Regulation of amylase and chymotrypsinogen expression by dexamethasone and caerulein in serum-free-cultured pancreatic acinar AR4-2J cells

Influence of glucose

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The direct effects of dexamethasone and caerulein on two pancreatic enzymes, amylase and chymotrypsin, were determined in AR4-2J cells cultured under serum-free conditions at two glucose concentrations (1.0 and 4.5 g/l). In the absence of any hormone, the higher glucose concentration resulted in a 1.6–1.8-fold increase in the basal levels of amylase and chymotrypsinogen. Dexamethasone (50 nM) increased the biosynthesis and mRNA levels of both enzymes at both glucose concentrations. However, dexamethasone had a more pronounced effect on amylase biosynthesis (5-fold induction) than on chymotrypsinogen biosynthesis (1.8-fold induction). The parallel increases in mRNA and protein indicated the existence of pre-translational regulation. This is in contrast with what was observed in serum-containing media, where a translational regulation of amylase biosynthesis took place, probably under the control of both glucose and some serum factors. By contrast, caerulein (10 nM) exerted a more specific action on chymotrypsinogen. The increases in chymotrypsinogen mRNA were 2.2- and 2.1-fold, and increases in chymotrypsin activity were 1.6- and 2.9-fold at 1.0 and 4.5 g of glucose/litre respectively. Thus the regulation by caerulein occurred mainly through the enhancement of chymotrypsinogen transcription and/or mRNA stabilization.

INTRODUCTION

Studies *in vivo* have shown that glucocorticoids and cholecystokinin (CCK) are involved in the regulation of the biosynthesis of secretory proteins in the rat pancreas [1–6]. Glucocorticoids exhibited a selective effect on the pancreatic amylase gene, whereas CCK decreased the biosynthesis of amylase and increased that of serine proteases. It is not, however, established whether the effects observed *in vivo* were directly due to the hormone tested and the molecular mechanisms involved in such regulation are not clearly understood. *In vitro* pancreatic systems, such as the acinar cell line AR4-2J [7–11], have been developed to gain better insight into regulation in pancreatic acinar cells.

AR4-2J cells are able to synthesize and secrete digestive enzymes and to respond to dexamethasone [7] and CCK [8]. Concerning the regulatory effects of dexamethasone, varying results have been reported: specific stimulation of amylase biosynthesis at the transcriptional level [9], and stimulation of amylase and chymotrypsinogen biosynthesis at both transcriptional and translational levels [11]. The influence of fetal serum components and/or the concentration of glucose in the culture medium might be responsible for these contradictory results. Indeed, glucose deprivation in the culture medium has been found to decrease the mRNA levels of amylase and chymotrypsinogen [12]. As far as CCK and caerulein (a decapeptide structurally similar to the CCK C-terminal sequence) are concerned, their specific effects on the biosynthesis of these enzymes and the underlying mechanisms have not yet been established *in vitro*.

We have analysed the effects of caerulein and dexamethasone

on the levels of chymotrypsin and amylase activities, and on their mRNAs. For this purpose, AR4-2J cells were cultured in the absence of serum in order to omit the possible interference of serum factors, and at different glucose concentrations. Dexamethasone was found to increase the biosynthesis and the mRNA levels of both enzymes, whereas caerulein exerted a more specific effect, mainly on chymotrypsinogen. Glucose and serum were involved in the regulation of both enzymes.

MATERIALS AND METHODS

Materials

Synthetic caerulein was kindly provided by Dr. De Castiglione (Farmitalia Carlo Erba, Milan, Italy). BTEE (*N*-benzoyl-L-tyrosine ethyl ester) was from Fluka (Buchs AG, Switzerland), and all other chemicals were from Sigma (St. Louis, MO, U.S.A.). Culture media were obtained from Gibco Laboratories (Grand Island, NY, U.S.A.).

