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The Gall Bladder Cholecystokinin Receptor Exists in Two Guanine Nucleotide-Binding Protein-Regulated Affinity States

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SUMMARY

To study proximal events in cholecystokinin (CCK) action on bovine gall bladder smooth muscle, we used the hormone analogue D-Tyr-Gly-[(Nle^{28,31})CCK-26–32]-phenethyl ester (OPE), which has unique biological properties. This fully efficacious agonist differs from native CCK by not expressing supramaximal inhibition of cell shortening, yet it clearly interacts with the same receptor molecule. This was demonstrated in binding and affinity labeling studies, where both peptides label the same M_r 70,000–85,000 protein and both fully compete for binding of the other ligand. Further, its relatively high affinity for the low affinity CCK receptor permits the clear demonstration of two affinity states of a CCK receptor on a membrane preparation and makes possible evaluation of the molecular basis of these affinity states and their regulation. Analysis of homologous and heterologous binding curves performed with both CCK and OPE peptides and radioligands demonstrated the presence of two affinity states, with CCK being able to distinguish them (K_{d_1} = 0.48 ± 0.04 nM and K_{d_2} = 56.5 ± 7.4 nM) and OPE recognizing them equally (K_{d_1} = 0.94 ± 0.31 nM and K_{d_2} = 0.96 ± 0.23 nM). In the presence of nonhydrolyzable GTP analogues, there was a shift in distribution of receptors toward the low affinity state, with the total number of receptors and their absolute affinities for each peptide remaining constant. Thus, the gall bladder CCK receptor is a single molecule capable of assuming two interconvertible affinity states, regulated by a guanine nucleotide-binding protein. Two full agonists are capable of interacting with this molecule to yield different biological responses via different molecular events.

CCK is the major hormonal stimulant of gall bladder contraction. Its effect can be demonstrated *in vivo* (1), as well as in muscle strips (2) and dispersed smooth muscle cells (3). Although much information exists about smooth muscle function in general (4) and about some of the biochemical events mediating contraction of the gall bladder smooth muscle cell (5), little is known about the proximal events stimulated by CCK in this cell. Recently, the CCK receptor present on this cell has been characterized functionally by radioligand binding (6, 7) and biochemically by affinity labeling (7–9). In addition, more distal events stimulated by CCK in this cell, at the level of calcium fluxes and molecular phosphorylation (10), have been studied.

Most of the work on proximal cellular events stimulated by this hormone has been performed on the pancreatic acinar cell. A very useful tool for the study of these events is a recently described phenethyl ester analogue of CCK, OPE, which has unique properties (11). Whereas native hormone has a complex concentration-response relationship, with increasing concentrations stimulating secretion to a maximum and further increases actually inhibiting secretion, this analogue is similarly efficacious in stimulating secretion and exhibits no supramaximal inhibition of secretion (11, 12). We know it does this via interaction with the pancreatic CCK receptor, based on direct binding and affinity labeling studies (11). Of particular interest is the suggestion that two agonists, native hormone and OPE, can work through a single receptor on the pancreatic acinar cell in molecularly distinct ways (11).

Because the pancreatic acinar cell and the gall bladder muscularis smooth muscle cell both express type A CCK receptors with similar structural specificities for agonists (CCK-8 ≫ CCK-8-desulfate > CCK-4) and antagonists (13), it was particularly interesting to evaluate the effects of this unique CCK analogue on the gall bladder cell. Despite the differences in cellular responses to CCK, with the acinar cell secreting and the gall bladder cell contracting, OPE had analogous effects on both cells. The results of binding studies with the gall bladder membranes were of particular interest. Using this tissue and radioligands based on both native CCK and OPE, it was possible to demonstrate both high and low affinity CCK receptors on a membrane preparation. This allowed access to intracellu-

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ABBREVIATIONS: CCK, cholecystokinin; OPE, D-Tyr-Gly-[(Nle^{28,31})CCK-26–32]-phenethyl ester; DTGNN, D-Tyr-Gly-[(Nle^{28,31})CCK-26–33]; Gpp(NH)p, guanosine 5′-(β,γ-imido)triphosphate; GTPγS, 5′-(γ-thio)triphosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

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lar sites of potential regulation and, indeed, the affinity states of the CCK receptor were shown to be interconvertible and regulated by a guanine nucleotide-binding protein.

