Structurally Related Peptide Agonist, Partial Agonist, and Antagonist Occupy a Similar Binding Pocket within the Cholecystokinin Receptor

RAPID ANALYSIS USING FLUORESCENT PHOTOAFFINITY LABELING PROBES AND CAPILLARY ELECTROPHORESIS*

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The molecular basis of ligand binding to receptors provides important insights for drug development. Here, we explore domains of the cholecystokinin (CCK) receptor that are critical for ligand binding, using a novel series of fluorescent photolabile probes, receptor proteolysis, and rapid high resolution separation of peptide fragments by capillary electrophoresis. Each probe incorporated the same fluorophore and a photolabile p-benzoylphenylalanine at the amino terminus of the pharmacophoric domain (residue 24 of CCK-33) of CCK analogues representing full agonist, partial agonist, and antagonist of this receptor. Each was used to label the CCK receptor expressed on Chinese hamster ovary (CHO)-CCKR cells, with the labeled domain then released by cyanogen bromide cleavage. Capillary electrophoresis with laser-induced fluorescence detection achieved an on-capillary mass sensitivity of 1.6 attomoles (10⁻¹⁸ mol), with an excellent signal-to-noise ratio. Each of the biologically divergent, but structurally similar probes saturably and specifically labeled the same receptor domain, consistent with conservation of “docking” determinants. This had an apparent mass of 2.9 kDa, most consistent with the first extracellular loop domain. An additional probe having its site of covalent attachment in a different region of the probe (residue 29 of CCK-33) labeled a distinct receptor fragment with differential migration on capillary electrophoresis (third extracellular loop). Identification of the specific receptor residue(s) covalently linked to the amino-terminal probes must await further fragmentation and sequence analysis.

Remarkable diversity exists for structures capable of high affinity binding and activation of G protein-coupled receptors. Understanding the molecular determinants of ligand binding provides important insights that may be useful in structure-based drug design and in understanding the basic mechanisms of receptor activation. Photoaffinity labeling provides the most direct way to identify sites of contact between residues in ligands and a receptor (1); however, traditionally this involves use of large amounts of radioactivity and inefficient and time-consuming purification procedures. In this work, we developed a simple, rapid, and efficient procedure for analyzing sites of ligand-receptor binding, and applied this to a series of structurally related ligands representing agonists, partial agonist, and antagonist of the CCK receptor.

Capillary electrophoresis (CE), initially performed by Jorgensen and Lukacs (2), has emerged in recent years as a versatile and powerful tool for the analysis of biological molecules, because of its unparalleled efficiency for separation, small injection volumes, and low mass detection limits (3, 4). We recently developed a CE method for the separation of peptides cleaved from hydrophobic membrane proteins, such as bacteriorhodopsin, a possible model for the study of G protein-coupled receptors (5). In the present work, we have optimized and extended this to apply to extremely sparse, wild-type receptor molecules. We achieved this through the use of the combination of CE and detection by laser-induced fluorescence (LIF). The high resolving power and small injection volumes associated with CE and the high sensitivity of LIF have achieved detection limits of attomole (10⁻¹⁸ mol) (6, 7), zeptomole (10⁻²ⁱ mol) (8–11), and even low yoctomole (10⁻²⁴ mol) (12–14) levels, corresponding to detecting fewer than six molecules in a peak fraction.

We have applied this methodology to a series of novel probes of the cholecystokinin (CCK) receptor, and have demonstrated that the amino terminus of the pharmacophoric domain of peptide analogues, representing a full agonist, partial agonist, and antagonist of the CCK receptor, resides in proximity to the same receptor domain, most likely representing the first extracellular loop, whereas the mid-region of a related agonist probe resides adjacent to a distinct receptor domain, representing the region in the third extracellular loop just outside of the seventh transmembrane domain (15). This procedure can be applied to analysis of ligand-receptor binding of other members in the G protein-coupled receptor superfamily and can be extended to rapidly yield molecular details of ligand-receptor interaction.

