CCK-B receptors produce similar signals but have opposite growth effects in CHO and Swiss 3T3 cells

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Detjen, Katharina, David Yule, Min-Jen Tseng, John A. Williams, and Craig D. Logsdon. CCK-B receptors produce similar signals but have opposite growth effects in CHO and Swiss 3T3 cells. Am. J. Physiol. 273 (Cell Physiol. 42): C1449–C1457, 1997.—Rat cholecystokinin-B (CCK-B) receptors were transfected into Chinese hamster ovary (CHO)-K1 (CHO-CCK-B) and Swiss 3T3 (Swiss 3T3-CCK-B) cells, and the effects of receptor activation on cell proliferation and intracellular signaling were investigated. CCK octapeptide (CCK-8) treatment had no effect on cell growth in quiescent CHO-CCK-B cells but inhibited DNA synthesis, proliferation, and colony formation when the cells were grown in fetal bovine serum (FBS). In contrast, CCK-8 stimulated DNA synthesis in quiescent Swiss 3T3-CCK-B cells and had no effect when the cells were grown in FBS. These differences in growth responses were not due to differences in the level of receptor expression, as similar numbers of receptors were present in both cell types. To determine whether the different growth effects were due to differences in receptor coupling to common second messenger pathways, we investigated the effects of CCK-8 on several known intracellular signals. In both cell types, CCK-8 stimulated increases in intracellular Ca\(^{2+}\) concentration and polyphosphoinositide hydrolysis with similar potencies and efficacies. CCK-8 also stimulated arachidonate release from both cell types, although the potency was higher in the CHO cells. Adenosine 3',5'-cyclic monophosphate generation was observed at high agonist concentrations in both cell types and was much greater in cells with higher receptor density. In summary, receptor activation had opposite effects on growth parameters in CHO and Swiss 3T3 cells, but only minor differences were observed in the characteristics of CCK-B receptor coupling to specific second messengers in the two cell types. Thus cellular context is a principal determinant of the biological effects of CCK-B receptor activation, and differences in biological responses may occur independently of major differences in receptor coupling.

Gastrin has been shown to mediate both short- and long-term actions in a variety of gastrointestinal tissues. Gastrin activates cholecystokinin-B (CCK-B) receptors, which are classic seven-transmembrane-spanning receptors coupled to heterotrimeric G proteins (33). Gastrin acts in the short term as a secretagogue on gastric parietal cells (2), and the mechanisms involved in this secretory effect have been well studied. CCK-B receptor effects on secretion are believed to be due to G\(_q\)-mediated activation of phosphoinositide-specific phospholipase C, leading ultimately to increases in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]) (2, 3, 34). Gastrin acts in the long term to stimulate cell proliferation in the stomach and intestine (16). Gastrin also has been reported to stimulate cell proliferation in tissue culture cell models transfected with CCK-B receptors (15, 26) and a variety of cancer cell lines, including pancreatic (30), gastric (13), colonic (29), and small lung tumor (10). In contrast, we recently observed that activation of exogenously expressed CCK-B receptors can inhibit the growth of MIA PaCa-2 and PANC-1 human pancreatic cancer cell lines (4). Therefore, it seems apparent that activation of these receptors can influence cell growth either positively or negatively, depending on the cell context. However, it is unclear whether these cell-specific differences in growth responses are due to differences in receptor coupling or to other mechanisms intrinsic to the different cells.

To develop model systems for investigating mechanisms involved in growth effects of gastrin, we transfected rat CCK-B receptors into Chinese hamster ovary (CHO) and Swiss 3T3 cells, both of which have been widely utilized for receptor expression and growth regulation studies. The effects of CCK-B receptor activation on both growth and second messenger generation were then determined in these two cell models. We found that activation of CCK-B receptors inhibited the growth of CHO cells. In contrast, activation of CCK-B receptors stimulated the growth of Swiss 3T3 cells. The inhibitory effect observed in CHO cells did not require overexpression of receptors, as it occurred in CHO cells bearing fewer receptors than were present in the Swiss 3T3 cells. In either cell type, CCK-B receptors coupled to increases in [Ca\(^{2+}\)], phosphoinositide hydrolysis, arachidonate release, and, to a lesser extent, adenosine 3',5'-cyclic monophosphate (cAMP) formation. Thus cellular context is a major determinant of the biological effects of CCK-B receptor activation, and differences in growth responses may occur independently of differences in receptor coupling.