Cell culture

The AR4-2J cell line, derived from an azaserine-induced pancreatic rat tumour [13], was kindly provided by Dr. C. D. Logsdon (University of California, San Francisco, CA, U.S.A.). The cells were maintained at 37 °C in a water-saturated atmosphere of 5% CO₂ and 95% air as subconfluent cultures in Dulbecco's modified Eagle's medium containing glucose (1 g/l), fetal calf serum (10%, v/v) and antibiotics. AR4-2J cells were routinely plated at 6 × 10⁴ cells/cm², cell numbers being determined using a Coultronics ZM cell counter. After overnight attachment of the cells, the culture medium was replaced by fresh medium (zero time) containing either dexamethasone (50 nM) as

Abbreviations used: CCK, cholecystokinin; BTEE, *N*-benzoyl-L-tyrosine ethyl ester.

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in [9,11] or caerulein (10 nM). Equivalent additions of caerulein, but not of dexamethasone, were made every 24 h because of possible proteolysis of the caerulein. In serum-free experiments, the cells were rinsed after the attachment period and the medium was then buffered with 20 mM-Hepes, pH 7.2.

Biochemical assays

At the selected time (48 h), AR4-2J cells were scraped off, centrifuged (1000 *g* for 5 min), further disrupted by freezing and thawing, and finally homogenized with a Turrax homogenizer (3 × 15 s at 2000 rev./min, 4 °C). The resulting cell homogenate and a sample of the culture medium were then assayed for amylase activity on soluble starch [14] and for chymotrypsin activity using BTEE as a substrate [15]. No chymotrypsin activity with BTEE could be detected in either medium or cell homogenates before the addition of activating trypsin. On the basis of the results obtained in preliminary studies, enzyme activation was routinely performed by incubation with 10 µg of trypsin/ml at 4 °C for 90 min, and activity was determined using 0.5 mM-BTEE. Under these conditions, enzyme activity was directly proportional to the amount of culture medium or cell homogenate used in the assay. Furthermore, total inactivation of the enzyme by Tos-Phe-CH₂Cl ('TPCK'), the chymotrypsin-active-site-directed inhibitor, confirmed the specificity of the enzymic determination. The absence of inactivation of secreted amylase and chymotrypsinogen during the 48 h stimulations was verified. For this, samples of culture media were removed every 8 h and enzyme activities were measured. The total activity secreted over a 48 h period could be derived from intermediate values, indicating that the kinetics of enzyme secretions were linear for at least 48 h. Amylase and chymotrypsin activities were expressed as µmol of substrate hydrolysed/min (units) per mg of cell DNA in the culture dish. Proteins and DNA in cell homogenates were determined as described by Bradford [16] and Burton [17] respectively.

Quantification of mRNA

Total RNA was prepared by the guanidinium thiocyanate method [18] and further purified by sedimentation through a layer of 5.7 M-CsCl. A 702 bp cDNA clone encoding rat pancreatic amylase mRNA [19] and a 621 bp cDNA clone encoding rat pancreatic chymotrypsinogen [20] were used to determine the

concentrations of individual mRNAs by slot-blot hybridization analysis. RNA samples were denatured [21] and spotted at five different concentrations (from 1 to 5 µg) on to a nitrocellulose filter [22] with a slot-blot apparatus (Bio-Rad). The filters were then baked at 80 °C for 2 h and hybridized [21] with ³²P-labelled nick-translated cDNA probes (Amersham nick translation kit, specific radioactivity = 2 × 10⁷ c.p.m./µg) at 42 °C for 16 h. After hybridization, the filters were washed [22] and further exposed to X-ray film. The radioactivity of each spot was determined by Čerenkov counting using a Packard Tri-Carb 4000 scintillation counter.

Statistics

Statistical analyses were carried out using Student's *t* test and, when appropriate, the paired *t* test.

RESULTS

Effects of dexamethasone and caerulein on cell growth

Table 1 shows the effects of dexamethasone and caerulein on the accumulation of DNA in serum-free cultures of AR4-2J cells. No statistically significant difference was observed between control and caerulein-treated cells, indicating that the CCK-like peptide did not exert any important mitogenic effect for at least 48 h. On the contrary, dexamethasone significantly inhibited cell proliferation, as reported for AR4-2J cells grown in 10% fetal serum [7].

