NMR Structural Comparison of the Cytoplasmic Juxtamembrane Domains of G-protein-coupled CB₁ and CB₂ Receptors in Membrane Mimetic Dodecylphosphocholine Micelles*

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The fourth cytoplasmic domain, the so-called C-terminal juxtamembrane segment or helix VIII, has been identified in numerous G-protein-coupled receptors and exhibits unique functional characteristics. Efforts have been devoted to studying the juxtamembrane segment in order to understand the biological importance of the segment in G-protein activation of the cannabinoid CB₁ and CB₂ receptors. Recent biochemical data revealed that the CB₁ C-terminal juxtamembrane peptide fragment CB₁(401–417) can directly activate the G-protein and also showed that the specificity of the signal transduction activation by the C-terminal juxtamembrane region is unique to the CB₁ receptor but not to the CB₂ receptor (Mukhopadhyay, S., and Howlett, A. C. (2001) Eur. J. Biochem. 268, 499–505). However, there is experimental work, not yet reported, on the conformational analyses and structural comparison between the respective helix VIII segments of the two receptors. In the present study, we have examined the conformational specificities of the cytoplasmic helical domains for both cannabinoid receptors. Three-dimensional structural features of two synthetic CB₁ and CB₂ peptides, CB₁I397-G418 and CB₂I298-K319, respectively, in membrane mimetic DPC micelles were studied using a combined high resolution NMR and computer modeling approach. Comparisons of the NMR-determined structures of the two peptides as well as their correspondent mutant peptides revealed their conformational properties and salt bridge dissimilarity, which might help us to understand the different structural roles of the fourth cytoplasmic helices in the function and regulation of CB₁ and CB₂ receptors.

The cannabinoid (CB)³ receptor subtypes, CB₁ and CB₂, have been cloned and classified into the class A rhodopsin-like family of the seven transmembrane G-protein-coupled receptors (GPCRs) (1, 2). These subtypes have also been identified as important drug discovery targets for numerous potential therapeutic applications, including antiinflammation, analgesics, glaucoma treatment, and immune suppression (3, 4). The CB₁ receptor is located in the central and peripheral nervous system (1, 5, 6), whereas the CB₂ receptor is distributed peripherally in non-neuronal tissues, particularly in immune cells (2, 6). The CB₁ receptor interacts with the pertussis toxin-sensitive Gᵢ family of G-proteins to inhibit adenylate cyclase (7) and to regulate N-type Ca²⁺ channels and inwardly rectifying K⁺ channels in the central nervous system (8–10). The CB₂ receptor is expressed in high quantities in the human spleen and tonsils and exhibits 44% amino acid identity to the CB₁ receptor throughout the whole protein. The CB₂ receptor is likely involved in the signal transduction processes in the immune system (6).

Studies have been carried out to understand the three-dimensional structure of the CB receptors and their mechanisms of action by using computer molecular modeling and NMR approaches (11–17). Bramblett et al. (11) first analyzed the secondary structure of the CB₁ receptor based upon calculated hydrophobicity and variability profiles to predict the regions of α-helicity and then predicted the three-dimensional structure model of the CB₁ receptor showing an extracellular N-terminal, seven transmembrane helical segments, three intracellular loops, and three extracellular loops, as well as a juxtamembrane helical C-terminal domain. Xie et al. (17) applied computer homology-based multiple sequence and conserved residue alignments to construct a human CB₂ three-dimensional structure model based on the recently available x-ray crystal structure of bovine rhodopsin (18), the first three-dimensional atomic structure in the GPCR family.

Efforts have also been devoted to investigating the CB receptor pharmacology and biological functions. In particular, attention has been focused on the C-terminal juxtamembrane domain in order to understand the biological importance of the domains in CB₁ and CB₂ activation. Mukhopadhyay et al. systematically investigated the biological functions of the CB₁ juxtamembrane region peptide CB₁ 401–417 (19–21). They concluded that the C-terminal juxtamembrane fragment peptide CB₁ 401–417 can directly activate the Gᵢ protein and, in addition, the specificity of the C-terminal juxtamembrane region’s affinity to Gᵢ and Gᵥ proteins is unique to the CB₁ receptor (19–21) but not to the CB₂ receptor (21). On the other hand, Feng and Song (22) investigated the role of two cysteine residues in the C-terminal juxtamembrane region of human CB₂ with site-directed mutagenesis. They found that the C313A and C320A mutations markedly reduced functional coupling to adenylate cyclase but had no effect on ligand binding and agonist-induced receptor desensitization. Their results suggested that the conserved cysteine residues in the C-termi-

