Conformational Ensembles: The Role of Neuropeptide Structures in Receptor Binding

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Neuropeptides are a major source of neurochemical diversity, with functions as wide ranging, for example, as the modulation of the action of pheromone and feeding behavior in mammals (Yang et al., 1985; Sakurai et al., 1998), the regulation of cardiac stimulation and pacemakers in mollusks (Payza, 1987; Simon et al., 1992), the stimulation or inhibition of ovulation contractions in locust (Wang et al., 1995a,b), and the modification of lobster pattern-generating circuits (Dickinson et al., 1990). Currently the largest, most widely distributed, and most diverse family of neuropeptides are the FMRFamide-like peptides (FLPs). FLPs are thought to be present in all animals (Greenberg and Price, 1992) and have amino acid sequence similarities to the “parent” peptide Phe-Met-Arg-Phe-NH₂ (FMRFa), which was first discovered in the clam (Price and Greenberg, 1977).

FLPs, like other neuropeptides, are encoded by precursor proteins that are processed into mature peptides in the secretory pathway (Sossin et al., 1989). Some FLP precursor proteins produce multiple copies of the same peptide. The most dramatic example of multiple copies is from Aplysia, with 28 copies of FMRFamide and one copy of FLRFamide (Tausigg and Scheller, 1986). Other precursor proteins encode several different FLPs, which tend to be longer than four amino acids and have common C-terminal amino acid sequences and variable N-terminal extensions. Large numbers of different FLPs are often present in a given animal. In nematodes, for example, Ascaris suum has at least 20 different FLPs (Cowden et al., 1989; Cowden and Stretton, 1993, 1995; Edison et al., 1997), and Caenorhabditis elegans has 20 FLP genes (Rosoff et al., 1992; Wilson et al., 1994; Bargmann, 1998; Nelson et al., 1998; Chris Li, personal communication). If all of the C. elegans genes were expressed and fully processed, they would produce at least 56 different FLP peptides (Bargmann, 1998; Nelson et al., 1998).

The functional role of multiple diverse peptides is currently ambiguous. A deletion in C. elegans of a FLP precursor protein encoding for eight closely related peptides produces five distinct phenotypes (Nelson et al., 1998), but it is unknown whether individual peptides or a “bouquet” (Greenberg and Price, 1992) is responsible for the specific phenotypes. Substitutions of a single amino acid in an FLP from locust produces opposite G-protein-mediated responses (Wang et al., 1995a,b). Replacement of an aspartic acid by tyrosine lowers the receptor binding affinity of a molluscan FLP (Payza, 1987; Payza et al., 1989). In contrast, two similar FLPs have essentially identical effects on the crust stomatogastric ganglion (Weimann et al., 1993), and a group of six peptides from the same precursor protein in Ascaris muscle (Davis and Stretton, 1996).

Here we report the conformational properties of individual peptides from a group of related FLPs from mollusks using nuclear magnetic resonance (NMR) spectroscopy and correlate the conformations with previously published receptor binding affinities (Payza, 1987; Payza et al., 1989). The organization of this paper is as follows. The technical NMR details are primarily limited to Materials and Methods. The Results section has four parts: NMR chemical shifts, NMR distance measurements, NMR pH dependence, and conformational models. Each NMR section has an introduction to the parameter with a description of its significance to the present study. The conformational model section presents a three-dimensional reverse turn structure that is most consistent with the NMR data and the known conformational properties of the amino acids comprising the peptides. The
Discussion section begins with a summary of published results from receptor binding studies of the same set of peptides (Payza, 1987; Payza et al., 1989). We compare experimental conditions between the NMR and receptor binding experiments and show that the NMR conformations are directly correlated with binding affinities. The strong correlation leads to a model in which the active conformation of a peptide is only a fraction (up to 100%) of the ensemble of conformations in solution. We end by discussing the biological significance of the conformational ensemble model and propose a set of experiments to further test its validity.

MATERIALS AND METHODS

Peptide synthesis. Peptides were synthesized by Alfred Chung (University of Florida Interdisciplinary Center for Biotechnology Research Protein Chemistry Core) at the 0.25 mm scale. After the synthesis of the conserved PFLRF-NH$_2$ C terminus, the reaction was split for the individual synthesis of the different N-terminal extensions. The peptides were made with a 432A Peptide Synthesizer (Applied Biosystems, Foster City, CA) using standard 4-(fluoromethyl)carbamyl chemistry and were cleaved from the solid support with trifluoroacetic acid in the presence of appropriate scavengers. The scavengers were extracted with t-butyl methyl ether, and the peptides were diluted with acetic acid and freeze-dried. Peptides were purified by HPLC. All peptides used for NMR were at least 95% pure, as estimated by analytical HPLC and mass spectrometry.