Experimental Procedures

Reagents

Synthetic native CCK-8 (CCK-26–33) was purchased from Peninsula (Belmont, CA). CCK analogues DTGNN, ^{125}I-D-Tyr-Gly-[(Nle^{28,31},pNO$_2$-Phe^{33})CCK-26–33], and OPE were synthesized by solid-phase and solution techniques and were radioiodinated oxidatively, as we described (11). Radioligands were purified by high pressure liquid chromatography to yield specific radioactivities of 2000 Ci/mmol.

The CCK receptor antagonist L-364,718 was kindly provided by Dr. R. Freidinger of Merck Sharp and Dohme Research Laboratories (West Point, PA) (14). Collagenase type II, Gpp(NH)p, and GTPγS were from Sigma Chemical Company (St. Louis, MO), and bacitracin was from Aldrich Chemical Company (Milwaukee, WI).

Tissue Preparations

Dispersed muscle cell preparation. Muscle cells were isolated from the fundus of the bovine gall bladder by a modification of the method described by Bitar and Makhlouf (15). In brief, the muscularis was promptly dissected free of serosa and mucosa at the local abattoir where the gall bladders were collected, and was transported to the laboratory in an iced oxygenated medium of the following composition: 25 mM HEPES (pH 7.3), 14 mM glucose, 120 mM NaCl, 0.6 mM $MgCl_2$, 1.5 mM $CaCl_2$, 2.6 mM KH_2PO_4, 1% essential amino acids, 0.2% bovine serum albumin, and 0.1% bacitracin. Muscularis strips were subsequently cut into fine pieces and incubated in 15 ml of collagenase-containing (0.2%) medium at 31° for 60 min. Thereafter, this was applied to a 500-μm nylon mesh, and the tissue remaining above the filter was reincubated in fresh medium for an additional 40 min. Filtration was then repeated, and the intact tissue was washed with 50 ml of collagenase-free medium. Muscle cells were then allowed to disperse in 10 ml of collagenase-free medium, and this time the cells were collected by filtration through a 200-μm nylon mesh. The overall yield ranged from 1 to 2 × 10^6 cells/animal, with viability, based on trypan blue exclusion, being always greater than 90%.

Plasma membrane preparation. Enriched plasma membranes from bovine gall bladder muscularis were prepared according to the method we developed (7, 9), with minor modifications. Namely, a step gradient of 1.18, 0.84, and 0.43 M sucrose was used in place of a linear gradient, and the band at the 0.43/0.84 M sucrose interface was collected. These membranes were washed, resuspended in binding medium consisting of 20 mM Tris (pH 6.0), 130 mM NaCl, 5 mM $MgCl_2$, 1 mM EGTA, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM 1,10-phenanthroline, 0.01% soybean trypsin inhibitor, and 0.2% bovine serum albumin, and stored at −70° until use. Binding was stable for 2 to 3 months under these conditions and did not differ between freshly prepared and frozen preparations.

Biological Activity Studies

The gall bladder muscularis smooth muscle cell preparation was used to characterize the biological activities of various peptides. Suspensions of cells (0.4 ml, containing approximately 10^5 cells/ml) were incubated with the peptides to be tested, at 25°. Reactions were terminated by the addition of the fixative, acrolein, at a final concentration of 1%. Results were assessed in a blinded manner, with cell lengths determined on coded tubes. The lengths of 100 cells in successive microscopic fields from each tube were measured using an optical micrometer in a phase-contrast microscope (Zeiss, West Germany). The response was expressed as the decrease in cell length as a percentage of control (no peptide added). The peptide concentration-response curves were obtained at the time points of maximal shortening for each ligand.

Binding Studies

Receptor binding characterization was performed using the gall bladder muscularis plasma membrane preparation and the native CCK-like radioligand ^{125}I-DTGNN or its analogue ^{125}I-OPE. Membranes (20–50 μg) were incubated with 20 pM radiolabel and the appropriate peptide concentration in 0.5 ml of binding medium for 90 min at 24°, conditions previously demonstrated to allow steady state binding (7). Separation of bound from free radioligand was achieved by a rapid filtration technique, utilizing a Skatron cell harvester (Lier, Norway) with receptor-binding filtermats. The amount of radioactivity retained on the filters after washing with ice-cold, protein-containing medium was measured using a γ-counter.

Nonsaturable binding, determined in the presence of 1 μM CCK-8 or 10 μM OPE, was always <12% of total binding. It was similar in the presence of the CCK receptor antagonist L-364,718 (0.1 μM). Values reported represent saturable binding.

Membrane protein content was determined by the fluorescamine assay, using bovine serum albumin as standard (16).