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§ The abbreviations used are: CB, cannabinoid; CB₁I397-G418, synthetic peptide encompassing Ile₃⁹⁷–Gly₄₁₈; CB₁I298-K319, synthetic peptide encompassing Ile₁⁹⁷–Lys₃₁₉; GPC₁, G-protein-coupled receptor; DPC, dodecylphosphocholine; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser enhancement spectroscopy; TOCSY, total correlation spectroscopy.
nal juxtamembrane region play different roles among GPCRs. These early investigations on the juxtamembrane regions have underscored the biological importance of the fourth cytoplasmic domain and provided a tentative hypothesis on its roles in CB₁ and CB₂ receptor function. However, only limited structural information has been reported regarding the structural and conformational specificities of the C-terminal juxtamembrane domains of the cannabinoid receptors along the transmembrane region of the GPCRs.

In the present study, we presented our recent NMR studies and computer structural calculations of two C-terminal juxtamembrane or fourth cytoplasmic domains of CB₁ and CB₂ receptors, CB₁I397-G418 and CB₂I298-K319, bound to membrane mimetic DPC micelles. Both polypeptides show the stable α-helical secondary structure in the membrane-like environment. Structural comparisons of the two peptides are correlated with the structure-function relationship of C-terminal juxtamembrane domains. In addition, the structural properties of these two target peptides were examined against their correspondent mutant peptides. The results from the present study, in conjunction with published works (19–21), support the hypothesis that the amphiphatic helix nature of both juxtamembrane domains (or helix VIII fragments) and the salt bridge feature could potentially play significant roles in the function and regulation of the CB₁ and CB₂ cannabinoid receptors.

MATERIALS AND METHODS

Peptide Synthesis—Two peptides, CB₁I397-G418 of the sequence IYLSPKDLRHAFLRSMFPSCEG and CB₂I298-K319 of the sequence IY-ALRSAKDLRHAFLRSMFPSCEG and CB₂I298-K319, were synthesized through solid-state phase synthesis at the biotechnology center of the University of Connecticut. Synthesis was performed on an Applied Biosystem 433A peptide synthesizer, using Fmoc (N-(9-fluorenylethoxycarbonyl) chemistry on p-hydroxymethylphenoxymethyl polystyrene resin at room temperature. O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate, was used for activation, and the resin was washed with 1-methyl-2-pyrrolidinone. Two corresponding peptides, CB₁I397-G418(R401A) of IYLASKDLRHAFLRSMFPSCEG and CB₂I298-K319(E305Q) of IY-ALRSAKQDLRHAFLRSMFPSCEG, were synthesized by Genemed (Genemed Synthesis, Inc., San Francisco, CA). The peptides were purified by high performance liquid chromatography and verified by mass spectrometry analysis.

NMR Experiments—The NMR samples of CB₁I397-G418, the CB₁I397-G418(R401A) mutant, CB₂I298-K319, and the CB₂I298-K319(E305Q) mutant were prepared by adding solution of DPC-d₃₈ (Cambridge Isotope Laboratories, Andover, MA) in sodium phosphate buffer (pH 5.7) containing 10% D₂O (99.9%; Isotec, Inc.) to the powder (Cambridge Isotope Laboratories, Andover, MA) in sodium phosphate buffer (pH 5.7) containing 10% D₂O (99.9%; Isotec, Inc.) to the powder sample with various molar ratios of DPC micelles. All NMR spectra were recorded at 298 K on a Bruker DMX-600 spectrometer equipped with a 5-mm QXI probe with z-gradient. The two-dimensional, phase-sensitive NOESY spectra were recorded with a mixing time of 100 ms. The TOCSY experiments were acquired according to standard pulse sequence (Malcom Levitt decoupling sequence17) (24) with 70- and 90-ms spin-lock periods. All two-dimensional spectra through the sequential assignments were made by choosing the residues Ala and Tyr as the logical starts for the assignment process. The Tyr residue has typical cross peaks between the NH, αH, βH, and aromatic protons in the NOESY spectrum, and there is only one Tyr residue in each of the two synthetic peptides. For example, on the basis of the connection of the αH and βH peaks of preceding residue (i + 1) from the βHαH(CO)/NH spin system with the αH and βH of adjacent residue (i) from the NHαHβH, the corresponding residues were identified. A similar analogy was applied to assign adjacent residues and repeated until all residues were assigned. Fig. 2 and Table I summarize the chemical shift assignments of proton resonance for both peptides.