Table 1. Effects of dexamethasone and caerulein on the proliferation of AR4-2J cells cultured for 48 h under serum-free conditions in 1.0 g of glucose/l

Results are means ± S.D. (*n* = 6). * *P* < 0.01 compared with unstimulated cells (Student's *t* test)

Treatment	Total DNA (µg/ml)
None	65 ± 4
Caerulein (10 nM)	73 ± 5
Dexamethasone (50 nM)	48 ± 4*

Fig. 1. Time-course of secretion of amylase (a) and chymotrypsinogen (b)

Secretion was measured in unstimulated cells (●) and in cells stimulated with 50 nM-dexamethasone (*) or 10 nM-caerulein (○), in the presence of 1.0 g of glucose/l. Values are means ± S.D. of six experiments. Amylase secretion at 48 h in cells treated with dexamethasone was significantly greater than that of control cells (*P* < 0.001). Chymotrypsinogen secretion by caerulein- and dexamethasone-treated cells at 48 h was significantly different from that by control cells (*P* < 0.01).

Table 2. Effects of hormones on enzyme activities and mRNA levels in AR4-2J cells cultured under serum-free conditions

The various determinations were performed after a 48 h treatment. When not specified in parentheses, $n = 9$. Values are means $\pm$ S.D. mRNA levels at 4.5 g of glucose/l were compared with those of controls at 1.0 g/l. Enzyme activity is given in units/mg of DNA and mRNA level is given in arbitrary units.

Enzyme	Glucose (g/l)	Control	Dexamethasone (50 nM)	Caerulein (10 nM)
Chymotrypsin	1.0			
cellular		2.8 $\pm$ 0.5	7.5 $\pm$ 0.9	3.1 $\pm$ 0.5
secreted		8.8 $\pm$ 0.5	13.0 $\pm$ 0.3	15.5 $\pm$ 0.3
cellular plus secreted		11.6 $\pm$ 0.8	20.5 $\pm$ 0.9	18.6 $\pm$ 0.6
cellular mRNA		1	1.8 $\pm$ 0.3 (7)	2.2 $\pm$ 0.2 (7)
Amylase	1.0			
cellular		200 $\pm$ 28	1100 $\pm$ 120	200 $\pm$ 52
secreted		3000 $\pm$ 840	15000 $\pm$ 1600	4000 $\pm$ 594
cellular plus secreted		3200 $\pm$ 710	16100 $\pm$ 1400	4200 $\pm$ 527
cellular mRNA		1	5.5 $\pm$ 0.3 (3)	1.0 $\pm$ 0.2 (3)
Chymotrypsin	4.5			
cellular		2.7 $\pm$ 0.2	5.6 $\pm$ 0.1	3.0 $\pm$ 0.4
secreted		16.0 $\pm$ 2.0	37.0 $\pm$ 2.0	52.0 $\pm$ 2.0
cellular plus secreted		18.7 $\pm$ 1.8	42.6 $\pm$ 1.7	55.0 $\pm$ 1.9
cellular mRNA		1 $\pm$ 0.1 (3)	2.7 $\pm$ 0.4 (7)	2.1 $\pm$ 0.1 (7)
Amylase	4.5			
cellular		190 $\pm$ 9	600 $\pm$ 74	205 $\pm$ 30
secreted		5000 $\pm$ 180	16500 $\pm$ 652	7000 $\pm$ 480
cellular plus secreted		5190 $\pm$ 154	17100 $\pm$ 592	7205 $\pm$ 416
cellular mRNA		1.0 $\pm$ 0.1 (3)	3.7 $\pm$ 0.2 (3)	1.0 $\pm$ 0.1 (3)

Fig. 2. Effects of dexamethasone and caerulein on the cellular content of amylose and chymotrypsinogen mRNA in AR4-2J cells cultured in 1.0 g of glucose/l.

After treatment with the appropriate hormone for 48 h, total cellular RNA was prepared, slotted and probed with 32 P-nick-translated probes specific for amylose and chymotrypsinogen. For clarity, results are single concentrations of RNA (1 μ g/slot). Lane 1, untreated cells; lane 2, caerulein-treated cells; lane 3, dexamethasone-treated cells. C and A indicate chymotrypsinogen and amylose respectively.