Affinity Labeling Studies

Affinity labeling was performed to identify the membrane proteins that bound to native CCK and to OPE. For this, binding was performed as above, except using greater amounts of radioligand (300 pM) and membrane protein (100 μg/tube). Probes included the photolabile analogue ^{125}I-D-Tyr-Gly-[Nle^{28,31},pNO$_2$-Phe^{33})CCK-26–33, which behaves like native hormone (17), and the chemically cross-linkable analogue, ^{125}I-OPE. Photoaffinity labeling of the native CCK-binding protein was performed after incubation of radioligand and membranes in the dark, with subsequent photolysis for 30 min at 4° using a 200-W medium pressure lamp (Hanovia; Ace Glass Co., Vineland, NJ) with a Pyrex filter (17). Under these conditions, essentially all covalent attachment is through the photolabile nitrophenylalanyl residue within the theoretical receptor-binding domain of this ligand (17). The OPE-binding protein was labeled using bifunctional chemical cross-linking with 5 μM m-maleimidobenzoyl N-hydroxysuccinimide ester for 5 min at 4°. For this, ligand-bound membranes were washed and resuspended in HEPES buffer, pH 7, without bovine serum albumin.

Labeled membranes were pelleted by centrifugation, solubilized with 4% sodium dodecyl sulfate, and run on 9% sodium dodecyl sulfate-polyacrylamide slab gels, using the buffer system of Laemmli (18). Labeled proteins were visualized by autoradiography, and apparent molecular weight values were determined by interpolation on a plot of log M_r versus mobilities of standard proteins [myosin (M_r = 200,000), β-galactosidase (M_r = 116,000), phosphorylase B (M_r = 92,500), bovine serum albumin (M_r = 66,000), ovalbumin (M_r = 45,000), and carbonic anhydrase (M_r = 29,000)].

Analysis of Data

Binding data were analyzed using a weighted least-squares nonlinear regression method (LIGAND program) (19). Results are expressed as mean ± standard error of at least four sets of experiments. Statistical significance was evaluated using Student's t test for paired values, with $p < 0.05$ being considered significant.

Results

Biological activity studies. As shown in Fig. 1, the resting lengths of the dispersed bovine gall bladder smooth muscle cells had a normal distribution around a mean of 74.8 ± 4.9 μm (cell lengths ranged from 35 to 210 μm). When exposed to CCK-8, the cells shortened and the distribution shifted to the left, with the mean cell length being reduced by 26.6 ± 2.1% in response to the optimal CCK concentration of 1 nM, similar to the percentage of cell shortening previously observed by Severi *et al.* (3).

Like other gastrointestinal smooth muscle cells (15), the gall

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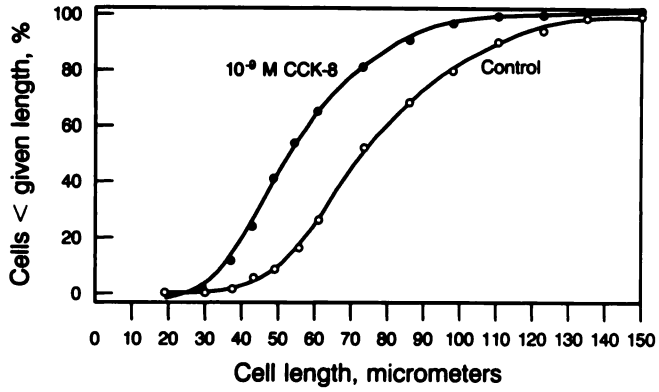

Fig. 1. Dispersed bovine gall bladder smooth muscle cells had a mean length of 74.8 ± 4.9 μm, with a normal distribution about the mean. Exposure to 1 nM CCK-8 for 30 sec produced cell shortening, with the curve of distribution of cell lengths moving to the left.

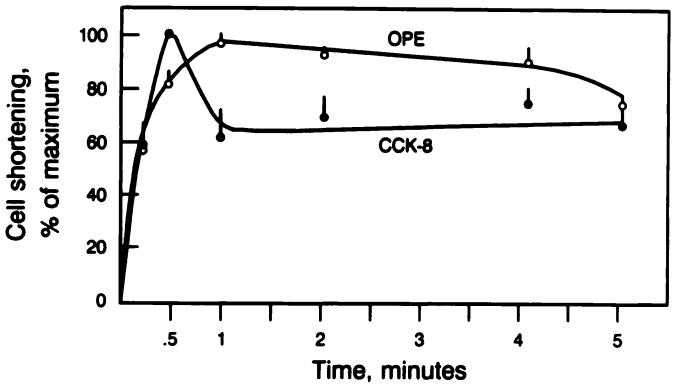

Fig. 2. The time courses of gall bladder muscle cell shortening in response to 1 nM CCK-8 and 1 μM OPE differed. Native hormone stimulated maximal shortening in 30 sec, with rapid relaxation thereafter. OPE-stimulated cells shortened maximally after 60 sec and subsequently relaxed much more slowly. Results are expressed as percentages of maximal shortening, with the maximal response being similar after both peptides.