NOE Interactions—NOESY spectra were used not only to determine the sequential assignments but also to obtain the NOE distance constraints and determine the peptide secondary structure. The NOESY spectrum of the peptide CB₁I397-G418 in DPC micelles revealed an α-helical conformation on the basis of the observed typical helix-indicating NOE patterns of d₃₈N(i,i+1), d₈N(i,i+1), and d₈(déš(i,i+1)) cross-peaks as shown in Fig. 3A. The NOE data showed that there are two helical segments formed, namely helix a from residues II₃₉₇ to Leu₄₀⁶ and helix b from Ser₄⁰₂ to Phe₄₁₃ in the target peptide CB₁I397-G418. Additional NOE interactions in this peptide were detected between Ala₃₉₉(αH) and Leu₄₀⁶(αH), Ala₃₉₉(βH) and Leu₄₀⁵(αH), and Ala₃₉₉(αH) and Leu₄₀⁵(αH). These NOE data provided the basis for the distance-constrained structural calculations for determining the relative orientation between helix a and helix b of the CB₁ C-terminal juxtamembrane peptide. Additionally, our NMR data shows that only the dₑš(i,i+1) NOE was observed for the residue Pro₄₁⁴ in CB₁I397-G418, indicating that this proline residue is in the trans conformation (25). The residues after proline in structures generated from DIANA were then subjected to extensive energy minimization using the Sybyl program (26) under the Kollman all-atom force field and Kollman charges to fold structures that were consistent with the available experimental distance constraints. Finally, 10 representative three-dimensional structures from the two peptides were selected with the lowest total energies and the criteria of no NOE violations >0.30 Å and a root mean square difference for bond deviations from ideality of <0.01 Å.

RESULTS

One-dimensional proton experiments of peptide CB₂I298-K319 was carried out as a function of DPC concentrations in order to examine the peptide-micelle interaction and to achieve the best concentration of DPC micelles as illustrated in Fig. 1. Fig. 1A showed that the spectrum of the peptide in pure water (H₂O/D₂O, 90:10) displays sharp peaks with a narrow line shape, indicating a homogenous peptide solution. Upon the addition of DPC into the solution, the changes of chemical shifts and the increase in the peak line width were detected, revealing the presence of a conformational exchange of peptides in the micelle solution. At a 12.5 molar ratio of [DPC]/[CB₂I298-K319] (Fig. 1D), the conformational exchange was observed, indicating a millisecond to microseconds time scale equilibrium between the free and micelle-bound states of peptide CB₂I298-K319. The sharp peak shape in Fig. 1P indicated that most of the peptide is on the micelle-bound state at the concentration of 200 mM DPC solution (100 molar ratio of [DPC]/[CB₂I298-K319]).
Fig. 1. Amidic and aromatic region of the one-dimensional proton NMR spectra of CB1,298-K319 (2 mM) at 298 K. A, 50 mM sodium phosphate buffer at pH 5.7. B, 10 mM DPC ([DPC]/[CB1,298-K319] = 5). C, 15 mM DPC ([DPC]/[CB1,298-K319] = 7.5). D, 25 mM DPC ([DPC]/[CB1,298-K319] = 12.5. E, 80 mM DPC ([DPC]/[CB1,298-K319] = 40. F, 200 mM DPC ([DPC]/[CB1,298-K319] = 100).

Fig. 2. Expanded, two-dimensional, phase-sensitive NOESY spectra of the peptides CB1,397-G418 (A) and CB1,298-K319 (B) in DPC micelles, recorded with 100 ms of mixing time at 298 K and pH 5.7. Single letter amino acid abbreviations are used with position numbers.
CB1I397-G418 appear to be in a random coil. On the other hand, Fig. 3B (mutant peptide) revealed different NOE patterns as depicted in the observed NOEs, i.e. dαN(i,i+3), dαN(i,i+3), and dαβ(i,i+3), in comparison with the non-mutant peptide (Fig. 3A). The data indicated that the peptide CB2I298-K319(E305Q) mutant gave a similar NOE pattern, in which the residue Asp300 was mutated to Ala, can form one continuous helical conformation ranging from Ile307 to Phe313, without a right angle bend at the fifth residue as the peptide CB1I397-G418. That is expected, because the residue proline in CB1I397-G418 terminates the helical conformation. Furthermore, Fig. 3D showed that the CB2I298-K319(E305Q) mutant gave a similar NOE pattern, which indicated that the peptide CB2I298-K319(E305Q) mutant retains the secondary structure. The secondary structures of four peptides (CB1I397-G418 and its mutant and CB2I298-K319 and its mutant) were further confirmed by the secondary shift analysis of the Hα resonances (26, 27), which is illustrated in Fig. 3.