Dexamethasone-induced enzyme activities and mRNA levels in AR4-2J cells (1 g of glucose/l)

Enzyme activities. The effect of dexamethasone treatment on enzyme activities was progressive and linear from 8 h onwards, both in cells (results not shown) and in the secretion (Fig. 1). The cell enzyme contents after 48 h of treatment were about 5-fold that of controls for amylose, and 2.7-fold for chymotrypsinogen at 1 g of glucose/l (Table 2). The differential effect of dexamethasone on amylose and chymotrypsinogen secretion was also clearly detected in the time course of stimulation by the hormone (Fig. 1). A comparable strong induction of amylose synthesis (6-fold) was observed in the presence of serum (results not shown).

mRNA concentrations. In order to determine whether the hormone-induced production of the enzymes resulted from

changes in the levels of the corresponding mRNAs, total RNA from AR4-2J cells was assayed for amylose and chymotrypsinogen mRNAs by slot-blot analysis. Fig. 2 shows a typical autoradiogram of slot-blotted RNA hybridized to specific cDNA probes. During stimulation by dexamethasone, the increases in the mRNA level of both enzymes were detectable from 8 h of incubation (results not shown) onwards. By 48 h there was a 5.5-fold increase in amylose mRNA and a 1.8-fold increase in chymotrypsinogen RNA (Table 2). Therefore the observed increases in mRNA levels closely paralleled those in enzyme activities. Table 3 shows that when cell lines were grown in the presence of serum, the magnitude of mRNA induction was lower, 1.5-fold for chymotrypsinogen and 2-fold for amylose. Thus upon serum addition into the culture medium there was no longer a direct correlation between amylose synthesis and the corresponding mRNA level.

Caerulein-induced enzymic activity and mRNA levels in AR4-2J cells (1 g of glucose/l)

Enzyme activities. Caerulein had little effect on amylose activity levels in either cell homogenates or culture media (1.3-fold enhancement) whether the cells were cultured in the absence (Table 2) or in the presence (results not shown) of fetal serum. By contrast, both Fig. 1 and Table 2 show that the CCK-like peptide progressively increased chymotrypsinogen secretion (1.7-fold after 48 h; $P < 0.01$). No change was detected in the cellular concentration of glucose (4.5 g/l) was also investigated.

mRNA concentrations. No change in amylose mRNA occurred upon caerulein stimulation, whereas chymotrypsinogen mRNA was increased 2.2-fold in serum-free (Table 2) as well as in serum-containing (Table 3) media.

Effects of high glucose concentrations on hormone-induced production of amylose and chymotrypsinogen

All of the above experiments were carried out with a glucose concentration of 1.0 g/l of culture medium, which is similar to

Table 3. mRNA levels in serum-cultured AR4-2J cells

mRNA levels were determined after a 48 h cell treatment. Numbers of measurements are given in parentheses.

Enzyme	Glucose (g/l)	mRNA level (arbitrary units)		
		Control	Dexamethasone (50 nM)	Caerulein (10 nM)
Chymotrypsinogen	1.0	1.0	1.5 ± 0.4 (3)	2.2 ± 0.3 (5)
Amylase	1.0	1.0	2.0 ± 0.5 (3)	1.0 ± 0.2 (3)
Chymotrypsinogen	4.5	1.0	2.0 ± 0.3 (3)	1.9 ± 0.1 (3)
Amylase	4.5	1.0	6.6 ± 1.0 (3)	1.0 ± 0.1 (3)

that in normal serum. However, in order to further explain some discrepancies observed in previously reported studies on dexamethasone treatment of AR4-2J cells [9,11], a higher concentration of glucose (4.5 g/l) was also investigated.

Enzyme activities and mRNA levels in the absence of hormonal stimulation. After a 48 h culture period in a medium containing 4.5 g of glucose/l but lacking any hormone, the intracellular amylase and chymotrypsinogen contents, as well as the levels of the corresponding mRNAs, were not modified compared with incubation with 1 g of glucose/l (Table 2). By contrast, amylase and chymotrypsinogen secretions were increased 1.6- and 1.8-fold respectively.