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1 CE, capillary electrophoresis; LIF, laser-induced fluorescence; CCK, cholecystokinin; Bodipy, Bodipy®FL, 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionic acid; Bpa, p-benzoyl-phenylalanine; Bodipy-Bpa24 agonist, Bodipy-Gly-Bpa-Gly-Gly-Tyr(SO3)-Nle-Gly-Trp-Nle-Asp-Phe-NH2; Bodipy-Bpa24 partial agonist, Bodipy-Gly-Bpa24 agonist, Bodipy-Gly-Asp-Tyr(SO3)-Nle-Bpa-Trp-Nle-Asp-Phe-NH2; HPLC, high pressure liquid chromatography; CHO, Chinese hamster ovary; KRH, Krebs-Ringer-Hepes.
**Experimental Procedures**

**Materials**—Synthetic cholecystokinin-octapeptide (CCK) was purchased from Peninsula Laboratories (Belmont, CA). Ethylene glycol and phenylethylamine were synthesized by Fluks (Ronkonkoma, NY); HPLC-grade methanol and acetonitrile were from Fisher (Itasca, IL); cyano bromide (CNBr) was from Pierce; formic acid and HCl were from EM Science (ChMXmall, NJ); soybean trypsin inhibitor was from Worthington Biochemical Co.; and polydimethylallylamine chloride and polybrene (hexadimethrine bromide) were from Aldrich. All buffers for CE were made in glass-distilled, deionized water, and filtered through a 0.45-μm membrane (Gelman, Ann Arbor, MI). Bare fused silica capillaries were from Polymicro Technologies (Phoenix, AZ) and C<sub>x</sub>-silica from Baker (Phillipsburg, NJ).

**Synthesis of CCK Agonist Probes**—Four fluorescent CCK receptor ligand probes were synthesized by solid- and solution-phase techniques and purified by reversed-phase HPLC, as we have reported for other peptide receptor ligands (16–18). The design of each probe was based on well established structure-activity information for CCK analogues (17–20). The only difference from the fully characterized analogues was the addition of a fluorescent bodipy moiety with a glycine spacer at the amino terminus of the peptide, a position known to be fully tolerant of such a modification (19). These probes represent the following: Bodipy-Bpa<sup>24</sup> agonist, Bodipy-Gly-Bpa-Gly-Tyr(SO<sub>3</sub>)<sub>2</sub>-Nle-Gly-Trp-Nle-Asp-Asp-Asp-NH<sub>2</sub> BODIPY-Bpa<sup>24</sup>-partial agonist, Bodipy-Gly-Bpa-Gly-Asp-Tyr(SO<sub>3</sub>)<sub>2</sub>-Nle-Gly-Asp-phenethyl eiter; Bodipy-Bpa<sup>24</sup> antagonist, Bodipy-Gly-Bpa-Gly-Tyr(SO<sub>3</sub>)<sub>2</sub>-Nle-Gly-(N-Trp)-Nle-Asp-phenethyl ester; and Bodipy-Bpa<sup>24</sup> agonist, Bodipy-Gly-Asp-Tyr(SO<sub>3</sub>)<sub>2</sub>-Nle-Gly-Nle-Asp-Phe-NH<sub>2</sub>. Each peptide was synthesized to the level of the photolabile Bpa residue, deprotected, and purified to homogeneity by HPLC. Then the remainder of the synthesis was performed in solution using N-hydroxysuccinimide active esters and Fmoc (N-(9-fluorenyl)methoxycarbonyl) protection. Completeness of conjugation was followed by HPLC, and deprotection was performed in 20% piperidine/dimethylformamide. Each peptide was then purified by reversed-phase HPLC and characterized by amino acid analysis and mass spectrometry.

An additional probe was synthesized that was fully analogous to the Bodipy-Bpa<sup>24</sup> agonist, except that the fluorophore was replaced with a des-amino-tyrosyl moiety that is amenable to radioiodination (radioiodinatable-Bpa<sup>24</sup> agonist, des-amino-Tyr-Gly-Bpa-Gly-Asp-Tyr(SO<sub>3</sub>)<sub>2</sub>-Nle-Gly-Trp-Asp-Phenethyl eiter; and Bodipy-Bpa<sup>24</sup> agonist, Bodipy-Gly-Asp-Tyr(SO<sub>3</sub>)<sub>2</sub>-Nle-Gly-Nle-Asp-Phe-NH<sub>2</sub>). This was prepared in a manner fully analogous to that described above. This was radioiodinated oxidatively and purified to a specific radioactivity of 2,000 Ci/mmol using reversed-phase HPLC, in a manner analogous to that previously reported (21).

