Glucocorticoids Increase Cholecystokinin Receptors and Amylase Secretion in Pancreatic Acinar AR42J Cells*

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We recently reported in AR42J pancreatic acinar cells that glucocorticoids increased the synthesis, cell content, and mRNA levels for amylase (Logsdon, C. D., Moessner, A., Williams, J. A., and Goldfine, I. D. (1985) J. Cell Biol. 100, 1200-1208). In addition, in these cells glucocorticoids increased the volume density of secretory granules and rough endoplasmic reticulum. In the present study we investigate the effects of glucocorticoids on the receptor binding and biological effects of cholecystokinin (CCK) on AR42J cells. Treatment with 10 nM dexamethasone for 48 h increased the specific binding of $^{125}$I-CCK. This increase in binding was time-dependent, with maximal effects occurring after 48 h, and dose-dependent, with a one-half maximal effect elicited by 1 nM dexamethasone. Other steroid analogs were also effective and their potencies paralleled their relative effectiveness as glucocorticoids. Analyses of competitive binding experiments conducted at 4°C to minimize hormone internalization and degradation revealed the presence of a single class of CCK binding sites with a $K_d$ of $\sim 6$ nM and indicated that dexamethasone treatment nearly tripled the number of CCK receptors/cell with little change in receptor affinity. Treatment with 10 nM dexamethasone increased both basal amylase secretion and the amylase released in response to CCK stimulation. In addition, dexamethasone increased the sensitivity of the cells to CCK. The glucocorticoid decreased the concentration of CCK required for one half-maximal stimulation of amylase secretion from 35 ± 6 to 8 ± 1 pm. These data indicate, therefore, that glucocorticoids induce an increase in the number of CCK receptors in AR42J cells, and this increase leads to enhanced sensitivity to CCK.

The use of in vitro preparations of the acinar pancreas such as isolated cells (1) and acini (2, 3) has led to much information about the short-term regulation of acinar cells by hormones and neurotransmitters (4, 5). However due to the lack of suitable model systems, our understanding of the long-term regulation of pancreatic acinar cells has been limited to in vitro studies. We have recently investigated AR42J cells as a model system for studies of the long-term regulation of pancreatic acinar cells. This cell line is derived from a chemically induced transplantable tumor of the exocrine pancreas (6), is acinar in origin, contains significant amounts of amylase, and can be grown in continuous culture (7). Recently, we reported that in AR42J cells glucocorticoids increase the expression of the amylase gene, the numbers of secretory organelles, and the level of amylase secretion (8). These studies indicated, therefore, that AR42J cells may be useful for long-term studies of pancreatic acinar cell functions.

Cholecystokinin (CCK) is the major hormonal regulator of pancreatic acinar cell function. CCK has documented effects on enzyme release, growth, the amino acid transport, membrane potential, phospholipid turnover, and ion fluxes (9) and, in addition, may also regulate cell growth (10, 11). However, the factors that regulate CCK receptors and CCK action have not yet been defined. One limitation has been the lack of a continuous acinar cell line in tissue culture that has functional CCK receptors.

The present study describes the receptor binding and biological effects of CCK on AR42J cells. We report that these cells have specific CCK receptors with properties similar to normal pancreatic CCK receptors and that the occupancy of these receptors regulates amylase secretion. Moreover, we find that glucocorticoids induce an increase in the number of CCK receptors, which leads in turn to an increase in the sensitivity of the cells to CCK.