bladder muscularis cells shortened in response to CCK-8 in a biphasic manner, shortening maximally within 30 sec after exposure to the peptide and relaxing thereafter (Fig. 2). In contrast, the CCK analogue OPE stimulated cells to shorten maximally after 60 sec and they subsequently relaxed much more slowly. Peptide concentration-response curves were obtained at the time points of maximal shortening for that ligand.

CCK-8 stimulated cell shortening in a concentration-dependent manner; shortening was measurable at a concentration as low as 0.1 pM (6.7%) and maximal at 1 nM (26.6%) (Fig. 3). Supramaximal concentrations of CCK-8 produced a smaller amount of shortening than 1 nM CCK-8 (21.1% at 10 nM, 16.2% at 100 nM), thus demonstrating supramaximal inhibition of the contractile response to native hormone. In contrast, whereas the phenethyl ester analogue OPE also stimulated cell shortening in a concentration-dependent manner, reaching a maximum at 1 μM peptide (25.1%), this analogue exhibited no supramaximal inhibition of shortening (24.7% at 10 μM, 24.9% at 50 μM). Note that the efficacies of both peptides were similar, with similar degrees of maximal shortening, but the potency of CCK-8 was approximately 100 times that of OPE.

Binding studies. Both radioligands, ^{125}I-DTGNN and ^{125}I-OPE, bound to the gall bladder muscularis plasma membranes in a specific, high affinity manner (Figs. 4 and 5). Binding of

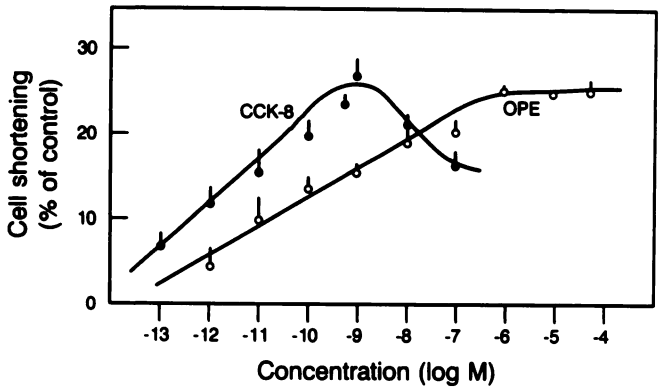

Fig. 3. Concentration-response curves for gall bladder muscle cell shortening in response to CCK-8 and OPE are shown. Data were derived from the times of maximal shortening for each ligand (30 sec for CCK-8, 60 sec for OPE), with the percentage decrease from mean control cell length plotted. Both peptides stimulated shortening in a concentration-dependent manner, with CCK-8 but not OPE expressing supramaximal inhibition of this effect. Peptides were equally efficacious, but OPE was less potent than native hormone.

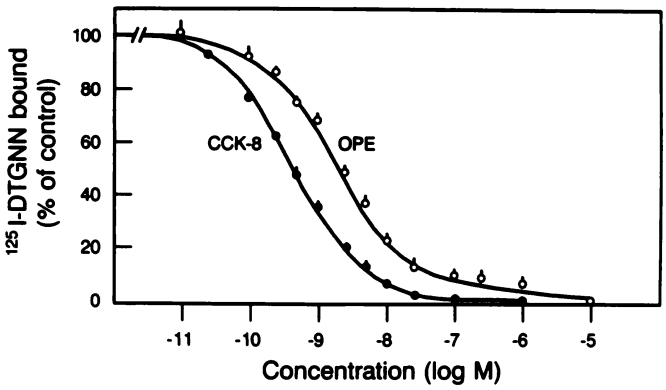

Fig. 4. Both CCK-8 and OPE competed for the binding of the CCK-like radioligand ^{125}I-DTGNN to gall bladder muscularis membranes. Analysis of binding data can be seen in Table 1.