**EXPERIMENTAL PROCEDURES**

**Materials**

125\(^I\)-labeled cholecystokinin octapeptide (CCK-8), 125\(^I\)-cAMP radioimmunoassay kits, and \(^{3}H\)arachidonate (100 Ci/mmol) were obtained from DuPont NEN (Boston, MA). 125\(^I\)-cAMP radioimmunoassay kits were also obtained from Diagnostic Products (Los Angeles, CA). myo-[3\(^{3}H\)]inositol (19.1 Ci/mmol), \(^{3}H\)thymidine, and the “cell proliferation kit” were obtained from Amersham (Arlington Heights, IL). Fura 2-acetoxymethyl ester (fura 2-AM) and fura 2 free acid were purchased from Molecular Probes (Eugene, OR). Analytical grade 1-X8 resin (Ag 1-8, 100–200 mesh) and protein assay reagent were obtained from Bio-Rad (Rockville Center, NY). Restriction endonucleases were purchased from Gibco (Grand Island, NY). Sulfated CCK-8 was purchased from...
Bachem (Torrance, CA). Trichloroacetic acid was purchased from J. T. Baker (Phillipsburg, NJ). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin, and amphotericin B were obtained from Gibco. Tissue culture plasticware (24- and 6-well plates and 10-cm petri dishes) were obtained from Sarstedt (Newton, NC). CHO-K1 cells were obtained from the American Type Culture Collection (Rockville, MD), and Swiss 3T3 cells were obtained from Dr. G. Johnson (National Jewish Center for Immunology and Respiratory Medicine, Denver, CO). Soybean trypsin inhibitor type I-S, bovine serum albumin fraction V, and all other chemicals were obtained from Sigma Chemical (St. Louis, MO).

Generation and Characterization of CCK-B Receptor-Expressing Cell Lines

Construction of CCK-B receptor expression vector and development of stable cell lines. A full-length cDNA encoding the rat CCK-B (33) receptor was kindly provided by S. A. Wank (National Institutes of Health, Bethesda, MD). The receptor cDNA was subcloned into the pET8 expression vector as previously described (36). CHO-K1 and Swiss 3T3 cells were routinely cultured in DMEM supplemented with 5% FBS in a humidified atmosphere of 5% CO₂. For transfection, cells were grown to 30–40% confluence in 60-mm dishes and transfected with 3 µg of Pvu I-linearized plasmid DNA using lipofectin reagent (Gibco-BRL). G-418-resistant colonies were selected and screened for CCK-B receptors by binding of ¹²⁵I-CCK-8. Two or more clones were tested in all assays to control for clonal variation.

Measurement of CCK-B receptor binding. Ligand binding was carried out using cells plated in 24-well dishes at 1 × 10⁶ cells/well the day before the binding assay. For binding assays, ¹²⁵I-CCK-8 (10 nM) was added to N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-Ringer buffer (HR buffer) that contained (in mM) 137 NaCl, 4.7 KCl, 0.56 MgCl₂, 1 Na₂PO₄, 1.28 CaCl₂, 10 HEPES (pH 7.4), supplemented with 0.1 mg/ml soybean trypsin inhibitor. Cells were incubated to equilibrium (18 h at 4°C) and then washed twice with ice-cold phosphate-buffered saline (PBS). The cells were then scraped and counted in a gamma counter. Nonspecific binding was determined in the presence of 100 nM CCK-8. Protein contents were determined on samples after counting. Binding affinity and capacity were calculated using the LIGAND analysis program (21).