EXPERIMENTAL PROCEDURES

Materials—Purchased materials and suppliers are as follows: bovine plasma albumin (fraction V) from Miles Laboratories and Reheis; soybean trypsin inhibitor (type 1-s), HEPS, dexamethasone, aldosterone, corticosterone, progesterone, and estradiol benzoate from Sigma; penicillin, streptomycin, and amphotericin B, fetal calf serum, and trypsin from the Cell Culture Facility, University of California San Francisco, San Francisco; 3,5-diaminobenzoic acid from Aldrich; minimal Eagle’s medium essential amino acid supplement from Grand Island Biological Co.; protein reagent from Bio-Rad; $^{125}$I-Bolton-Hunter reagent from New England Nuclear; pure natural porcine cholecystokinin (CCK33) from the Gastrointestinal Hormone Laboratory, Karolinska Institute, Stockholm, Sweden; and the carboxyl-terminal tetrapeptide of CCK (CCK4) from Research Plus, Denville, NJ. Synthetic cholecystokinin octapeptide (CCK8) was a gift from Squibb, New Brunswick, NJ. All other chemicals and reagents were of analytical grade.

Cell Culture—AR42J cells (kindly provided by Dr. Y. Kim, Veteran’s Administration Hospital, San Francisco) were maintained as subconfluent cultures in Dulbecco’s modified Eagle’s medium containing penicillin, streptomycin, and 10% fetal bovine serum (8). Cells were plated at 4 × 10^5 cells/ml into 16-mm wells (2 cm² surface area) in microcluster plates. All experiments were carried out on cells which had been plated for 48 h and were in the log phase of growth. During time-course studies, the medium was changed daily. Steroid

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1 The abbreviations used are: CCK, cholecystokinin; DEX, dexamethasone; 9a-fluoro-11β,17α-dihydroxy-16-methylpregna-1,4-diene-3,20-dione; estradiol benzoate, 1,5,5,10-estratetraen-17β-ol-3 benzoate; HEPS, N2-hydroxyethylpiperazine-N2-ethanesulfonic acid; HR, HEPS-buffered Ringer; $^{125}$I-labeled Bolton-Hunter reagent, N-succinimidyl-3-(4-hydroxy-5-$^{125}$I)iodophenyl)propionate.
hormones were added at the beginning of the culture period except during time course studies when dexamethasone was added after various periods of culturing.

Amylase Secretion—The method utilized for the measurement of cholecystokinin-induced amylase release has been reported previously (8). Briefly, the cells were washed twice with 1 ml of HR containing 130 mM NaCl, 4.7 mM KCl, 1.0 mM NaH₂PO₄, 1.85 mM MgCl₂, 1.28 mM CaCl₂, 5.6 mM glucose, and 10 mM HEPES (pH 7.40) which was enriched with minimal Eagle’s medium amino acid supplement, 0.1 mg/ml soybean trypsin inhibitor, 5 mg/ml bovine plasma albumin, and gassed with 100% O₂. The cultures were then incubated at 37 °C for 15 min and a sample of medium was taken for determination of the initial amylase concentration. Various concentrations of CCK were added to the HR containing washed cells at 4 °C. After incubation for 40 min, the cell layer was removed, and the medium was counted to determine the total radioactivity. Binding was normalized per initial amount.

The radioactivity associated with the cells was measured in a scintillation counter and an aliquot of the incubation medium was taken as described above, after which the cells were incubated in 1 ml of 0.2 M acetic acid with 0.5 M NaCl (pH 2.5) for 5 min at 4 °C. The acid-washing experiments conducted at a final concentration of 10 nM dexamethasone was conducted. AR42J cells, cultured under control conditions, were incubated with 30 pM ¹²⁵I-CCK at 22 °C. Binding was one-half maximal after 15 min and maximal within 45–60 min (Fig. 1). The average specific binding for control cultures was 4.7 ± 0.4% of the total radioactivity/10⁶ cells (n = 10). Nonspecific binding (labeled hormone plus an excess of unlabeled hormone) was 0.38 ± 0.03% of the total radioactivity/10⁶ cells. Degradation of ¹²⁵I-CCK by AR42J cells, as determined by the appearance of radioactivity soluble in trichloroacetic acid, did not exceed 3% of the total.

