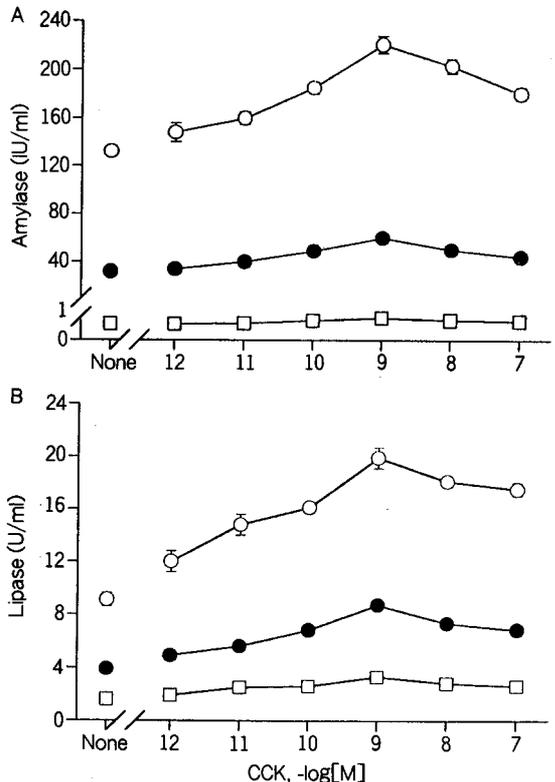
Dose-response of acinar cells to CCK in lipase release

At the 24 hr-culture, basal lipase releases of acinar cells cultured in serum-containing media, serum-free media, and confluent monolayer cells were 9.1±0.5 U/ml, 3.9±0.1 U/ml, and 1.6 ±0.1 U/ml, respectively (Fig. 2B). Dose-response of CCK on lipase release had a similar biphasic pattern to that of amylase release. However, the stimulation of CCK on lipase release was greater than amylase release. Lipase release of CCK-treated cells cultured in serum-containing media was 19.9±0.8 U/ml (218% of untreated control) and that cultured in serum-free media was 8.7±0.2 U/ml (223% of control). Confluent monolayer cells increased lipase release from 1.6±0.1 U/ml at basal cumulative release of untreated state to 3.3±0.1 U/ml,

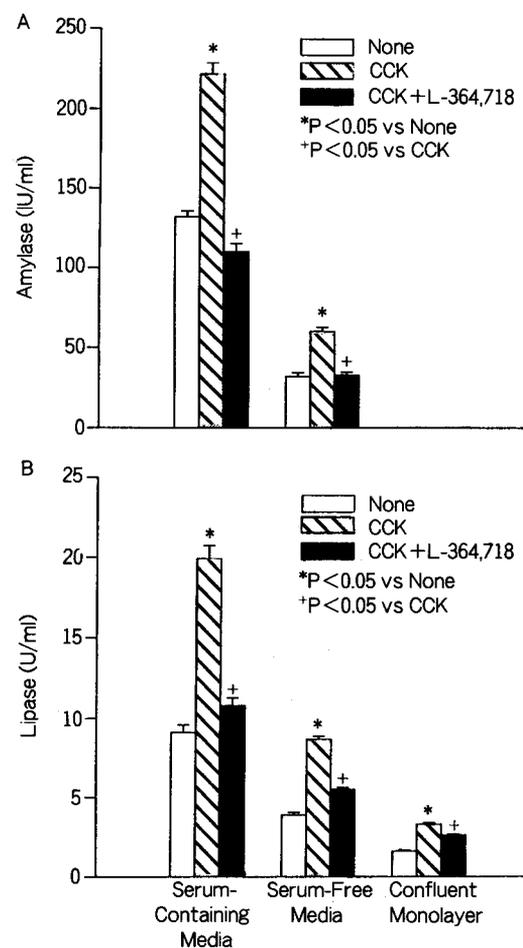which was 206% of the untreated control at 10^{-9} M CCK treatment.

Effect of L-364, 718 on basal and CCK-stimulated releases of amylase and lipase from acinar cells

In order to determine the effect of L-364, 718 on CCK-stimulated acinar cells, 10^{-6} M of L-364, 718 was treated 15min prior to the addition of CCK (10^{-9} M) and the release of amylase and lipase were determined after 24 hr. L-364, 718 blocked the CCK stimulation on the releases of both amylase and lipase of acinar cells cultured in serum-containing media,

Fig. 2. *Dose-response of acinar cells to CCK for the releases of amylase (A) and lipase (B). Acinar cells were treated with CCK (10^{-12} M - 10^{-7} M) and cultured either in serum-containing media (○) or in serum-free media (●) for 24 hr. CCK-treated confluent monolayer cells (□) were cultured in serum-containing media for 24 hr. Each point represents mean±s.e.m. from 6 separate experiments.*

serum-free media, and those of the confluent monolayer cells (Fig. 3). CCK-stimulated amylase release of acinar cells cultured in serum-containing media, serum-free media, and confluent monolayer cells were 221.0 ± 7.0 IU/ml, 60.0 ± 2.5 IU/ml and 1.46 ± 0.04 IU/ml, which decreased to 110.2 ± 5.1 IU/ml (50% of CCK stim-

ulation), 33.0 ± 2.0 IU/ml (55% of the stimulation) and 0.89 ± 0.01 IU/ml (60% of the stimulation) by the addition of L-364, 718 (Fig. 3A). Lipase releases of CCK-treated acinar cells cultured in serum-containing media, serum-free media, and confluent monolayer cells were 19.9 ± 0.8 U/ml, 8.7 ± 0.2 U/ml and 3.3 ± 0.1 U/ml respectively. Pretreatment of L-364, 718 decreased these stimulation on lipase release to 10.8 ± 0.5 U/ml (54% of CCK stimulation) released by the cells cultured in serum-containing media, 5.5 ± 0.1 U/ml (63% of the stimulation) released by those cultured in serum-free media, and 2.6 ± 0.1 U/ml (78% of the stimulation) released by the confluent monolayer cells (Fig. 3B). However, L-364, 718 (10^{-6} M) itself had no effect on basal releases of amylase and lipase of acinar cells cultured in serum-containing media at 24 hr-culture (Table 1).