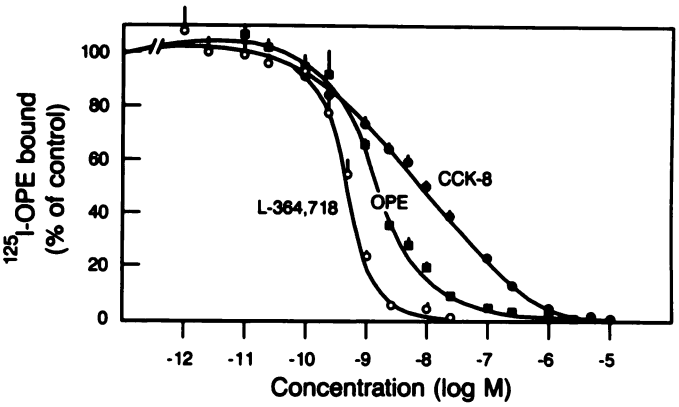

Fig. 5. Both agonists, OPE and CCK-8, as well as the type A CCK receptor antagonist L-364,718 competed for binding of ^{125}I-OPE to gall bladder muscularis membranes. Analysis of binding data can be seen in Table 1.

both radioligands was competed for in a concentration-dependent manner by both CCK-8 and OPE, suggesting that they were both binding to the same receptor molecules. In addition, the CCK receptor antagonist L-364,718, which is known to compete for binding of native CCK (14), also fully competed for the binding of radiolabeled OPE (Fig. 5).

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Binding data are summarized in Table 1. Estimates of binding parameters were obtained from groups of four data sets, pairing each radioligand with each competitor. When data from homologous competition curves (the same radioligand and competing ligand) were analyzed, CCK appeared to recognize only a single state of the receptor (K_d = 0.44 ± 0.03 nM), and OPE appeared to also recognize only a single state of the receptor (K_d = 1.09 ± 0.19 nM). CCK, however, appeared to have fewer binding sites than OPE (1672 ± 222 versus 3984 ± 331 fmol/mg of protein; $p < 0.0005$), a finding that is not possible because CCK-8 fully displaces ^{125}I-OPE binding. This is better assessed by analyzing all of the data, including the heterologous curves. Using this method, CCK was shown to recognize binding sites with two different affinities, high (K_{d_1} = 480 ± 40 pM; B_{max_1} = 1858 ± 245 fmol/mg of protein, representing 51.9 ± 4.8% of total sites) and low (K_{d_2} = 56.5 ± 7.4 nM; B_{max_2} = 1721 ± 221 fmol/mg of protein, representing 47.9 ± 4.7% of total sites). OPE, however, still appeared to bind to a single site (K_d = 1.0 ± 0.3 nM), with the two-site model not fitting the data significantly better (K_{d_1} = 0.96 ± 0.23 and K_{d_2} = 0.94 ± 0.31 nM) ($p > 0.05$). These data are also, therefore, consistent with OPE binding to two sites with approximately equal affinities, with native CCK being able to more clearly distinguish between these two sites.

Effect of guanine nucleotides on binding. The ability of guanine nucleotides to alter the binding of a hormonal agonist has been predictive of the coupling of the receptor for that hormone to a guanine nucleotide-binding protein. The nonhydrolyzable GTP analogue Gpp(NH)p inhibited the binding of the CCK-like radioligand to gall bladder membranes in a concentration-dependent manner (Fig. 6). However, in concentrations as high as 0.2 mM, Gpp(NH)p did not affect the total specific binding of ^{125}I-OPE. The same phenomenon was observed with GTPγS (data not shown). Even though ^{125}I-OPE binding was not affected by Gpp(NH)p, in the presence of this nonhydrolyzable GTP analogue CCK-8 displacement of labeled OPE changed significantly (Fig. 7). The IC_{50} for CCK inhibition of ^{125}I-OPE binding to the gall bladder membranes was higher in the presence of Gpp(NH)p. Analysis of these data (Table 1) revealed no change in the affinity of either state of the receptor but marked changes in receptor capacities. The capacity of the high affinity receptor was decreased from 1858 ± 245 to 680 ± 125 fmol/mg of protein, whereas that of the low affinity site was increased from 1721 ± 221 to 2815 ± 445 fmol/mg of protein. The total number of sites was not different under the two conditions [3580 ± 324 versus 3494 ± 376 in the presence of Gpp(NH)p; $p > 0.05$]. Thus, assuming that the number of receptors was conserved, in the absence of guanine nucleotide 51.9 ± 4.8% of the sites were of high affinity, whereas in the presence of Gpp(NH)p this was reduced to 19.3 ± 3.7% ($p < 0.002$).