Comparison of the NOESY spectra of the CB1I397-G418 and CB2I298-K319 peptides showed many similarities between the CB1 and CB2 peptide fragments. Within the respective a and b portion of each peptide, unique NOEs were observed between Ala300(aH) and Leu405(aH), Ala300(aH) and Leu405(aH), and Ala300(aH) and Leu405(aH) in peptide CB1I397-G418, whereas peptide CB2I298-K319 had NOEs between Ala300(aH) and Ile306(aH), Ala300(aH) and Ile306(aH), and Ala300(aH) and Ile306(aH). Based on the existence of helical substructures in both peptides, the respective NOE patterns suggest that there may

| Residue | NH | αH | βH | Other |
|---------|----|----|----|-------|
| CB1I397-G418 | | | | |
| Ile397 | 3.78 | 1.89 | yH, 1.47, 0.91; δH, 0.86 | |
| Tyr398 | 8.56 | 4.37 | 2.99, 2.87; 2.6H, 7.02; 3.5H, 6.73 | |
| Ala399 | 8.20 | 4.21 | 1.25 | |
| Leu400 | 7.88 | 4.28 | 1.63, 1.61; yH, 1.49; δH, 0.89, 0.84 | |
| Arg401 | 8.49 | 4.32 | 1.97; yH, 1.75, 1.62; δH, 3.12; NH, 7.45 | |
| Ser402 | 8.84 | 4.01 | 3.88, 3.85; yH, 1.35, 1.30; δH, 1.57, eH, 2.89; NH, 7.58 | |
| Lys403 | 8.39 | 4.06 | 1.80, 1.70 | |
| Asp404 | 7.66 | 4.57 | 2.80, 2.76; yH, 1.55, δH, 0.89, 0.85 | |
| Leu405 | 7.96 | 4.04 | 1.82, 1.74; yH, 1.71, 1.50; δH, 3.19, 3.14; NH, 7.59 | |
| Arg406 | 8.32 | 3.89 | 1.91, 1.84 | |
| His407 | 8.04 | 4.28 | 3.32 | 2H, 7.28; NH, 8.53 | |
| Ala408 | 8.16 | 4.03 | 1.47 | |
| Phe409 | 8.33 | 4.14 | 3.23, 3.10 | |
| Arg410 | 8.06 | 3.90 | 1.86, 1.83; yH, 1.71, δH, 3.17; NH, 7.53 | |
| Ser411 | 7.74 | 4.12 | 3.81, 3.73 | |
| Met412 | 7.33 | 4.07 | 1.40, 1.70; yH, 2.19, 2.13; eH, 2.34 | |
| Phe413 | 7.59 | 4.81 | 2.88, 2.48; 2.6H, 6.98; 3.5H, 7.10; eH, 7.14 | |
| Pro414 | 7.90 | 4.45 | 2.27; yH, 1.90, δH, 3.48 | |
| Ser415 | 8.42 | 4.40 | 3.87, 8.83 | |
| Cys416 | 8.20 | 4.44 | 2.92 | |
| Glu417 | 8.29 | 4.33 | 1.90 | yH, 2.10, 2.34 | |
| Gly418 | 7.98 | 3.71 | | |