Analysis of Second Messenger Generation

Measurement of [Ca²⁺]. Analysis of [Ca²⁺] changes were conducted on cells grown to 30–50% confluence on glass coverslips. Cells were incubated with 5 µM fura 2-AM at 37°C for 30 min and then washed in HR buffer. Cover slips containing cells were transferred to a closed chamber, mounted on the stage of a Zeiss Axiovert inverted microscope, and continuously superfused at 1 ml/min with HR buffer at 37°C. Measurement of emitted fluorescence and calibration of these signals to yield a measurement of [Ca²⁺], were performed using an Attofluor digital imaging system (Rockville, MD), exactly as described previously (36).

Measurement of [³H]inositol phosphates. For measurement of total inositol phosphates, cells were cultured in 24-well plates at 1 × 10⁶ cells/well for 24 h in the presence of 3 µCi of myo-[³H]inositol. The cells were then washed twice with HR buffer containing 10 mM Li⁺. Incubation with various concentrations of CCK-8 was conducted in the same buffer for 30 min and terminated by addition of an equal volume of 20% ice-cold trichloroacetic acid (TCA). After centrifugation at 2,000 g for 20 min, 0.9 ml of each supernatant was washed twice with water-saturated diethyl ether, neutralized with 100 µl of KHCO₃, and diluted with 2.5 ml of water. Analysis of total [³H]inositol phosphates was carried out by the method described by Berridge (1).

Measurement of [³H]arachidonic acid release. Measurement of arachidonic acid release was conducted as previously described (36). Briefly, cells grown to near confluence in six-well plates were incubated with 1 µCi of [³H]arachidonic acid for 24 h. The cells were then washed with PBS and incubated in HR buffer with or without CCK-8 (10 nM) for 30 min. The incubation medium was then removed and counted in a scintillation counter.

Measurement of CAMP production. Measurement of cAMP generation was conducted as previously described (36). CHO and Swiss 3T3 cells grown in six-well dishes were incubated with indicated concentrations of CCK-8 for 1 h in HR containing 1 mM 3-isobutyl-1-methylxanthine. The incubation was stopped by addition of 20% ice-cold TCA, and cells were scraped from the plate. The TCA was neutralized, and the cAMP was extracted and assayed by radioimmunoassay using kits purchased from DuPont NEN (NEK-033) or Diagnostic Products, following the manufacturer's recommendations.

Analysis of Cell Growth Parameters

Measurement of DNA synthesis. DNA synthesis was estimated by measurement of [³H]thymidine incorporation into TCA-precipitable material. For studies in exponentially growing CHO cells, cells cultured in 5% FBS were treated for 8 h with the indicated concentrations of CCK-8, and during the last 1 h of the treatment period [³H]thymidine was added to each well (final concentration, 1 µCi/ml). In experiments utilizing quiescent CHO cells, the cells were serum starved for 24 h and then treated with CCK-8 (10 nM) for 24 h and exposed to [³H]thymidine for the last 6 h. For studies in Swiss 3T3 cells, cells were made quiescent by incubation in serum-free medium supplemented with 10% conditioned medium overnight. Cells were then treated with the indicated concentrations of CCK-8 for 24 h and exposed to [³H]thymidine (0.5 µCi/ml) during the final h. After labeling, cells were washed twice with PBS and then twice with TCA (final concentration, 6%) and removed by dissolving in 0.3 ml of 0.1 N NaOH. Radioactivity was measured by liquid scintillation counting.

Nuclear labeling. Nuclear labeling was investigated using an Amersham cell proliferation kit that identifies cells synthesizing DNA by detecting incorporation of 5-bromo-2-deoxyuridine (BrdU). For determination of effects on cells growing in serum, either CHO or Swiss 3T3 cells expressing CCK-B receptors were treated with CCK-8 (10 nM) for 8 h, and BrdU was added during the last 1 h. For determination of effects on quiescent cells, cells were serum starved for either 24 h (CHO) or 36 h (Swiss 3T3). Cells were then treated with CCK-8 (10 nM) for 24 h (CHO) or 40 h (Swiss 3T3), and BrdU was added for the last 24 h. Subsequently, cells were fixed with acidic methanol and BrdU-labeled nuclei were detected, following the manufacturer's directions.