Affinity labeling studies. Affinity labeling demonstrated that both native CCK and OPE bound to the same gall bladder membrane protein (Fig. 8). As previously shown, the CCK-like radioligand ^{125}I-D-Tyr-Gly-[(Nle^{28,31}, pNO$_2$-Phe^{33})CCK-26–33] specifically labeled a M_r 70,000–85,000 glycoprotein on this tissue. This labeling was competed for by native CCK-8, as well as OPE. Using the OPE radioligand as probe, a protein of the same apparent size was labeled. This, too, was competed for by OPE itself and by CCK-8. In addition, the type A CCK receptor antagonist L-364,718 inhibited the labeling of this band by ^{125}I-OPE.

Discussion

In this work, the use of a phenethyl ester analogue of CCK (OPE) in studies of binding and biological activity, using membranes and dispersed cells from bovine gall bladder muscularis, has provided new insights into the characterization and regulation of the CCK receptor. This receptor is shown to represent a single molecule with two interconvertible affinity states, regulated by a guanine nucleotide-binding protein. Of further note is the observation that CCK and this analogue represent two fully efficacious agonists that interact with the same receptor molecule on this cell to yield different biological responses. The hypothesis is proposed that these agonists differentially activate cellular processes based on differential stabilization of a ternary complex of ligand-receptor-Gp.

The ability to demonstrate both high and low affinity states of the CCK receptor on a membrane preparation has made it possible to show that these represent two states of the same

TABLE 1
Analysis of binding data

	Analysis of homologous competition curves only	
	K_d	B_{max}
	nM	fmol/mg of protein
CCK	0.44 ± 0.03	1672 ± 222[a]
OPE	1.09 ± 0.19	3984 ± 331[a]

	Analysis of homologous and heterologous competition curves				
	K_{d_1}	K_{d_2}	B_{max_1}	B_{max_2}	Total sites
	nM		fmol/mg of protein		
Control					
CCK	0.48 ± 0.04	56.5 ± 7.4	1858 ± 245 (51.9 ± 4.8%)	1721 ± 221 (47.9 ± 4.7%)	3580 ± 324
OPE	0.94 ± 0.31	0.96 ± 0.23			
In the presence of 0.2 mM Gpp(NH)p					
CCK	0.47 ± 0.07[b]	46.5 ± 4.6[b]	680 ± 125[c] (19.3 ± 3.7%[d])	2815 ± 445[c] (80.7 ± 3.7%[d])	3494 ± 376[b]
OPE	0.72 ± 0.19[b]	0.88 ± 0.09[b]			

[a] $p < 0.0005$.
[b] NS, not significant.
[c] $p < 0.05$, compared with control.
[d] $p < 0.001$, compared with control.

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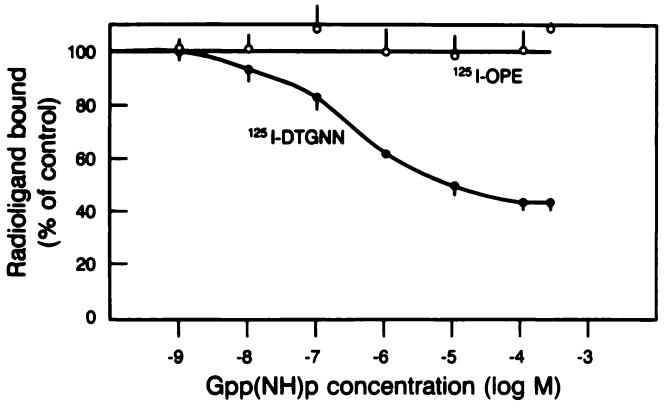

Fig. 6. The nonhydrolyzable guanine nucleotide analogue Gpp(NH)p inhibited the binding of the CCK-like radioligand ^{125}I-DTGNN to gall bladder muscularis membranes but had no effect on the binding of ^{125}I-OPE to this tissue.