| CB2I298-K319 | | | | |
| Ile306 | 3.78 | 1.89 | yH, 1.60, 1.47, 0.90; δH, 0.84 | |
| Tyr307 | 8.61 | 4.36 | 3.00, 2.87; 2.6H, 7.02; 3.5H, 6.73 | |
| Ala308 | 8.21 | 4.18 | 1.25 | |
| Leu309 | 7.92 | 4.27 | 1.61 | |
| Arg310 | 8.27 | 4.43 | 1.90, 1.72; yH, 1.59, 1.51; δH, 3.14; NH, 7.36 | |
| Ser311 | 8.09 | 4.05 | 3.85, 3.89 | |
| Gly312 | 8.74 | 3.96, 3.85 | 2.07; yH, 2.29 | |
| Gly313 | 7.88 | 4.29 | | |
| Ile314 | 8.08 | 3.72 | 1.94 | yH, 1.67, 1.10; δH, 0.83 | |
| Arg315 | 8.39 | 3.80 | 1.89, 1.83; yH, 1.64, 1.50; δH, 3.18, 3.14; NH, 7.57 | |
| Ser316 | 8.18 | 4.20 | 3.91, 3.89 | |
| Ser317 | 8.29 | 4.28 | 3.98, 3.81 | |
| Ala318 | 8.53 | 3.98 | 1.41 | |
| His319 | 8.34 | 4.19 | 3.33 | 2H, 8.52, 4H, 7.14 | |
| His320 | 8.31 | 4.37 | 3.39 | 2H, 8.47, 4H, 7.26 | |
| Cys321 | 8.14 | 4.11 | 3.05, 2.78 | |
| Leu322 | 7.98 | 4.14 | 1.77 | yH, 1.61, δH, 0.82, 0.80 | |
| Ala323 | 7.75 | 4.06 | 1.25 | 2H, 8.47, 4H, 6.65 | |
| His324 | 7.71 | 4.44 | 3.11, 2.97 | |
| Trp325 | 7.79 | 4.46 | 3.33, 3.17 | 2H, 7.19, 4H, 7.55; 5H, 6.94; 6H, 6.99; 7H, 7.37, NH, 10.53 | |
| Lys326 | 7.80 | 4.19 | 1.75 | yH, 1.29, 1.26; δH, 1.80, eH, 2.87 | |
| Lys327 | 7.78 | 4.02 | 1.72 | yH, 1.29, δH, 1.61, eH, 2.84 | |
be similarities in the relative orientations of helices a and b, in peptides CB1I397-G418 and CB2I298-K319. However, there are some significantly different NOE interactions observed in two NOESY spectra of the peptides. For example, the acidic residues each in the two peptides have NOE interactions with the different basic residues, e.g. Asp404(H)/His407(H) in CB1I397-G418 and Arg302(guanidine NH)/Glu305(H) in CB2I298-K319. However, no NOE interaction was observed between Arg302(guanidine NH) and Gln305(H) in the peptide CB2I298-K319 mutant.

Three-dimensional Structures of the C-terminal Juxtamembrane Domains—NMR-derived constraints for the peptides CB1I397-G418 and CB2I298-K319 were used as input for structure calculations as implemented in the software Sybyl/DIANA package. Ten of the 100 best scored structures of both peptides were then refined through energy minimization calculations with the Sybyl program. Fig. 4 shows the backbone superposition of the 10 lowest energy structures with no experimental violation >0.35 Å for CB1I397-G418 (A) and CB2I298-K319 (B), respectively. The averaged pairwise root mean square values of the superimposed ten lowest energy structures were found to be 0.38 Å for backbone atoms and 1.52 Å for heavy atoms on the well defined region of 2–4 and 6–17 residues in CB1I397-G418, and 0.40 Å for backbone atoms and 1.50 Å for all heavy atoms on the correspondent region of 2–4, 6–21 residues in CB2I298-K319.

The topology and defined residues of the α-helices calculated using the NOE distance constraints were consistent with those determined by the NMR NOE analysis of secondary structures mentioned above and then showed a “L-shaped” turn at the fifth residue (Arg) between two helices a and b. Figs. 3A and 4A show a break of the helical structure at the residue Arg401 in CB1I397-G418. A similar turn was also observed at the residue Arg302 for CB2I298-K319 (Figs. 3C and 4B). The respective breaks in each peptide resulted in the formation of two helical portions, a and b in which the two helical axes were almost perpendicular to each other (Fig. 4). The tight turns at Ala399-Lys403 in CB1I397-G418 or Ala300-Lys303 in CB2I298-K319.
Ser303 in CB₂I298-K319 were also identified in the cannabinoid receptor three-dimensional molecular modeling structure using homology calculations (15, 17). The measured distances of Ala399(H)/Leu405(NH), Ala399(H)/Leu405(NH), and Ala399(H)/Leu405(γH) in CB₁I397-G418 are 3.78, 4.75, and 4.13 Å, respectively. The corresponding distances of Ala300(NH)/Ile306(NH), Ala300(NH)/Ile306(H), and Ala300(H)/Ile306(γH) in CB₂I298-K319 are 4.75, 3.39, and 3.62 Å, respectively. Helix a is a largely conserved region showing a high homology between CB₁ and CB₂ peptides.

Fig. 5 displays the conformational similarity between the two peptide segments and the corresponding roles of the respective Arg residues in CB₁I397-G418 and CB₂I298-K319. Our results show that the side chain of Arg401 in CB₁I397-G418 (Fig. 5A) is in the extended conformation, whereas in the Arg302 of CB₂I298-K319 the side chain curves back and forms a salt bridge with the residue Glu305 (Fig. 5B). The presence of a salt bridge in the peptide CB₂I298-K319 is confirmed by the NOE interaction observed between Arg302(guanidine NH) and Glu305(γH) in CB₂I298-K319, but no NOE interaction between Arg302(guanidine NH) and Gln305(γH) in the peptide CB₂I298-K319(E305Q) mutant is observed.