Analysis of colony formation in soft agar. Colony formation was assayed as previously described (5). Cells were plated at 1,000 cells/dish into 35-mm dishes in agar (0.3% in DMEM supplemented with 5% FBS) and treated with the indicated
concentrations of CCK-8. After 2 wk, the dishes were examined and all colonies >0.5 mm in diameter were counted.

RESULTS

Effects of CCK-B Receptor Activation on Cell Growth

To test whether CCK-B receptors would stimulate the growth of Swiss 3T3 cells, the cells were transfected with the rat CCK-B receptor. Swiss 3T3 cells bearing CCK-B receptors bound ligand with an affinity similar to what has been reported previously in native cells (Table 1). When CCK-B receptor-bearing Swiss 3T3 cells growing exponentially in FBS were treated with CCK-8, there was no effect on DNA synthesis as measured by \( [3H] \) thymidine incorporation (data not shown). Likewise, no change in nuclear labeling was observed in the presence of FBS (Fig. 1). In contrast, stimulation of quiescent Swiss 3T3-CCK-B cells with CCK-8 (100 nM) led to a fourfold increase in the rate of DNA synthesis (Fig. 2). This increase in \( [3H] \) thymidine incorporation also corresponded to an increase in nuclear labeling (Fig 1). The stimulatory effect was concentration dependent, with significant stimulation noted at 0.1 nM, half-maximal effects at \( 2.0 \pm 0.8 \) nM (n = 4 experiments), and maximal effects at 10 nM CCK-8 (Fig. 2). Wild-type Swiss 3T3 cells showed no growth response, either stimulatory or inhibitory, to CCK-8 stimulation (data not shown).

We previously found that activation of CCK-A receptors could inhibit the growth of CHO cells (5). In the current study, we examined the effects of activation of CCK-B receptors in CHO cells. CHO cells expressing rat CCK-B receptors bound ligand with an affinity similar to Swiss 3T3 cells (Table 1). In contrast to the responses observed in the Swiss 3T3 cells, in CHO cells activation of CCK-B receptors led to a decrease in DNA synthesis.

Table 1. Characteristics of CCK-B receptor-expressing cell lines

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>K(_d), nM</th>
<th>B(_{max}), fmol/mg protein</th>
</tr>
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<tbody>
<tr>
<td>CHO-CCK-B cells</td>
<td></td>
<td></td>
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<tr>
<td>Higher density</td>
<td>0.25 ± 0.03</td>
<td>852 ± 72</td>
</tr>
<tr>
<td>Lower density</td>
<td>0.20 ± 0.06</td>
<td>329 ± 41</td>
</tr>
<tr>
<td>Swiss 3T3-CCK-B cells</td>
<td>0.24 ± 0.01</td>
<td>462 ± 81</td>
</tr>
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</table>

Values are means ± SE of 3–6 experiments. Competitive binding analysis was conducted with cells cultured to near confluence in 24-well dishes. For binding assays, cells were incubated to equilibrium (4 h at 24°C) in presence of \( ^{125}I \)-labeled cholecystokinin octapeptide (CCK-8; 10 pM). Cells were then washed, scraped, and counted in a gamma counter. Nonspecific binding was determined in presence of 100 nM CCK-8. Protein contents were determined from samples after counting. Binding affinity (K\(_d\)) and capacity (B\(_{max}\)) were calculated using LIGAND analysis program (21). CHO, Chinese hamster ovary; CCK-B, cholecystokinin-B.
synthesis (Fig. 3A). This decrease was detectable at 10 pM, inhibition was half-maximal at 0.07 ± 0.01 nM (n = 4), and a maximal inhibition of 58 ± 4% of control (n = 4) was observed at 3 nM CCK-8. The observed inhibition of [3H]thymidine incorporation corresponded to a reduction in the nuclear labeling of cells cultured in the presence of serum and CCK-8 (Fig. 1). When CHO-CCK-B cells were first made quiescent by serum starvation and then treated with CCK-8, no stimulatory effect was noted at any concentration between 0.01 and 100 nM CCK-8, and at 100 nM the [3H]thymidine incorporation was 83 ± 9% of control (n = 3). Likewise, no effect was observed on nuclear labeling under these conditions (Fig. 1). Effects of CCK-8 treatment on CHO cell numbers in exponentially growing cell populations cultured in the presence of 5% FBS were also examined (Fig. 3B). CCK-8 activation of CCK-B receptors led to an inhibition of CHO-CCK-B cell proliferation. The inhibition of cell proliferation was concentration dependent, with half-maximal inhibition occurring with 2.4 ± 0.3 nM CCK-8 and a maximal 92 ± 8% (n = 3) inhibition observed with 100 nM CCK-8.