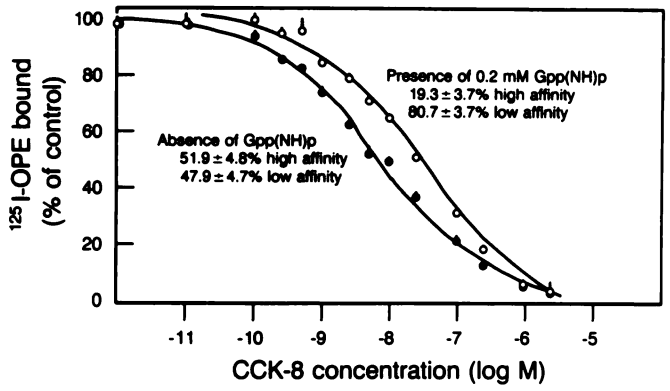

Fig. 7. CCK-8 competition for ^{125}I-OPE binding to gall bladder muscularis membranes was different in the absence and presence of 0.2 mM Gpp(NH)p. Although the total number of binding sites and their affinity characteristics did not change, the distribution of high and low affinity sites changed markedly (analysis in Table 1).

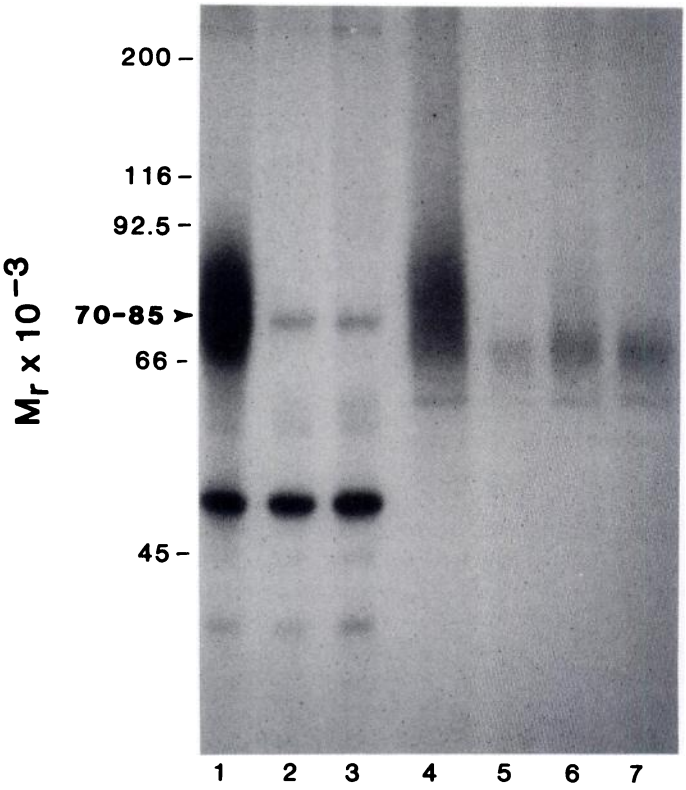

Fig. 8. Affinity labeling of CCK- and OPE-binding proteins on gall bladder muscularis membranes demonstrates their identity. *Lane 1*, the CCK-like radioligand ^{125}I-D-Tyr-Gly-[(Nle^{28,31},pNO$_2$-Phe^{33})CCK-26–33] specifically labeled an M_r 70,000–85,000 band in the absence of competing ligand. This was inhibited by the presence of 1 μM CCK-8 (*lane 2*) and 10 μM OPE (*lane 3*). Note that nonspecifically labeled bands were not affected by these peptides. The OPE probe, ^{125}I-OPE, used with the bifunctional cross-linker *m*-maleimidobenzoyl *N*-hydroxysuccinimide ester labeled a band of the same apparent size (*lane 4*), which was inhibited by the presence of 10 μM OPE (*lane 5*), 1 μM CCK-8 (*lane 6*), and 0.1 μM L-364,718 (*lane 7*). This radioligand generated different nonspecifically labeled bands.

molecule, rather than two distinct molecules. Previously, only a single affinity state of the CCK receptor had been observed on membranes from gall bladder (6, 7) or pancreas (20, 21), despite the clear demonstration of high and low affinity sites on intact pancreatic acinar cells (22, 23). This led to the proposal that the high affinity site present on those cells was lost in the preparation of membranes (24). More likely, based on the observations in this work, the low affinity state of CCK receptors on the membranes being studied was not recognized due to its extremely low affinity for native CCK radioligands.

The key to the current demonstration of two affinity states of the CCK receptor was the relatively high affinity of the OPE analogue for the low affinity state of the CCK receptor. Using the conditions described, the affinity of native CCK for this site was too low to be observed directly. This also clarifies why there appeared to be so many more OPE binding sites than CCK binding sites on these membranes when only homologous curves were analyzed. Although the OPE ligand had a sufficiently high affinity for both sites to recognize them both, it could not distinguish between them in homologous competition experiments (same radioligand and unlabeled competing ligand) because of similar affinities for both types of sites. To demonstrate that two affinity states of this receptor were present on membranes, the analysis had to include both homologous and heterologous competition curves, using both the OPE analogue and native hormone. Key to validation of this type of analysis was the demonstration that both ligands interacted with the same binding sites. This was done using both competition binding and affinity labeling approaches. The binding and affinity labeling by both types of probes were completely displaced by the opposite peptide, as well as by the CCK receptor antagonist L-364,718.