DISCUSSION

The present study established an adequate model for studying the functions of pancreatic acinar cells by observing secretory responses to CCK and its antagonist. In the preliminary experiment, secretory response of basal and

Fig. 3. Effect of L-364, 718 on CCK-stimulated releases of amylase (A) and lipase (B) from acinar cells. Acinar cells were incubated with or without CCK (10^{-9} M) for 24 hr. L-364, 718 (10^{-6} M) was treated 15 min prior to the addition of CCK (10^{-9} M). Releases of amylase and lipase were determined after 24 hr. Values are mean $\pm$ s.e.m. from 6 separate experiments. *: $P < 0.05$ vs untreated control, +: $P < 0.05$ vs CCK alone

Table 1. Effect of L-364, 718 on basal and CCK-stimulated releases of amylase and lipase from acinar cells

	Amylase (IU/ml)	Lipase (U/ml)
None	132.0 ± 3.5	9.1 ± 0.5
L-364,718	125.1 ± 4.0	9.4 ± 0.4
CCK	$221.0 \pm 7.1^*$	$19.9 \pm 0.8^*$
CCK+L-364,718	$110.2 \pm 5.1^+$	$10.8 \pm 0.5^+$

Acinar cells were treated with L-364, 718 (10^{-6} M) in the absence or presence of CCK (10^{-9} M) and cultured in the serum-containing media for 24 hr. Releases of amylase and lipase were determined in media. Values represent mean $\pm$ s.e.m. from 6 separate experiments.
*: $P < 0.05$ vs none (basal cumulative release from untreated acinar cells), +: $P < 0.05$ vs CCK alone

CCK-stimulated cells cultured in serum-containing media was determined depending on incubation time. We found that the increments of amylase and lipase in the medium were proportional to incubation time and CCK treatment. Thus, the releases of amylase and lipase were used as the parameters for secretory function of the cells in the present study. The reasons why we measured the releases of amylase and lipase for the parameters of secretory function are that (i) the cellular contents of pancreatic enzymes vary on the rate of synthesis and the degree of secretion, especially in the cultured acinar cells (Meda et al. 1986; Bruzzone et al. 1987; Hirschi et al. 1994) and (ii) other pancreatic enzymes (Hirschi et al. 1994) such as elastase, chymotrypsin, and trypsin cannot be measured in the present study since the culture medium contains soybean trypsin inhibitor (STI) for growth and the maintenance of pancreatic acinar cells (Logsdon, 1986) which may influence the activities of these enzymes.

The secretory response to CCK was similar in acinar cells cultured in serum-containing media or serum-free media; maximal stimulation was achieved with 10^{-9} M CCK as shown in other studies (Gardner and Jensen, 1981; Hirschi et al. 1994). A potent and specific CCK antagonist, L-364, 718 (Chang and Lotti, 1986) inhibited the CCK (10^{-9} M)-induced releases of amylase and lipase of cells cultured in serum-containing media or serum-free media. Serum-free media using highly nutritious Waymouth's medium MB752/1 was developed to avoid serum factors that may mask hormonal effects and suggested studying the regulation of the synthesis of amylase and other pancreatic enzymes in vitro (Brannon et al. 1985). Acinar cells were maintained viable in serum-free media for 4 to 5 days and were responsive to caerulein and secretin (Brannon et al. 1985). In the present study, basal releases of amylase and lipase of the cells cultured in serum-free media were lower than those cultured in serum-containing media even though the pattern of acinar response to CCK and cell viability were similar. In addition, we found that serum factors do not influence the secretory response of acinar cells to CCK at 24 hr-cul-

ture which was reported to be a critical response time for acinar cells to the secretagogues (Arias and Bendayan, 1991). However, confluent monolayer cells had little release of enzymes and exhibited less response to CCK than freshly isolated acinar cells cultured for 24 hr. This explained by the results that for enzyme secretion, secretory effects stimulated in the acinar cells were a smaller than those seen in perfused pancreas or dissected lobules (Kondo and Schulz, 1976; Williams et al. 1976). This decreased responsiveness by dispersed acinar cells to secretagogues may be attributed to the disruption of cell-to-cell communication and membrane structure associated with the dispersal of individual acinar cells. In the case of confluent monolayer cells, normal cell structure and intracellular communication may be less maintained than freshly isolated and shortterm cultured cells. In the morphological study using a monolayer patch of acinar cells whose morphology resembled that of cells in situ, the maintenance of diffe-rentiation in the pancreatic acinar cells in vitro appears to be independent from their secretory effect (Arias and Bendayan, 1991). Others reported that monolayer cells exhibited similar morphology to that of freshly isolated cells but contained heterogeneous granules resembled those seen in the embryonic pancreas (Preiser et al. 1975). Although we did not observe the ultrastructure of the cells in the present study, these slight alterations in morphological characteristics of monolayer cells (Preiser et al. 1975) may affect the secretory response of the cells.

In conclusion, it is suggested that (i) short-term culture of acinar cells may be suitable for studying the regulation of the synthesis and secretion of pancreatic enzymes, (ii) serum factors do not influence acinar response to the secretagogues and (iii) confluent monolayer of acinar cells loose their secretory function of amylase and lipase release in response to an agonist and antagonist.

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