Activation of CCK-A receptors on CHO cells was previously shown to decrease both anchorage-dependent and anchorage-independent growth, and these effects were mediated by separate receptor affinity states (5). To examine the effects of CCK-B receptor activation on CHO cell anchorage-independent growth, CHO-CCK-B cells were plated into soft agar in the presence of various concentrations of CCK-8 and cultured for 2 wk. Analysis of the number of colonies 0.5 mm in diameter or larger indicated a concentration-dependent decrease in colony formation (Fig. 3C). Significantly lower numbers of colonies were noted in cultures treated with 0.1 nM CCK-8, half-maximal inhibition was observed at 1.0 ± 0.5 nM (n = 6), and a maximal inhibition to 7 ± 5% (n = 6) of control was observed at 10 nM CCK-8.

Effects of CCK-B Receptor Activation on Signal Transduction Pathways

Activation of CCK-B receptors in native cells has been linked to stimulation of a variety of intracellular messenger pathways, including an increase in [Ca\textsuperscript{2+}], (3). This is similar to what has been observed for CCK-A receptors, for which it is well documented that this increase involves the release of Ca\textsuperscript{2+} from intracellular pools followed by the influx of extracellular Ca\textsuperscript{2+} (34, 36). For CCK-A receptors, the temporal pattern of [Ca\textsuperscript{2+}], signaling has been shown to depend on the concentration of agonist applied, such that at low concentrations...
physiological concentrations of agonists oscillations of $[\text{Ca}^{2+}]_i$ are seen, whereas at maximal doses a single large transient peak followed by a much smaller plateau is often observed. In the current study, superfusion of either CHO-CCK-B or Swiss 3T3-CCK-B cells with CCK-8 at low concentrations (25 pM–0.5 nM) predominantly resulted in an oscillatory $[\text{Ca}^{2+}]_i$ signal (Fig. 4). At concentrations above 0.5 nM, a single transient peak in $[\text{Ca}^{2+}]_i$ followed by a small plateau was observed. In CHO-CCK-B cells, a maximal concentration of 1 nM increased $[\text{Ca}^{2+}]_i$ from basal values of $78.2 \pm 9$ nM (4 experiments, 178 cells) to a maximal peak value of $244 \pm 37$ nM (4 experiments, 34 cells). In Swiss 3T3-CCK-B cells the basal $[\text{Ca}^{2+}]_i$ was $92.1 \pm 8.2$ nM (8 experiments, 120 cells), and stimulation with CCK-8 increased $[\text{Ca}^{2+}]_i$ to a maximum peak value of $428 \pm 43$ nM (3 experiments, 27 cells). Thus there were no qualitative differences between the effects of CCK-8 on $[\text{Ca}^{2+}]_i$ in the two cell types.

Activation of CCK-B receptors has previously been reported to increase polyphosphoinositide hydrolysis in guinea pig chief cells (22). In the current study, the effects of activation of CCK-B receptors on formation of $[^3\text{H}]$inositol phosphates were measured in CHO-CCK-B and Swiss 3T3-CCK-B cells. In both cell types labeled for 24 h with myo-$[^3\text{H}]$inositol, CCK-8 increased the formation of total $[^3\text{H}]$inositol phosphates in a concentration-dependent manner (Fig 5A). The potency of stimulation of polyphosphoinositide release was similar for both cell types (Table 1). An increase could be detected at 300 pM, was half maximal at $\sim 1$ nM, and reached a maximum at 10 nM (Fig. 5A, Table 2). The maximal effect was slightly larger in CHO cells with a higher receptor density. However, no difference was noted between the efficacy of CCK-8 in Swiss 3T3 cells and CHO cells with a lower receptor density (Fig. 5A).