**Cell and Membrane Preparations**—CHO-K1 cells that do not express endogenous type A or type B CCK receptors (21) were purchased from the American Type Culture Collection (Manassas, VA). The cell line stably expressing type A receptors (CHO-CCKR), which has been previously characterized (21) was obtained by transfecting these cells with rat type A CCK receptor cDNA. Receptors were expressed in density approximately 25 times that of the pancreatic acinar cell (125,000 sites per CHO-CCKR cell), bound ligands with appropriate structural specificity, and were appropriately coupled to signaling machinery (21). Cells were cultured on tissue culture plastic in Ham’s F-12 medium in a humidified incubator at 37 °C in an atmosphere of 5% CO<sub>2</sub> at room temperature for 15 min under nitrogen at a pressure of 300 psi in a Kontes pressure homogenizer (Kontes, NJ). Upon the release of the pressure, the broken cell suspension was collected and centrifuged at 1,000 × g for 10 min to remove intact cells and nuclei. The supernatant was removed, and the cells were suspended in cold phosphate-buffered saline, containing 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 138 mM NaCl, and 2.7 mM KCl.

Plasma membranes were prepared by nitrogen cavitation and discontinuous sucrose density gradient centrifugation. Briefly, cells (about 5 × 10<sup>6</sup>) cells from tissue culture vessels were suspended in 1 ml of phosphate-buffered saline containing 1 mM phenylmethylsulfonyl fluoride and 0.01% soybean trypsin inhibitor, and pelleted by centrifugation at 100,000 × g for 3 h, as described previously (22). Membranes at the interface between layers of 16 and 31% sucrose were separated by SDS-polyacrylamide gel electrophoresis, and visualized by autoradiography, as we previously described (15).

**Capillary Electrophoresis**—CE separation was performed on a Beckman P/ACE System 5500 with a laser-induced fluorescence detector (argon-ion laser, 5–4 mW, Beckman Instruments). The excitation wavelength on the CE was fixed at 488 nm, and the emission wavelength was 520 nm. Beckman System Gold software (version 8.1, Beckman) was used for system control and data collection and processing. All peak information was obtained through the System Gold software.

CE was performed using conditions recently developed for the separation of fragments obtained from bacteriorhodopsin (5). Briefly, a polycapillary-coated capillary (47 cm × 50-μm) was rinsed before sample injection under high pressure (20 psi, 138 kPa) for 2 min with separation buffer containing 1 mM acetic acid and 10 mM hexanesulfonic acid. Samples were introduced by low pressure (0.5 psi, 3.45 kPa; 16 nl volume) for 10 s. Electrophoresis was performed at 15 kV under reversed polarity (cathode to anode) for 30 min. After each run, the capillary was washed for 30 s with 70% acetonitrile with 30% 0.1% trifluoroacetic acid and followed by rinsing with the separation buffer for 2 min. Capillaries were re-coated after every 20 runs because of slight shifts in elution positions and broadening of peaks. For this reason, coinjection experiments were performed for every pair of conditions thought to represent the same fluorescently labeled peaks.

**Results**

**Synthetic CCK Receptor Probes**—Five photoflatable CCK analogues were synthesized and purified to homogeneity by reversed-phased HPLC. Structures were confirmed and quantified by amino acid analysis. Fig. 1 shows the structures of the fluorescent ligands used in the present study. Three of these probes, representing full agonist, partial agonist, and antagonist, had a photolabile Bpa at the amino terminus of the pharmacophoric domain of the peptide in the identical position of residue 24 of CCK-33 (standard numbering convention for CCK peptides).

The fourth probe was also a fluorescent full agonist, but with the Bpa residue positioned in the middle of the peptide pharmacophoric domain, in the site of Gly<sup>29</sup> of CCK-33. The fifth probe was a radioiodinatable-Bpa<sup>24</sup> agonist, fully analogous to one of the key fluorescent compounds in the series.