Enzyme activities and mRNA levels in dexamethasone- and caerulein-treated cells. The kinetics of the changes in the enzymic activities in response to dexamethasone and caerulein at a high glucose concentration (4.5 g/l) were comparable with those observed at 1.0 g of glucose/l (results not shown). Dexamethasone still enhanced amylase synthesis (3.3-fold) and mRNA levels (3.7-fold) as well as chymotrypsinogen synthesis (2.3-fold) and mRNA levels (2.7-fold) (Table 2). In both cases, the increases in mRNA levels were comparable with those in the corresponding proteins. The same correlation was observed in serum-cultured cells, although the increases in amylase synthesis and mRNA level were higher (6-fold) (Table 2). At the high glucose concentration, caerulein stimulated chymotrypsinogen synthesis by 2.9-fold and its mRNA level by 2.1-fold (Table 2). Upon serum addition, a comparable stimulation of the corresponding mRNA expression was observed (Table 3). By contrast, in both the presence and the absence of serum, amylase synthesis and mRNA concentrations were only slightly modified by caerulein (Tables 2 and 3).

DISCUSSION

The pancreatic acinar cell line AR4-2J is known to be an interesting model for studying *in vitro* the effects of hormones on the exocrine pancreas [7–11]. Amylase and chymotrypsinogen are two main secretory proteins, as in normal pancreatic acinar cells, and their respective mRNA levels have been reported to account for 42 % and 18 % of the total messenger pool [12]. The levels of these mRNAs decreased when glucose was lacking in the culture medium [12].

The regulation of amylase and chymotrypsinogen expression by dexamethasone in AR4-2J cells has previously been studied in serum-containing medium [9,11]. In the former study, the chymotrypsinogen mRNA level was found not to be affected, and no translational regulation of amylase biosynthesis was observed [9]. In order to explain this discrepancy, the effects of dexamethasone have been analysed in the present study under serum-free culture conditions at two glucose concentrations. When the glucose concentration in the culture medium was

increased from 1.0 g/l to 4.5 g/l in the absence of hormones, the activities of both amylase and chymotrypsin were increased (1.6- and 1.8-fold respectively) without any modification in the mRNA concentrations. This result indicated that glucose is probably involved in the regulation of the basal expression of these secretory proteins, and that such a regulation takes place apparently at the translational level.

We observed that 50 nM-dexamethasone enhanced the synthesis of both enzymes. However, the increase in amylase activity was greater than that in chymotrypsin activity at both glucose concentrations. Therefore, in the absence of serum, and independent of the glucose concentration, dexamethasone was able to induce not only the biosynthesis of amylase but also that of chymotrypsinogen, although to a lower extent. Concerning the mechanism of action of dexamethasone, we observed in time course studies that the enzyme activity levels in the presence of the hormone closely paralleled levels of the corresponding mRNA. All of these data suggest a pre-translational effect of dexamethasone on expression of both enzymes in the absence of serum. On the contrary, in parallel studies carried out in the presence of serum, and only at a low glucose concentration (1.0 g/l) equivalent to that in normal serum, we observed a 6-fold increase in amylase activity and a 3-fold increase in the corresponding mRNA. Hence the addition of serum to the culture medium resulted in an additional control of the enzyme biosynthesis at the translational level under the influence of glucose, thus explaining previously reported conflicting results [9,11].

In contrast with dexamethasone, caerulein only slightly affected amylase biosynthesis and did not change the level of amylase mRNA at any glucose concentration. However, chymotrypsin activity was increased (1.6- and 2.9-fold in the presence of 1.0 and 4.5 g of glucose/l respectively), and the mRNA was 2-fold increased at both glucose concentrations. In sharp contrast with dexamethasone, which is known to increase the number of secretory granules [7], and consequently the intracellular level of secretory enzymes, caerulein did not increase the cell storage compartment (results not shown). This explains the absence of accumulation of the newly synthesized chymotrypsinogen in cells and its progressive linear increase in secretion during caerulein treatment. The enhancement of chymotrypsinogen transcript by the secretagogue suggested the existence of a pre-translational regulation of gene expression and/or mRNA stabilization, which is in our case the main regulatory mechanism. On the other hand, kinetic studies and dose-response curves at high glucose concentrations indicated that the caerulein-induced increase in chymotrypsinogen synthesis was more important than the increase in the corresponding mRNA (results not shown). This reflected the co-existence of a translational control, as had been reported in previous experiments carried out *in vivo* [5,23]. In these studies, *in vivo*, the biosynthesis of chymotrypsinogen was found to be increased, whereas that of amylase was decreased. The results obtained in the present study suggest that the control exerted *in vivo* by caerulein on amylase is probably indirect and might depend essentially on other serum hormones.

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