Arachidonic acid is another potentially important intracellular messenger. The effects of CCK-B receptor activation on arachidonate formation in natively expressing cells are unknown. In the current study, CCK-8 led to a similar increase in arachidonate release in CHO-CCK-B cells and Swiss 3T3-CCK-B cells that were prelabeled with $[^3\text{H}]$arachidonate (Fig. 5B). The potency of CCK-8 was greater in the CHO cells bearing either high or low receptor densities, compared with the Swiss 3T3 cells.

Activation of CCK-B receptors on gastric parietal cells has been reported not to increase the intracellular concentration of cAMP (2). In the current study, CCK-8 activation of CCK-B receptors was able to induce a large accumulation of cAMP in CHO cells bearing high numbers of receptors and a small increase in Swiss 3T3 cells (Fig. 5C). However, no significant effect was noted in CHO cells with a low receptor density.

**Fig. 4.** Effects of CCK-8 on intracellular $\text{Ca}^{2+}$ concentration ($[\text{Ca}^{2+}]_i$) signaling in CHO and Swiss 3T3 cells bearing CCK-B receptors. Effects of different concentrations (A and C: 50 pM; B and D: 1 nM) of CCK-8 on $[\text{Ca}^{2+}]_i$ in individual Swiss 3T3-CCK-B cells (A and B) or CHO-CCK-B cells (C and D). Each trace is a recording from a representative cell; similar data were observed in at least 3 experiments, in each of which >10 cells were imaged.
DISCUSSION

In this study we observed that the cloned rat CCK-B receptor coupled to signal transduction pathways in a qualitatively similar manner in CHO and Swiss 3T3 cells. Agonist activation of these receptors potently increased phosphoinositide hydrolysis, \([\text{Ca}^{2+}]_i\), and arachidonate release. cAMP formation could also be detected in both cell types, but it required high concentrations of agonist and was much greater in cells bearing higher receptor densities. Quantitative responses to agonist in the two cell lines varied somewhat in terms of either efficacy or potency, depending on the specific pathway examined. However, these differences were not dramatic. In comparison to these minor quantitative differences, there were major differences in the functional responses in the two cell types. CCK-B receptor activation stimulated the growth of Swiss 3T3 cells and inhibited the growth of CHO cells. These data illustrate the cell type dependence of receptor signaling and function.

Exact quantitative comparisons between second messenger production in the two different cell types can be complicated by a variety of issues, including differences in receptor numbers. Two CHO cell clones were utilized in this study. One expressed significantly greater numbers of CCK-B receptors than did the Swiss 3T3 cell line, and the other expressed approximately equivalent numbers. Previous studies focused on the influence of receptor number on coupling to cellular signaling suggest that increased receptor number generally leads to increased efficacy and, to a lesser extent, increased potency (8, 32). Due to the phenomenon of “spare receptors,” which describes the situation when occupation of only a small fraction of receptors is sufficient to elicit a full biological response, it is often possible to achieve the same maximal response in cells bearing multifold different receptor numbers. In the current study, CCK-8 activation of CHO and Swiss 3T3 cells resulted in approximately equivalent maximal effects on \([\text{Ca}^{2+}]_i\), phosphoinositol hydrolysis, and arachidonate release, despite some variation in receptor number. This supports the hypothesis that there are significant spare receptors in terms of coupling to these pathways. In contrast, the maximal increase in intracellular concentrations of cAMP was significantly less in Swiss 3T3 cells and CHO cells bearing fewer CCK-B receptors than in CHO cells bearing a greater number of CCK-B receptors. This is similar to what has been reported for \(\beta_2\)-adrenergic receptors expressed in CHO cells, in

Fig. 5. Effects of CCK-8 on signaling molecule generation in CHO or Swiss 3T3 cells bearing CCK-B receptors (CHO<sub>high</sub> and CHO<sub>low</sub>, CHO clones with high and low CCK-B receptor densities, respectively). A: effects of CCK-8 on total phosphoinositide hydrolysis. Confluent cells were incubated with myo-[2-\text{H}]inositol for 24 h, after which they were exposed to indicated concentrations of CCK-8 for 30 min. Isolation of total \([\text{H}]\)inositol phosphates was performed as detailed in EXPERIMENTAL PROCEDURES. Data are expressed as multiples of basal inositol phosphate content and are means ± SE of 3–5 separate experiments, with each value measured in triplicate in each experiment.