Under the same conditions AR42J cells, which had been treated with 10 nM dexamethasone for 48 h, showed a similar time course of hormone binding, but binding at all time points was increased. After dexamethasone treatment, the average specific binding of ¹²⁵I-CCK at 60 min was 9.8 ± 0.5% of the total radioactivity/10⁶ cells, a greater than 2-fold increase over control (p < 0.05). In contrast, nonspecific binding was only slightly increased to 0.8 ± 0.04%/10⁶ cells. Dexamethasone treatment had no effect on the degradation of ¹²⁵I-CCK in the binding medium.

The possibility was considered that the increased association of CCK with dexamethasone-treated cells was due to increased hormone internalization. In order to investigate whether dexamethasone-treated cells had greater internalization of CCK, acid-washing experiments were conducted. This procedure removes hormone which is bound to receptors located at the cell surface, but not internalized hormone (16). In experiments where cells were incubated with 30 pM ¹²⁵I-CCK at 22 °C for 60 min and then washed at acid pH, 82 ± 2% of the radioactivity bound to control cells was acid-resistant and 75 ± 7% (n = 4) of the radioactivity bound to cells cultured in the presence of 10 nM dexamethasone was acid-resistant. These results indicated, therefore, that the primary effect of dexamethasone on the binding of CCK to AR42J cells was not due to an increase in CCK internalization.

Effects of Dexamethasone on the Affinity and Capacity of AR42J Cell CCK Receptors—In order to further evaluate the

RESULTS

125I-CCK Binding to AR42J Cells Grown in the Absence and Presence of Dexamethasone—In order to characterize both the interactions of CCK with the AR42J cells and the effects of glucocorticoids on these interactions, 125I-CCK receptor binding experiments were conducted. AR42J cells, cultured under control conditions, were incubated with 30 pM ¹²⁵I-CCK at 22 °C. Binding was one-half maximal after 15 min and maximal within 45–60 min (Fig. 1). The average specific binding for control cultures was 4.7 ± 0.4% of the total radioactivity/10⁶ cells (n = 10). Nonspecific binding (labeled hormone plus an excess of unlabeled hormone) was 0.38 ± 0.03% of the total radioactivity/10⁶ cells. Degradation of ¹²⁵I-CCK by AR42J cells, as measured by the appearance of radioactivity soluble in trichloroacetic acid, did not exceed 3% of the total.

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![Fig. 1. Time course of 30 pM ¹²⁵I-CCK binding to AR42J cells at 22 °C. Cells were cultured for 48 h in the absence (C) or presence (D) of 10 nM dexamethasone. Total ¹²⁵I-CCK binding to AR42J cells is expressed as the per cent of total radioactivity that is bound per 10⁶ cells. Nonspecific binding was determined with labeled hormone in the presence of 100 nM unlabeled CCK. Values are the mean ± S.D. of triplicate samples from a representative of 2 experiments.](image-url)
effects of dexamethasone treatment on the association of CCK with AR42J cells, saturation binding analyses were conducted. Because hormone internalization and degradation can complicate the interpretation of saturation binding analysis, these experiments were carried out at 4 °C where these processes are markedly reduced. When AR42J cells, which had been cultured under control conditions, were incubated with 30 pm 125I-CCK at 4 °C, binding was one half-maximal after 10 min and maximal within 45 min (data not shown). Under the same conditions, AR42J cells which had been treated with 10 nM dexamethasone for 48 h, showed a similar time course of hormone association, but an increased level at steady state. Under these binding conditions, no degradation of 125I-CCK in the binding medium was detected for cells grown either in the presence or absence of dexamethasone.