**Detection Sensitivity of CE-LIF**—Fig. 2 shows a series of capillary electropherograms of the Bodipy-Bpa<sup>24</sup> agonist obtained with 16 μl aliquots at various concentrations. The limit of detection with a 3-mW argon ion laser at 488 nm (defined as the mass of analyte injected to give a peak height no less than three times the standard deviation of the baseline; signal-to-noise ratio) was 1.6 attomoles. Similar detection limits (1.5–2.0 attomoles) were also achieved for each of the other Bodipy-conjugated probes used in this study. Of note, the...
cells incubated with this probe (Fig. 3E). When affinity labeling of the CHO-CCKR cell membranes was performed at 4 °C for 1 h, peaks migrating in a similar position were also observed, although having a decreased fluorescence intensity to reflect the slower kinetics of binding at this temperature (Fig. 3F). These were absent under these conditions when using 100-fold molar excess CCK during binding (Fig. 3G). At 4 °C, when affinity labeling was extended to 2.5 h, fluorescent ligand attached to CCK receptor fragments was better detected (see below and Fig. 7).

It should be noted that a peak migrating at 7 min, just before the fluorescent ligand peak, in Fig. 3, C, D, and E, and in the following figures probably represents the degradation of the probe when the sample was incubated at room temperature, because it was not present when affinity labeling was performed at 4 °C (Figs. 3, F and G, and 7). This peak was present in both the experimental and control-competition affinity-labeling samples, and also in affinity-labeled samples from non-CCK receptor-bearing preparations, indicating that it was not the type of saturable binding expected of a receptor fragment adduct. Probe degradation may also have contributed to the dual probe-receptor fragment adduct peaks that were often observed. Alternatively, these could also reflect the covalent labeling of the same receptor fragment at different positions.

Fig. 4 shows capillary electropherograms of CNBr-cleaved CCK receptor fragments labeled by each of three fluorescent probes, Bodipy-Bpa24 agonist (Fig. 4A), Bodipy-Bpa24 partial agonist (Fig. 4C), and Bodipy-Bpa24 antagonist (Fig. 4E). The elution profiles for each were almost identical, with elution times in the 13- to 14-min range for the best candidate peaks to represent receptor fragment adducts. These migrated more slowly than the respective free probes (the second major peak in each profile). Like the labeling of the CCK receptor fragment with the agonist probe (Figs. 3D and 4B), labeling of CCK receptor fragments by the partial agonist probe and the antagonist probe were fully saturable, being completely eliminated in the presence of 100-fold molar excess of CCK (Fig. 4, D and F). The detected covalent receptor fragment adduct peaks in Fig. 4, A, C, and E represented 9.2, 3.1, and 5.0 attomole, respectively, based on extrapolation of areas under the fluorescent peaks (sum of relevant areas) to those of injections of standard amounts of probe.

Fig. 5 illustrates representative CE elution profiles of competition experiments using the nonfluorescent receptor ligand, CCK, to interfere with the affinity labeling of CHO-CCKR cell membranes with the Bodipy-Bpa24 agonist probe. The fluorescence intensity of the CCK receptor fragment-probe adduct decreased as the concentration of competing CCK increased.
The probe-bound CCK receptor fragments started to decrease when CCK was present in a concentration as low as 0.1 nM, and they were completely eliminated with 0.1 μM CCK. The last panel in Fig. 5 shows quantitation of results of experiments of a series of these experiments. This was analogous to competition binding analysis of a CCK radioligand to membranes from these cells (21). Such competitive inhibition was also typical of experiments performed with the partial agonist and antagonist probes (data not shown).

To address whether all three probes having the Bpa in position of residue 24 were attached to the same CCK receptor fragment, combinations of pairs of samples were coinjected for analysis by CE. As shown in Fig. 6, coinjection of CCK receptor fragments attached to the agonist probe and that with the...
partial agonist probe (Fig. 6A) did not produce additional peaks; neither did the coinjection of CCK receptor fragments attached to the agonist probe and that with the antagonist probe (Fig. 6B), nor did that of fragments with the partial agonist probe and that with the antagonist probe (Fig. 6C). Like CE of separate injection of each probe attached to CCK receptor fragments, the peaks eluted in a consistent position, suggesting all three ligands share the same binding domain of the CCK receptor.