Side Chain Orientations of Key Residues—The NMR-based computations of the structural conformations show that the cationic hydrophilic side chains, such as Lys406, Arg406, and Arg410 in peptide CB₁I397-G418 and Arg407, His311, Lys318 in peptide CB₂I298-K319 tend to orient on the same side of each correspondent helix. Our data also show that the orientation of these cationic side chain residues in the helix b portion (or C-terminal juxtamembrane domain) of the CB₁ or CB₂ receptors is opposite to helix a as shown in Fig. 5.

Cysteine residues are recognized as important residues in the C-terminal juxtamembrane regions of GPCRs. A number of studies (19, 22, 28, 29) have indicated that the cysteines in the juxtamembrane region become palmitoylated in order to interact with the membrane. This palmitoylation/depalmitoylation process plays an important role in the functional coupling of GPCRs.
One the other hand, Cys\textsuperscript{416} in CB\textsubscript{1}I397-G418 is in the random coil of the C-terminal peptide.

**Discussion**

We have determined the three-dimensional structures of two peptides corresponding to the extended fourth cytoplasmic juxtamembrane domains within the CB\textsubscript{1} and CB\textsubscript{2} receptors by means of proton NMR measurements and NMR-derived, distance-constrained dynamics simulation. Our NMR results reveal that the C-terminal juxtamembrane domains of both CB\textsubscript{1} and CB\textsubscript{2} acquire helical conformations in membrane mimetic DPC micelles. These findings are consistent with the results of circular dichroism measurements of the peptides (15, 19).

Our NMR NOE-constrained structural analyses of the extended CB\textsubscript{1} and CB\textsubscript{2} juxtamembrane peptides identify an L-shaped turn at the arginine residue in each peptide leading to two helical portions, a and b (Fig. 3, A and C, and Figs. 4 and 5). The results were confirmed by the observed NOEs between Ala\textsuperscript{399}(\textbeta H) and Leu\textsuperscript{405}(\textNH), Ala\textsuperscript{399}(\textbeta H) and Leu\textsuperscript{405}(\textalpha H), and Ala\textsuperscript{399}(\textbeta H) and Leu\textsuperscript{405}(\gamma H) for peptide CB\textsubscript{1}I397-G418, and by the NOEs between Ala\textsuperscript{390}(\textNH) and Ile\textsuperscript{396}(\textalpha H), Ala\textsuperscript{390}(\textNH) and Ile\textsuperscript{396}(\alpha H), and Ala\textsuperscript{390}(\textNH) and Ile\textsuperscript{396}(\gamma H) for peptide CB\textsubscript{2}I298-K319. Helix a in both peptides represents the highly conserved N-terminal region, which can be considered as the C-terminal region of the transmembrane domain helix VII in both cannabinoid receptors. Thus, if helix a is aligned along the helix VII axis, the L-shaped turn will then make the helix b portion, i.e. the C-terminal juxtamembrane domains (or helix VIII), almost parallel with the membrane surface. The connection between helices a and b corresponds to the turn between the transmembrane helix VII domain and the juxtamembrane helix VIII observed in the crystal structure of bovine rhodopsin (18). Such a right angle bend at the fifth residue of the CB\textsubscript{1}I397-G418 was also confirmed by our further NMR study of the peptide CB\textsubscript{1}I397-G418(R401A) mutant for which a continuing helical conformation was detected without the right angle bend at the fifth residue.