NMR spectroscopy. NMR data were collected at 600 MHz using a Varian Unity 600 (Palo Alto, CA) at the University of Florida Center for Structural Biology. Approximately 1–3 mM samples were prepared with 90% H$_2$O, 10% D$_2$O, and 0.3 mM 3-trimethylsilyl (2,2,3,3-$^2$H$_4$) propionic acid for an internal chemical shift standard (0.0 ppm). Unless otherwise indicated, the pH (uncorrected for D$_2$O) of each sample was adjusted to acid for an internal chemical shift standard (0.0 ppm). Unless otherwise indicated, peptides were purified by HPLC. All peptides used for NMR were at least 95% pure, as estimated by analytical HPLC and mass spectrometry.

Data were obtained with the transmitter centered on the H$_2$O peak, which was reduced using presaturation or by excitation sculpting (Callihan et al., 1996). Proton spectral widths were between 10 and 15 ppm. One-dimensional data were generally collected with 16,384 points; two-dimensional data were collected with 2048 or 4096 complex points in the acquisition, and indirect dimensions, respectively. One-dimensional $^1$H pH titrations were from pH 2 to 5.6 at roughly 0.2 pH increments; the pH was adjusted by adding small volumes of concentrated KOH or HCl to the samples. Two-dimensional total correlation spectroscopy (ROESY) (Bothner-By et al., 1984) data were collected using an MLEV-17 mixing sequence applied for 60 msec with an 8.8 kHz radio frequency (rf) field strength. Rotating Overhauser effect spectroscopy (ROESY) (Bothner-By et al., 1984) data were collected using continuous wave irradiation during the mixing time with an rf field strength of 2.2–5 kHz and mixing times ranging from 150 to 300 msec. Quadrature detection in the indirect dimensions was achieved by the method of States and coworkers (1982).

Data were processed using the computer software NMRPipe (Delaglio et al., 1995) by first eliminating the residual water peak using spectral deconvolution followed by appodization with squared cosine functions, zero-filling to twice the original data size, Fourier transformation, and baseline correction. Data were sequentially assigned using standard methods (Wuthrich, 1986) using NMRView (Johnson and Blevins, 1994). Peak positions and intensities were determined using the automatic peak-picking routine in NMRView (Johnson and Blevins, 1994). Intensities were also checked by extracting one-dimensional slices through the peaks of interest.

RESULTS

NMR data were collected for the naturally occurring and modified peptides shown in Table 1 (Linacre et al., 1990; Price et al., 1990; Saunders et al., 1991; Lutz et al., 1992; Kellett et al., 1994). The series of peptides were chosen because of detailed binding studies that revealed large differences in receptor binding affinities as a function of amino acid sequence (Payza, 1987; Payza et al., 1989). We used standard NMR methods to assign each resonance (peak) in the NMR spectrum to a particular atom in the peptide. Two-dimensional total correlation spectroscopy (ROESY) (Bothner-By et al., 1984) data were collected using an MLEV-17 mixing sequence applied for 60 msec with an 8.8 kHz radio frequency (rf) field strength. Rotating Overhauser effect spectroscopy (ROESY) (Bothner-By et al., 1984) data were collected using continuous wave irradiation during the mixing time with an rf field strength of 2.2–5 kHz and mixing times ranging from 150 to 300 msec. Quadrature detection in the indirect dimensions was achieved by the method of States and coworkers (1982).

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Table 1. Peptides from this study and their measured NMR parameters