An additional fluorescent probe, the Bodipy-BPA29 agonist, having its site of covalent attachment in a different region of the probe, labeled a distinct receptor fragment with differential CE migration, as shown in Fig. 7. This probe, attached to CCK receptor fragments, migrated at 6–8 min (Fig. 7A) and also competed with 100-fold molar excess of nonfluorescent CCK (Fig. 7B). Interestingly, these distinct fragments migrated quite differently from the covalent adducts described above. As shown by the coinjection experiments, these fragments migrated much faster than the fragments attached to the Bodipy-Bpa24 agonist (Fig. 7C). This was also observed with partial agonist and antagonist probes (data not shown). This suggests that the Bodipy-BPA29 agonist site of attachment to the CCK receptor was quite different from that of the other probes or at least has a markedly different charge-to-mass ratio allowing it to migrate quite differently in free solution CE.

To better understand the identity of the receptor fragments labeled by the photolabile probes, the products of CNBr digestion of the labeled CCK receptor that had been purified on an SDS-polyacrylamide gel were separated by NuPAGE gel electrophoresis and detected by autoradiography. The fragments theoretically generated by CNBr cleavage of the CCK receptor are shown in Fig. 8A. We recently demonstrated that photoaffinity labeling of the CCK receptor through position 29 of a CCK analogue probe covalently labeled His347 and Leu246 in the third extracellular loop just outside of the seventh transmembrane domain of the receptor (15), whereas labeling through position 33 of a similar probe covalently labeled Trp39 in the amino-terminal tail just outside of the first transmembrane domain of the receptor (1). Autoradiography of a representative SDS-polyacrylamide gel separating the products of photoaffinity labeling the CHO-CCKR cell membranes is shown in Fig. 8B. Consistent with the photoaffinity labeling of this receptor using a series of other CCK analogue probes (1, 15, 20, 26, 27), the radioiodinated-Bpa24 agonist satura- 

FIG. 5. Concentration-dependent inhibition of affinity labeling of the CCK receptor fragments with Bodipy- 
Bpa24 agonist in the presence of CCK. Affinity labeling was performed with 1 nM Bodipy-Bpa24 agonist in the presence of increasing concentrations of nonfluorescent competing CCK. CE conditions were the same as those described in Fig. 2. The bottom right panel displays data from the quantitation of the Bodipy-Bpa24 agonist-receptor adducts from a minimum of three independent experiments (means ± S.E. is shown with some errors within the displayed data points).
because the calculated mass (M+H)+ of the covalent probe-
fragment adduct is m/z 4601.3, and no shift with deglycosyla-
tion would be expected. The calculated mass (M+H)+ of the
covalent probe second loop fragment adduct is m/z 4147.5,
assuming that the consensus sequence for N-linked glycosyla-
tion (NxS/T) is not used. If this fragment were glycosylated, its
mass could better approach the observed mass, but a shift in its
migration would have then been expected after deglycosyla-
tion. No such shift was observed.

**FIG. 6.** CE electropherograms of co-injected CNBr-cleaved
samples from affinity labeling of CHO-CCKR cell membranes
with different Bodipy-conjugated probes. CE conditions were
the same as those described in Fig. 2. Results displayed are
typical of eight independently performed experiments.

**FIG. 7.** CE electropherograms of CNBr-cleaved fragments of the CCK
receptor on CHO-CCKR cell membranes affinity labeled with the
Bodipy-Bpa29 agonist probe in the absence ([panel A]) and presence
([panel B]) of competing nonfluorescent 0.1 μM CCK. In panel C, products
of affinity labeling with this probe were co-injected with Bodipy-Bpa24
agonist, demonstrating a clear difference in CE migration of labeled
fragments. This was also true of fragments labeled by partial agonist
and antagonist probes (not shown). The affinity labeling was performed
for 2.5 h at 4 °C instead of room temperature. CE conditions were
the same as those described in Fig. 2. Results displayed are typical of six
independently performed experiments.

**DISCUSSION**

An understanding of the molecular basis of ligand binding to
a receptor and of the conformational changes involved in
achieving the active or inactive states of a receptor will be
powerful tools to aid in the rational design of novel therapeu-
tics. Most insights contributing to our current understanding of
this area have been low resolution or indirect, such as those
provided by receptor mutagenesis studies and by structure-
activity relationships for receptor ligands (28). Photofluorifer
labeling of active site domains and residues provides more
direct information regarding the ligand binding pocket (29).
This technique, however, has not been applied to a broad sam-
ping of receptors or to a large series of ligands or sites within
a ligand for a specific receptor because of inherent difficulties.
These include the need to synthesize special ligand probes that
incorporate photolabile residues as well as an indicator moiety,
such as a radiolabel or a fluorophore. Additionally, such sites of
potential covalent labeling of the receptor should ideally in-
clude complementary chemistries and varied positions within
the ligand. Beyond the specialized preparation of such probes,
the sparsity and physicochemical characteristics of the highly
hydrophobic serpiginous G protein-coupled receptors and the
typical inefficiency of establishing a covalent bond between the
ligand and receptor make the ultimate purification of the re-
ceptor and its labeled fragments, and characterization of the
specific site of covalent attachment quite difficult and time
consuming to accomplish.