B: effects of CCK-8 on release of arachidonate. Cells were grown to near confluence in 6-well plates and then incubated with 1 µCi of [\text{H}]arachidonic acid for 24 h. Cells were then washed with phosphate-buffered saline and incubated in HEPES-Ringer buffer with indicated concentrations of CCK-8 for 30 min. Incubation medium was then removed and counted in a scintillation counter. Data are expressed as multiples of basal arachidonate release and are means ± SE of 3–6 separate experiments, with each value measured in triplicate in each experiment.

C: effects of CCK-8 on cAMP generation. Cells were grown to near confluence in 6-well plates. Cells were then stimulated with indicated concentrations of CCK-8 for 30 min. Incubation medium was then removed, cells were solubilized, and cAMP was extracted and measured as described in EXPERIMENTAL PROCEDURES. Data are expressed as multiples of basal value and are means ± SE of 3–4 separate experiments, with each value measured in triplicate in each experiment.
which, over a 200-fold range of receptor expression, transfectants displayed increasing efficacy of agonist-stimulated cAMP accumulation in response to agonist (8). These results suggest that, in terms of coupling to $G_\text{S}$ and cAMP generation, there are no spare CCK-B receptors. The potency of CCK-B stimulation of [Ca$^{2+}$], and phosphoinositol hydrolysis was approximately equivalent for both cell types. However, the potency of CCK-B for arachidonate release was much greater in the CHO cells bearing either high or low receptor levels, compared with the Swiss 3T3 cells. Thus both the receptor expression level and the cellular context were important determinants of receptor coupling to specific signaling pathways.

Previously, we reported that CCK-A receptors expressed in CHO cells also coupled to increased phosphoinositol hydrolysis, [Ca$^{2+}$], arachidonate release, and cAMP (36). Comparing the two studies, we found no major differences between the coupling of CCK-A and CCK-B receptors in CHO cells. CCK-B receptors have also been shown to increase [Ca$^{2+}$], and polyphosphoinositide hydrolysis in natively receptor-expressing gastric cells (2, 3). In contrast, in guinea pig pancreatic acini, which endogenously possess both CCK-A and CCK-B receptors, only occupation of the CCK-A receptor has been reported to stimulate activation of phospholipase C (35). The current data suggest that the observed lack of coupling to phospholipase C may be due to the relatively small number of CCK-B receptors present in the guinea pig pancreatic cells.

Important cell-specific differences have previously been noted in the influence of CCK-B receptor activation on cellular cAMP levels. Activation of CCK-B receptors found on guinea pig pancreatic acinar cells (35) or rabbit parietal cells (2) does not lead to formation of cAMP. Activation of CCK-B receptors has been reported to inhibit adenylate cyclase activity in membranes prepared from the pancreatic AR42J cell line (24). In other reports, CCK-B receptor activation has been shown to increase cAMP in certain tumor cells (14). In the present study, the ability of the CCK-B receptor to increase cAMP generation in two different cell models supports the hypothesis that the CCK-B receptor, like the CCK-A receptor, is capable of coupling to increased cAMP. However, cAMP generation in the current study was highly dependent on receptor expression levels. Thus it is likely that the level of receptor expression in native cells is too low for generation of a significant increase in cellular cAMP concentration.