The homology/multiple sequence alignment (17) showed that part of the C-terminal juxtamembrane domains of both CB receptors include positively charged residues such as Arg, Lys, and His separated by hydrophobic residues. Such nonconsecutive cationic residues impart an amphipathic character to this helical peptide fragment. The structures of these two fragments were obtained from NMR NOE analyses (Figs. 2 and 3) and the constrained structural calculation (Fig. 4). It can be argued that such an amphipathic α-helical structure that includes a multiple positively charged motif is a requirement for its productive interaction of the receptor with G-proteins (19, 31). The amphipathic characteristic of C-terminal juxtamembrane domain is by no means unique to the cannabinoid receptors. Other members of the GPCR superfamily such as rhodopsin and the β-adrenergic receptor, the human P2Y1 receptor, the dopamine receptor, the histamine receptor, the endothelin receptor, and others were found through sequence analysis to possess an amphipathic helical property. In addition, our studies show that a different salt bridge may be formed in the corresponding CB1 or CB2 polypeptide fragments. Such conformational and structural differences may help to explain why the specificities of the signal transduction activation by the C-terminal juxtamembrane region is unique to the CB\textsubscript{1} receptor but not the CB\textsubscript{2} receptor. However, more detailed biological and biophysical studies are required to further elucidate the roles of the fourth cytoplasmic helix domain in G-protein binding. In addition, more rigorous studies of the relative conformations and orientations of the Helix VIII segments with respect to a membrane or other helix domains should be carried out to directly test the hypothesis in a much more extended peptide fragment or in an intact receptor system. This work is currently under way by using recombinant protein engineering.
and isotope-edited NMR spectroscopy. Ultimately, the structures observed would lead to direct biochemical tests that could serve to validate the proposed hypothesis.

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REFERENCES

1. Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., and Bonner, T. I. (1990) Nature 346, 561–564
2. Munro, S., Thomas, K. L., and Abu-Shaar, M. (1993) Nature 365, 61–65
3. Pertwee, R. G. (1997) Pharm. Sci. 3, 539–545
4. Izzo, A. A., Mascolo, N., and Capasso, F. (2000) Trends Pharmacol. Sci. 21, 281–282
5. Tssea, K., Brown, S., Sanudo-Pena, M. C., Mackie, K., and Walker, J. M. (1998) Neuroscience 83, 393–411
6. Galiegue, S., Mary, S., Marchand, J., Dussossoy, D., Carriere, D., Carayon, P., Bouaboula, M., Shire, D., Le Fur, G., and Casellas, P. (1995) Eur. J. Biochem. 232, 54–61
7. Howlett, A. C. (1985) Mol. Pharmacol. 27, 429–436
8. Pacheco, M., Childers, S. R., Arnold, R., Casiano, F., and Ward, S. J. (1991) J. Pharmacol. Exp. Ther. 257, 170–183
9. Mackie, K., and Hille, B. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 3825–3829
10. Bouaboula, M., Bourrie, B., Rinaldi-Carmona, M., Shire, D., Le Fur, G., and Casellas, P. (1995) J. Biol. Chem. 270, 13973–13980
11. Bramblett, R. D., Panu, A. M., Ballesteros, J. A., and Reggio, P. H. (1995) Life Sci. 56, 1971–1982
12. Mahmoudian, M. (1997) J. Mol. Graph. Model. 15, 149–153, 179
13. Shire, D., Calandra, B., Gauldson, P., Delpech, M., Kerneis, A., Rinaldi-Carmona, M., Barth, F., Le Fur, G., and Ferrara, P. (1998) in 1998 Symposium on the Cannabinoids, Le Grande Motte, France, July 23–25, 1998, p. 86, International Cannabinoid Research Society, Burlington, VT
14. Gauldson, P., Calandra, B., Legoux, P., Kerneis, A., Rinaldi-Carmona, M., Barth, F., Le Fur, G., Ferrara, P., and Shire, D. (2000) Eur. J. Pharmacol. 401, 17–25
15. Choi, G., Lundin, J., and Xie, X.-Q. (2002) J. Pept. Res. 60, 169–177
16. Ulfers, A. L., McMurry, J. L., Kendall, D. A., and Mierke, D. F. (2002) Biochemistry 41, 11344–11350
17. Xie, X.-Q., Chen, J.-Z., and Billings, E. M. (2003) Proteins Struct. Funct. Genet. 53, 307–319
18. Paleczewski, K., Kumasaka, T., Horii, T., Behnke, C. A., Motohashi, H., Fox, B. A., Le Trong, I., Teller, D. C., Okada, T., Stenkamp, R. E., Yamamoto, M., and Miyano, M. (2000) Science 289, 739–745
19. Mukhopadhyay, S., Cowisik, S. M., Lynn, A. M., Welsh, W. J., and Howlett, A. C. (1999) Biochemistry 38, 3447–3455
20. Mukhopadhyay, S., McIntosh, H. H., Houston, D. B., and Howlett, A. C. (2000) Mol. Pharmacol. 57, 162–170
21. Ulfers, S. L., and Howlett, A. C. (2001) Eur. J. Biochem. 268, 499–505
22. Feng, W., and Song, Z. H. (2001) FEBS Lett. 501, 166–170
23. Piotti, D., Schenkel, V., and Sklenar, V. (1992) J. Biomol. NMR 2, 661–665
24. Bax, A., and Davis, D. G. (1985) J. Magn. Reson. 65, 355–360
25. Wuthrich, K. (1986) NMR of Proteins and Nucleic Acids, John Wiley & Sons, Inc., New York
26. Tripos Inc. (2003) SYBYL Version 6.9.2, Tripos Inc., St Louis, MO
27. Ovchinnikov Y. A., Abdulaev, N. G., and Bogachuk, A. S. (1988) FEBS Lett. 230, 1–5
28. Ovchinnikov, N., Panetta, R., Kumar, U., Rocheville, M., and Patel, Y. C. (1998) J. Biol. Chem. 273, 21416–21422
29. Ernst, O. P., Meyer, C. K., Marin, E. P., Henklein, P., Fu, W. Y., Sakmar, T. P., and Hofmann, K. P. (2000) J. Biol. Chem. 275, 1937–1943
30. Shirai, H., Takahashi, K., Katada, T., and Inagami, T. (1995) Hypertension 25, 726–730
NMR Structural Comparison of the Cytoplasmic Juxtamembrane Domains of
G-protein-coupled CB₁ and CB₂ Receptors in Membrane Mimetic
Dodecylphosphocholine Micelles
Xiang-Qun Xie and Jian-Zhong Chen