125I-CCK binding to acini was competitively inhibited by increasing concentrations of unlabeled CCK8 at 4 °C. The affinity and capacity of binding at steady state were then determined by computer analysis and the saturable components were replotted as bound versus free, or as bound divided by free versus bound ratios (Fig. 2). The data from five separate experiments conducted on cells from control cultures indicated a single order of binding sites having a Kd of 5.3 ± 0.4 nM and a binding capacity of 47 ± 8 fmol/106 cells. In the same experiments, cells which had been treated for 48 h with 10 nM dexamethasone had a slightly higher Kd of 6.6 ± 0.6 nM (not significant) and a higher binding capacity of 151 ± 17 fmol/106 cells (p < 0.05). Thus, there was little change in the affinity of the CCK receptors after dexamethasone treatment, but the number of receptors/cell was increased almost 3-fold. There were approximately 43,000 CCK receptors/cell under control conditions, and 122,000 receptors/cell after dexamethasone treatment.

Steroid Specificity and Time Course of Effect on CCK Binding—The effect of various steroids on 125I-CCK binding with AR42J cells was then investigated. Cells plated for 48 h in the presence of either dexamethasone, corticosterone, aldosterone, or progesterone showed dose-dependent increases in 125I-CCK binding. Estradiol benzoate, in contrast, had no effect. Dexamethasone was the most potent steroid with a one half-maximal stimulation of a 125I-CCK binding occurring at 1 nM and maximal stimulation occurring at 100 nM (Fig. 3). The relative potencies of the steroids were dexamethasone > corticosterone > aldosterone > progesterone > estradiol benzoate and paralleled their relative effectiveness as glucocorticoids.

This effect of dexamethasone on the binding of 125I-CCK to AR42J cells required a prolonged incubation period. No effect was seen before 12 h and the increase in 125I-CCK binding was maximal after 48 h (Fig. 4). Specificity of the AR42J CCK Receptor and Effects of Dexamethasone—To further characterize the AR42J cell CCK receptor, the abilities of several CCK analogs to inhibit 125I-CCK binding were examined. Binding to AR42J cells was competitively inhibited by increasing concentrations of the various unlabeled CCK molecules (Fig. 5). For AR42J cells grown under control conditions, CCK8 was the most potent competitor, and CCK33 was nearly equipotent. In contrast, desulfated CCK8 was 30-fold, and CCK4 and 300-fold, less potent. The binding of 125I-CCK to AR42J cells treated for 48 h with 10 nM dexamethasone was also inhibited by the CCK analogs, and the relative potencies were unchanged compared to control cells (Table 1).

Effects of Dexamethasone on CCK-stimulated Amylase Release—As previously reported (11), AR42J cells spontaneously secreted amylase into the incubation medium (Fig. 6). Furthermore, amylase secretion was linear for at least 50 min either in the unstimulated state or after stimulation with CCK (data not shown). In control cultures, CCK induced a 2.6-fold increase in amylase secretion, from 100 ± 10 to 260 ± 20 milliunits/40 min (n = 5) (Fig. 6a). Dexamethasone pretreatment led to enhanced secretion of amylase. In cells pretreated with 10 nM dexamethasone for 48 h, basal and CCK-stimulated amylase secretion were increased ~5.5-fold to 550 ± 60 milliunits/40 min basal release and 1440 ± 140 milliunits/40 min after stimulation with CCK (n = 5). These data indicate an increased secretory ability of the AR42J cells after treatment with dexamethasone.

Upon closer examination, when the amylase released during 40 min was studied as a function of the concentration of CCK8, the same maximal 2.6-fold increase was seen using either control cells or cells which had been pretreated with dexamethasone, even though the basal amylase release was only 18% as great in control cells (Fig. 6a). In order to better understand the effects of the glucocorticoids, the data for amylase release was normalized based on the initial content.

**Fig. 2. Competition inhibition data for 125I-CCK binding to AR42J cells grown in the absence (○) or presence (●) of 10 nM dexamethasone for 48 h.** Cells were incubated in the presence of 30 pm 125I-CCK for 60 min at 4 °C. The binding data were plotted as either bound versus free ligand or bound/free versus bound. Results are the mean ± S.E. of 5 experiments.
Glucocorticoids Increase CCK Receptors in AR42J Cells

**Fig. 3.** The effect of various steroid hormones on the binding of $^{125}$I-CCK to AR42J cells. Cells were cultured in the presence of various concentrations of the different hormones for 48 h and then incubated with 30 pm $^{125}$I-CCK for 1 h at 22 °C. Values are expressed as per cent control and are the mean ± S.E. of 3-5 experiments.