Also consistent with the interpretation that the type A CCK receptor represents a single molecule capable of assuming two affinity states are the observations on pancreatic acini that one molecule has been affinity labeled under the various conditions that would kinetically favor labeling of the high or low affinity site[1] and that the same molecule has been labeled on membranes as well as acini (25). Indeed, ample precedent exists for the rigorous demonstration that a single receptor molecule can exist in multiple affinity states (26).

Several characteristics of a membrane preparation have been shown to affect the demonstration of different states of a receptor (27, 28). Among these are receptor and Gp concentrations (27, 29). Clearly, the receptor density on the bovine gall bladder membranes is lower than that on rat pancreatic membranes (7). Perhaps these membranes are also less rich in Gp,

[1] L. J. Miller, unpublished observations.

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and this may even be limiting in the demonstration of changes in affinity by the proposed ternary complex of CCK-receptor-Gp. Buffer composition can similarly affect the demonstration of different affinity states of a receptor. Preparation of hepatic membranes in the absence of calcium has been helpful in highlighting the presence of two states of the α_1-adrenergic receptor (28). The current work, also, was performed in the absence of calcium in the buffer.

The ability to demonstrate both affinity states of the CCK receptor on a membrane preparation has also made it possible to investigate the role of intracellular proteins in regulation of this receptor. The demonstration in this work that Gpp(NH)p converted high affinity binding sites for CCK to low affinity sites is consistent with observations made with many other receptors that interact with guanine nucleotide-binding proteins (30). As is typical of such receptors, the occupancy of the CCK receptor with native hormone has been shown to result in the subsequent association of receptor and Gp (31). By analogy, that ternary complex likely represents the high affinity state of the receptor, with the binding of GTP or a nonhydrolyzable analogue of it to this complex leading to lowering of the affinity between ligand and receptor, with dissociation of ligand as well as Gp.

This type of behavior, with agonist dissociation in the presence of analogues of GTP, has even been used as being predictive of Gp-associated receptors. In this work, this was directly demonstrated using a native CCK-like radioligand and increasing concentrations of Gpp(NH)p. Most unusual, however, was the observation that a fully efficacious agonist that acts via the same receptor, such as OPE, did not display this. Even millimolar concentrations of Gpp(NH)p and GTPγS had no effect on the membrane binding of the OPE radioligand. Although the binding of antagonists to a Gp-coupled receptor would typically be unaffected by the nucleotide exposure (32), that is explained by an antagonist not inducing the conformational change in the receptor that leads to stabilization of the ternary complex and initiation of the activation cascade. This type of behavior, in fact, has been reported with the non-peptide type A CCK receptor antagonist L-364,718 (33).

Perhaps OPE, too, does not stabilize this ternary complex. However, being an agonist, unlike the antagonist L-364,718 it must activate an effector cascade in the cell. Although there is the possibility that a ternary complex of OPE-receptor-Gp does exist transiently and thereby initiates the activation cascade, it is also possible that another heretofore unrecognized mediator is activated by the OPE-occupied receptor. In favor of the latter possibility are observations that maximally stimulatory concentrations of phenethyl ester analogues of CCK stimulate little or no cellular responses in phosphatidylinositol hydrolysis (34, 35), events that have been shown to be mediated by the release of the α subunit of the Gp bound to GTP from a stable ternary complex (36).

In this work we show that, in the gall bladder muscularis smooth muscle cell, like in the pancreatic acinar cell (11), two different full agonists can work through a single receptor molecule in molecularly distinct ways. We propose that CCK, but not its analogue OPE, activates the traditionally described cascade beginning with Gp association to the receptor and leading to phospholipase C activation, phosphatidylinositol hydrolysis, and protein kinase C activation. We believe that it is this difference in a series of biochemical events that contributes to the supramaximal inhibition of action observed with CCK but not with OPE. Both peptides are fully efficacious agonists, presumably mediated by events that are either less dependent on a stabilized ligand-receptor-Gp ternary complex or independent of it. Further investigation will be necessary to identify the messengers involved in this process.

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