We have recently begun the systematic application of this
approach to the natural peptide agonist-binding domain of the
CCK receptor using a series of radiolabeled photolabile
probes (1, 15). In the first of this series to be successful, we
incorporated a Bpa residue into the position of the carboxyl-
terminal residue of CCK and demonstrated the specific cova-
lent labeling of the Trp29 residue predicted to reside just out-
side of the first transmembrane domain (1). Recently, we have
also been able to demonstrate a second residue-residue inter-
action between a photolabile residue in the mid-region of a CCK analogue (residue 29 of CCK-33) and His347 and Leu348 in the third extracellular loop outside of the seventh transmembrane segment (15). As noted above, these efforts required the use of large amounts of radioactivity and the incorporation of extensive, difficult, and inefficient purification procedures. These sites of contact are being used as constraints in an evolving model of the peptide agonist-occupied CCK receptor (1, 15). However, the difficulties described above for the photolabeling with radiolabeled probes led to our development of the novel approach reported here.

In this work, we have developed a rapid and sensitive method for the localization of ligand-binding domains of G protein-coupled receptors using photoaffinity labeling with fluorescent photolabile probes, receptor proteolysis, and capillary electrophoresis with laser-induced fluorescence detection. We have applied this to a novel series of peptide probes of the CCK receptor, representing structurally related full agonist, partial agonist, and antagonist, with each having its site of covalent attachment to the receptor through a photolabile Bpa residue at the amino terminus of the ligand pharmacophore. Despite very different biological effects at the CCK receptor, each probe covalently labeled the same CNBr fragment of the receptor. In contrast, another structurally related full agonist peptide probe having its Bpa sited in the middle of the pharmacophoric domain labeled a distinct CNBr fragment of the receptor. These insights were achieved rapidly, without extensive purification of the receptor or its fragments, relying on the unique fluorescent signal provided by the probe and the low attomole sensitivity provided by CE-LIF. The Bodipy fluorophore incorporated into the probes was particularly well suited for this approach, because it is insensitive to exposure to acid, which is necessary for the CNBr cleavage of the receptor. The photolabile Bpa residue incorporated into the probes also possesses near ideal characteristics for the efficient establishment of covalent bonds to a domain in approximation to this residue in the probe upon photolysis, using conditions that are not destructive or damaging to the receptor. The conditions for the capillary electrophoresis of the hydrophobic peptide fragments of the hydrophobic serpiginous receptors were care-
fully established and optimized using bacteriorhodopsin as a model protein (5).

It is particularly interesting that each of the series of biologically divergent, yet structurally analogous, probes labeled the same receptor domain. This is consistent with each using the same docking determinants, placing the noncritical amino terminus of these probes in a similar position relative to the receptor. This receptor domain is clearly distinct from those recently demonstrated to be adjacent to the carboxyl terminus and mid-region of the pharmacophore of CCK (1, 15). A strong case is made for this to represent the first extracellular loop of the receptor, based on its apparent migration on an SDS-polyacrylamide gel and the absence of any apparent shift in its migration after its deglycosylation. Of interest, this domain has also been shown to be important for CCK binding in receptor mutagenesis studies (30).

As more extensive proteolysis and finer mapping techniques are applied to the labeled fragments, it will be possible to determine whether the same receptor residue is the site of labeling for each probe. This would not be surprising, because the key determinants for biological activity reside in other regions of the peptide pharmacophore (31). We are not aware of any other existing examples of the utilization of this type of series of biologically divergent probes having a similar site of photolability and covalent attachment to probe critical receptor domains. This should provide a powerful direct approach to probe the receptor domain occupied by the ligands with the receptor presumably in distinct conformations associated with active, inactive, and partially active states.

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