**Fig. 4.** Time course of the dexamethasone-induced increase in $^{125}$I-CCK binding to AR42J cells. AR42J cells were cultured in the presence of dexamethasone (10 nM) for up to 72 h. The cells were then incubated with 30 pm $^{125}$I-CCK for 1 h at 22 °C. Values are expressed as per cent total $^{125}$I-CCK bound/mg of protein and are mean ± S.E. of 3-5 experiments.

**Fig. 5.** Specificity of binding to the AR42J cell CCK receptor. Binding of 30 pm $^{125}$I-CCK was carried out at 22 °C for 60 min in the presence of various concentrations of CCK8, CCK33, desulfated CCK8 (dsCCK8), and CCK4. Values are expressed as per cent of control $^{125}$I-CCK bound/mg of protein and represent the mean ± S.E. of 5 experiments.

**Fig. 6.** Concentration dependence of amylase release stimulated by CCK8 for AR42J cells raised for 48 h in the absence (○) or presence (●) of 10 nM dexamethasone. Amylase release over 40 min is plotted as a function of the concentration of CCK8 in the medium. a, amylase activity released into the medium expressed per culture. b, amylase release expressed as the percentage of the total amylase activity initially present in the cultures. Results shown are the mean ± S.E. for 5 experiments.

### Table I

**Effect of dexamethasone pretreatment on the selectivity of the AR42J CCK receptor as shown by competitive inhibition of CCK binding**

<table>
<thead>
<tr>
<th>Analog</th>
<th>Control IC$_{50}$ (M)</th>
<th>Dexamethasone IC$_{50}$ (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCK33</td>
<td>$4.2 \times 10^{-9}$</td>
<td>$4.5 \times 10^{-9}$</td>
</tr>
<tr>
<td>CCK8</td>
<td>$2.8 \times 10^{-9}$</td>
<td>$3.0 \times 10^{-9}$</td>
</tr>
<tr>
<td>dsCCK8</td>
<td>$5.8 \times 10^{-8}$</td>
<td>$1.0 \times 10^{-7}$</td>
</tr>
<tr>
<td>CCK4</td>
<td>$1.0 \times 10^{-6}$</td>
<td>$9.0 \times 10^{-7}$</td>
</tr>
</tbody>
</table>

in the cells in Fig. 6b. Although, when expressed as percentage of initial content, control cells showed the same maximal responsiveness to CCK8 as dexamethasone-treated cells, they were less sensitive in terms of the threshold and one half-
maximally effective concentrations of CCK8. The concentration of CCK8 required for one half-maximal stimulation of amylose secretion was 35 ± 6 pm (n = 5) for control cells and 8 ± 1 pm (n = 4) (p < 0.05) for cells pretreated with dexamethasone. These findings indicated, therefore, that treatment with glucocorticoids decreased the concentration of CCK required for stimulation of amylose release.

**DISCUSSION**

The primary differentiated function of the exocrine pancreas is to secrete proteins. Protein secretion involves three major phases: 1) synthesis of secretory proteins, 2) intracellular transport and packaging, and 3) secretagogue-induced discharge. We have previously reported that glucocorticoids increase the differentiated state of pancreas-derived AR42J cells as evidenced by increases in the first two phases of protein secretion. In the present study we have further demonstrated that glucocorticoids increase the differentiated state of AR42J cells by influencing the third phase of protein secretion, secretagogue-induced discharge.