<table>
<thead>
<tr>
<th>Sequence</th>
<th>$I_{\text{NN}}/I_{\text{HN}}$ F4–L5</th>
<th>$\Delta \delta$ pH F4 (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFLRF$^a$d</td>
<td>0.0$^e$</td>
<td>NA$^e$</td>
</tr>
<tr>
<td>DPFLRF$^a$d</td>
<td>0.20$^e$</td>
<td>0.128$^e$</td>
</tr>
<tr>
<td>SDPFLRFa</td>
<td>0.48 (4°C)</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>0.51 (15°C)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1.58 (26°C)</td>
<td>ND</td>
</tr>
<tr>
<td>GDPFLRFa</td>
<td>0.45 (4°C)</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>0.72 (15°C)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1.54 (26°C)</td>
<td>ND</td>
</tr>
<tr>
<td>NDPFLRFa</td>
<td>0.24</td>
<td>0.33</td>
</tr>
<tr>
<td>pQDPFLRFa</td>
<td>0.28</td>
<td>0.35</td>
</tr>
<tr>
<td>GYPFLRFa$^d$</td>
<td>0.0</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA, Not applicable (no titrating group); ND, not done.
$a$One-letter amino acid code with “pQ” = pyroglutamic acid and with “a” = NH$_2$.
$b$Ratio of intensities of amide-to-amide ($I_{\text{NN}}$) and a-to-amide ($I_{\text{HN}}$) ROESY cross peaks between F4 and L5.
$c$Magnitude of the shift ($\Delta \delta$) in chemical shift (d) of the amide proton of F4 as a function of pH. The values represent the shift at highest pH minus the shift at lowest pH.
$d$Not naturally occurring.
$e$Data are for F2 and L3.
$f$Data are for F3 and L4.

Data are for F2 and L3.

DATA are for F3 and L4.

Chemical shifts

Chemical shifts are extremely sensitive probes of molecular conformation. Despite recent advances in empirical (Wishart and Sykes, 1994) and theoretical (Oldfield, 1995) understanding of chemical shifts in proteins, conformational averaging and interactions with solvents currently prevent a quantitative use of chemical shifts in small peptides. Each atom in each amino acid has a characteristic and known random coil chemical shift value when the amino acid is completely random and without structure (Wishart and Sykes, 1994). Useful qualitative information can be gleaned from groups of similar peptides by comparing their chemical shifts with each other and with random coil values (Wishart and Sykes, 1994).

Figure 1 shows differences between random coil values of the amide (H$^\text{N}$) and α (H$^\text{α}$) proton chemical shifts of the conserved PFLRF-NH$_2$ region of representative peptides from Table 1. The histograms of Figure 1 provide a “fingerprint” of the overall
The major feature of Figure 1 is that the peptides can be classified into DPFLRF-NH₂ (DP) and YPFLRF-NH₂ (YP) subgroups. It must be reemphasized that the shifts presented in Figure 1 are common to all peptides. Therefore, chemical shift data show that long-range conformational changes occur in these peptides as a result of the amino acid N terminal to the proline. A second feature of Figure 1 is that the deviations from random coil chemical shift values, in general, are larger for the DP than for the YP subgroup, suggesting that the DP peptides have higher populations of “nonrandom” conformations.

Internuclear distances
Chemical shifts are extremely sensitive to molecular conformation and thus provide a good probe for comparing similar molecules. However, details about particular conformations such as turns can best be obtained by direct measurement of internuclear distances. Two-dimensional ROESY experiments yield cross peaks between protons closer than 5 Å in space; the closer the protons, the stronger the peak.

Figure 2 shows the α-to-amide and amide-to-amide regions of ROESY spectra from GDPFLRF-NH₂ and GYPFLRF-NH₂, which are representative of other DP and YP peptides. One of the most notable features in Figure 2 is the large difference in the amide region (bottom panels) between the two peptides. The ROESY spectrum of GDPFLRF-NH₂ has strong cross peaks between the amide protons of F4 and L5 and weaker peaks between L5 and R6. In contrast, GYPFLRF-NH₂ has no measurable amide-to-amide cross peaks except between F7 and the NH₂ group (not included in Fig. 2), a feature present in all of the dozens of amidated peptides that we have studied (our unpublished observations). The pattern of ROESY amide-to-amide cross peaks in GDPFLRF-NH₂ is consistent with a reverse turn (Dyson et al., 1988a); the lack of similar ROESY cross peaks in GYPFLRF-NH₂ indicates that it is extended or randomly oriented.

The α-to-amide region (Fig. 2, top panels) shows two main features. First, GYPFLRF-NH₂ has significant heterogeneity associated with the two forms (cis and trans) of the Y–P peptide bond. GDPFLRF-NH₂, on the other hand, is predominantly trans, with <10% of the population in the cis form. All peptides examined in this study had trans X-Pro peptide bonds as the major species, as evidenced by ROESY cross peaks between Pro-Hα and X-Hα or X-Hβ (Dyson et al., 1988a,b). We estimate that ~90% of the DP and 70% of the YP subgroups have trans X-Pro peptide bonds. Second, the chemical shift dispersion, a good indicator of structure, is greater in GDPFLRF-NH₂ than in GYPFLRF-NH₂. Interactions between amide protons of adjacent amino acids are particularly sensitive to secondary structure: amide-to-amide distances are large in extended structures but short in turns or helical structures. In contrast, the distance between an α proton of one amino acid and the amide proton of the next amino acid is characteristically short for extended structures but long in turns or helical structures (Wüthrich, 1986; Dyson et al., 1988a,b). Thus, the ratio of the intensity of ROESY amide-to-amide and α-to-amide cross peaks (I_NN/I_αN) can provide a measure of the percentage of compact structure (Bradley et al., 1990). Table 1 lists I_NN/I_αN values for interactions between F4 and L5 for all of the peptides in this study. The I_NN/I_αN values range from 0.0 for all of the YP peptides to >1.5 for the DP peptides. These numbers will be more thoroughly examined in Discussion.