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Additions and Corrections

Vol. 279 (2004) 23882–23891

Acyl-CoA synthetase 2 overexpression enhances fatty acid internalization and neurite outgrowth.

Joseph R. Marzalek, Claire Kitidis, Ariya Dararutana, and Harvey F. Lodish

Page 23883, “Materials and Methods”: Under the section headed “Isolation and Construction of ACS1 and ACS2,” two errors occurred. First, the incorrect primer sequence was included for ACS1 antisense. It should read 5’-CCGGATCCTAGGGCCCAATCTTGATGGTGGAG-3’ not 5’-CCGGATCCTTAAATCTTGATGGTTGGAGTAC-3’. Second, the GenBank™ accession number referenced for the rat ACS2 sequence used in the study should be AY625254, not D10041. These two sequences are alternatively spliced variants of rat Acs16, each containing a different variant of exon 13.

Although these minor corrections do not change the data or interpretation of the data in the paper, they are important for accuracy and reproducibility of the results by other investigators.

Vol. 279 (2004) 54881–54886

A crucial role for exopolysaccharide modification in bacterial biofilm formation, immune evasion, and virulence.

Cuong Vuong, Stanislava Kocianova, Jovanka M. Voyich, Yufeng Yao, Elizabeth R. Fischer, Frank R. DeLeo, and Michael Otto

The thesis of Oliver Schweitzer (1997) described S. epidermidis ΔicaB mutants and pTXicaB constructs similar to those described in this article, suggested a role for icaB in biofilm formation and cell aggregation, and noted its localization on the cell surface, with secretion when overexpressed. The thesis can be obtained from the international OPAC catalogue http://opac.ub.uni-tuebingen.de/.

Vol. 280 (2005) 2361–2369

Pro-angiogenic signaling by the endothelial presence of CEACAM1.

Nerbol Kilic, Leticia Oliviera-Ferrer, Jan-Henner Wurmbach, Sonja Loges, Fariba Chalajour, Samira Neshat-Vahid, Joachim Weil, Malkanthi Fernando, and Suleyman Ergun

Dr. Neshat-Vahid's last name was misspelled. The correct spelling is shown above.

Vol. 280 (2005) 3605–3612

NMR structural comparison of the cytoplasmic juxtamembrane domains of G-protein-coupled CB1 and CB2 receptors in membrane mimetic dodecylphosphocholine micelles.

Xiang-Qun Xie and Jian-Zhong Chen

Page 3612: Add new Ref. 37, Wishart, D. S., Sykes, B. D., and Richards, F. M. (1992) Biochemistry 31, 1647–1651. The reference list will now include a total of 37 references. As a result, the following reference citation should be changed:

Pg. 3608, right column, line 11 from the top: “(26, 27)” should be “(37).”

Vol. 280 (2005) 3802–3811

Activation of the phagocyte NADPH oxidase by Rac guanine nucleotide exchange factors in conjunction with ATP and nucleoside diphosphate kinase.

Ariel Mizrahi, Shahar Molshanski-Mor, Carolyn Weinbaum, Yi Zheng, Miriam Hirshberg, and Edgar Pick

Pages 3803–3811: The word “on” was omitted from the running title. The correct running title should read as follows: NADPH Oxidase Activation Dependent on GEF, ATP, and NDPK.

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.