Glucocorticoids increase the number of CCK receptors on cultured AR42J cells in a time- and dose-dependent manner while the affinity of the CCK receptor was not significantly altered. In dexamethasone-induced cells, the relative affinities for various CCK analogs indicated that dexamethasone did not change the nature of the CCK receptor. The relative potencies of the various steroids employed, which are similar to those we have shown for the effects of steroids on AR42J cell amylose content (8), indicate that the effect on the CCK receptor is via interaction of the steroids with glucocorticoid receptors.

The present studies indicate that the AR42J cell CCK receptor has a specificity for CCK analogs that is similar to the rat acinar cell CCK receptor (17-20) and that the AR42J cell CCK receptor is functional in terms of the stimulation of enzyme secretion. Thus, this cell type should prove valuable for further investigations of CCK action and CCK receptor regulation. There are, however, a few differences between the AR42J CCK receptor and those found on normal pancreatic acinar cells. First, CCK binding to AR42J cells yields linear Scatchard plots indicating a single binding site, whereas, binding to rat pancreatic acini yields curvilinear Scatchard plots (19). Second, the regulation of amylose release by receptors on normal acini is biphasic with low concentrations of CCK stimulating, but with high concentrations inhibiting, enzyme release. In contrast the AR42J response to CCK was monophasic. Third, the relative specificities for CCK analogs were somewhat different than those found in normal acini, with the AR42J cells being less selective for sulfated analogs. Competitive binding studies conducted with either rat pancreatic acini (18, 19) or pancreatic membranes (20) have indicated that desulfated CCK8 is 200-300- and CCK4 is 10,000-fold less potent than CCK8, whereas, in the AR42J cells these analogs were 30- and 300-fold less potent, respectively.

Dexamethasone increased the secretory response of AR42J cells primarily by inducing a large increase in the cellular contents of amylose. However, when enzyme secretion was normalized to the percentage of initial cellular content, both control and dexamethasone-treated cells released amylose at a comparable maximal rate. A similar phenomenon has been reported for the effects of insulin on pancreatic acinar cells (21). In those studies, animals rendered diabetic by streptozotocin treatment showed both a reduced content of digestive enzymes and a reduced secretory response to CCK (21), and treatment of diabetic animals with insulin led to an increase in enzyme content and secretion. However, when enzyme secretion was normalized to the initial acinar content, both normal and diabetic acini were able to release enzymes at a comparable maximum rate in response to CCK. The observation that the ratio of secreted to total enzyme content remains fixed under varied conditions (and in the face of large differences in the content of enzyme) suggests that the secretary capacity of the cells is independent of changes in enzyme content.

Treatment of AR42J cells with dexamethasone also increased the sensitivity of the cells to the stimulation of amylose secretion by CCK. Most likely, this effect was due to the dexamethasone-induced increase in the numbers of CCK receptors. An increase in the number of receptors leads to a decrease in the concentration of hormone required to fill an equal number of receptors, and, since biological responses are generally proportional to the number of occupied receptors, there is a reduction in the concentration of hormone required to elicit an equal biological response (22). Treatment with dexamethasone led to an approximate 3-fold increase in CCK receptors/cell and a 4-fold increase in sensitivity.

In the present study, glucocorticoids increased the numbers of CCK receptors present on AR42J cells with no change in receptor affinity. Glucocorticoids have previously been shown to modulate the receptor numbers of a variety of other hormone receptors (23, 24). The present data could be explained by an effect either on CCK receptors synthesis or CCK receptor turnover. The effects of glucocorticoids on CCK receptor numbers occurred over the same prolonged time period and with the same concentrations of steroid as the previously reported effects of glucocorticoids on amylose synthesis, suggesting that glucocorticoids may increase the numbers of CCK receptors in AR42J cells by increasing CCK receptor synthesis.

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**REFERENCES**


*By definition, for a simple bimolecular interaction, [hormone-receptor] = 1/Kd × [hormone] × [receptor], where Kd is the equilibrium dissociation constant; therefore, in the absence of a change in Kd, increasing the number of receptors has the effect of increasing the number of occupied receptors at any given hormone concentration.*