In vivo, gastrin stimulates gastric and intestinal proliferation (16) and CCK stimulates pancreatic proliferation (31). Potentially, these in vivo effects may involve secondary release of growth regulatory substances, because both CCK and gastrin are known to increase the secretion of other bioactive molecules. However, evidence that direct activation of the CCK-A receptor mediates the effects of CCK on pancreatic acinar cell growth comes from the observations that CCK stimulates growth of cultured rat (11) and mouse (19) acinar cells in vitro. This evidence is compelling, as these cultures are highly enriched in acinar cells, and in these species pancreatic acinar cells do not express CCK-B receptors (33). In vitro, trophic actions of gastrin have been reported in primary cultures of gastrointestinal cells (20). However, these are mixed cultures consisting of several cell types, so that it is difficult to rule out the possibility of secondary effects. Direct trophic effects of gastrin have also been reported in a variety of pancreatic (30), colonic (29), gastric (13), and small lung tumor cells (25). Recently, however, it has become unclear whether the effects of gastrin on the growth of cancer cell lines are mediated through a classic CCK-B receptor or involve non-A, non-B CCK receptors such as those reported in pancreatic acinar AR42J cells (27) or Swiss 3T3 cells (28). More direct evidence for trophic actions of the CCK-B receptor comes from studies using transfected cell lines (15, 26). Therefore, there is little doubt that both CCK-B and CCK-A receptors can directly stimulate the growth of certain cells.

Likewise, both CCK-B and CCK-A receptors have been shown to inhibit the growth of specific cells. Activation of endogenous CCK receptors has been reported to inhibit the growth of lymphocytes (7), a transplantable rat pancreatic carcinoma (17), and two human gastrointestinal cancers (12). Activation of exogenously expressed CCK-B or CCK-A receptors inhibited growth of two pancreatic cancer cell lines (4), and activation of either CCK-B (current study) or CCK-A (5) receptors inhibits the growth of CHO cells. Thus the growth effects of CCK-B and CCK-A receptors do not appear to differ on the basis of receptor structure but are determined by the cell context.

The mechanisms involved in the stimulatory or inhibitory growth effects observed in the present study are unknown. We previously observed inhibition of CHO cell growth on activation of CCK-A and m3 muscarinic cholinergic receptors (5, 6). Thus inhibition of CHO cell
growth appears to be a general phenomenon for a group of G protein-linked receptors that are characterized by potently activating increases in [Ca\(^{2+}\)], and phosphoinositide hydrolysis. Interestingly, this same group of receptors has been shown to be capable ofagonist-induced cell transformation and growth stimulation in the context of NIH/3T3 cells (9). These observations support possible roles for Ca\(^{2+}\) and phosphoinositol hydrolysis in both the growth inhibitory and growth stimulatory responses. Alternatively, cAMP may be involved. CHO cells have previously been shown to be growth inhibited by cAMP (18). In contrast, cAMP may be reported to have the opposite effect on Swiss 3T3 cell growth (23). However, it was previously concluded that cAMP was not involved in the growth stimulatory effects observed on muscarinic cholinergic receptor activation in NIH/3T3 cells. Thus it remains possible that cAMP is involved in both the growth inhibitory and growth stimulatory effects of CCK receptor activation observed in this study. However, an argument against this hypothesis is the observation that CHO cells bearing few CCK-B receptors showed no increase in cAMP yet were clearly growth inhibited. Finally, it is also possible that the growth effects observed after CCK-B receptor activation are related to other, as yet unidentified, cell signals.

Our data are in contrast to those of Ito et al. (15), who reported that activation of exogenously expressed CCK-B receptors stimulated the growth of CHO cells. The explanation for this difference is not readily apparent. It is possible that the specific CHO cell clones utilized in these two studies differed in some aspect of growth regulation that is currently not understood.

In summary, CCK-B receptors expressed in CHO or Swiss 3T3 cells coupled to common second messengers in a qualitatively similar manner. Receptor expression levels were not a major factor in coupling to phosphoinositide hydrolysis, arachidonate release, or increased [Ca\(^{2+}\)], but appeared to be critically important in terms of receptor coupling to cAMP generation. CCK-B receptor activation stimulated or inhibited growth depending on the cell model. No major differences were noted in either the effects on cell growth or coupling to intracellular second messengers between CCK-B receptors observed in the current study and CCK-A receptors previously described (5, 36). Therefore, the biological actions of these two receptor subtypes observed in native tissues likely are influenced primarily by cellular context. These data indicate that differences in biological responses may occur independently of major differences in receptor coupling.

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