All of the data presented to this point have been collected at 4°C to match experimental binding conditions (see Discussion). For two of the peptides (GDPFLRF-NH₂ and SDPFLRF-NH₂),...
we collected ROESY data at higher temperatures. To our surprise, the $I_{NN}/I_{AN}$ values became larger as the temperature increased, suggesting that the turn population increases at room temperature.

**pH dependence**

The pH dependence of the chemical shifts of titratable groups in peptides can provide important data about hydrogen bonding (Bundi and Wüthrich, 1979). Within the pH range investigated in this study (1.9–5.5), the only titratable group in the peptides of Table 1 is aspartic acid (D). Generally, the peptide C terminus is titratable, but each peptide in this study has a C-terminal amide because all of the FLPs are amidated in vivo. Amide, $\alpha$, and side-chain protons within aspartic or glutamic acids undergo intrinsic shifts that, in general, contain little conformational information. Protons outside of titratable amino acids often show little or no dependence on pH. However, protons that interact closely through hydrogen bonding with carboxylic acid side-chains will exhibit chemical shift changes as a function of pH and thus provide direct evidence of long-range interactions.

Figure 3 shows the pH dependence of the amide protons of GDPFLRF-NH$_2$, pQDPFLRF-NH$_2$, and DPFLRF-NH$_2$. The other XDPFLRF-NH$_2$ ($X = S, N$) peptides were similar to GDPFLRF-NH$_2$ and are not shown. Each peptide shows the characteristic upfield shift of the aspartic acid (D) H$\alpha$ (Bundi and Wüthrich, 1979) and very large downfield shifts of the amide protons of the phenylalanine after the proline. These data clearly demonstrate strong hydrogen bonding interactions between the carboxylate side-chain of aspartic acid and the amide proton of phenylalanine-4 (F4) in GDPFLRF-NH$_2$. The smaller magnitude...
of the shifts in DPFLRF-NH₂ suggests more moderate interactions or a lower population of the hydrogen-bonded conformation. The magnitude of the shifts of F₄ (F₃ in DPFLRF-NH₂) over the entire pH range for each peptide studied are shown in Table 1. Series of one-dimensional ¹H spectra are drawn from lowest to highest pH (bottom to top), with some of the values of pH indicated. Minor peaks in the spectra correspond to cis D–P peptide bonds. All data were collected at 4°C.

Figure 3. Amide regions of one-dimensional pH titrations for GDPFLRF-NH₂ (top), pQDPFLRF-NH₂ (middle), and DPFLRF-NH₂ (bottom). The total magnitudes (highest pH minus lowest pH) of the changes in the phenylalanine chemical shift for each peptide studied are shown in Table 1. Series of one-dimensional ¹H spectra are drawn from lowest to highest pH (bottom to top), with some of the values of pH indicated. Minor peaks in the spectra correspond to cis D–P peptide bonds. All data were collected at 4°C.

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On the basis of data from pH titrations of bovine pancreatic trypsin inhibitor, Bundi and Wüthrich (1979) estimated that a 100% population of an amide to carboxylate hydrogen bond will give rise to a 0.4 ppm downfield shift. Assuming linearity, they claimed that a 0.04 ppm downfield amide shift corresponds to a 10% hydrogen-bonded population. Those estimates translate into ~80% of GDPFLRF-NH₂ and SDPFLRF-NH₂ and 30% of DPFLRF-NH₂ in hydrogen-bonded conformations.

Conformational models
The NMR data are all consistent with significant populations of reverse turns in the DP subgroup and predominantly extended or “random” conformations in the YP subgroup. From the NMR data alone, we are unable to specify a particular type of turn. However, on the basis of the following facts, we are confident that the peptides in the DP subgroup form type I reverse turns. First,
aspartic acid and proline are very commonly found in the first and second positions, respectively, of type I turns (Wilmot and Thornton, 1988). Second, crystal structures of proteins with DP in the first two positions of a type I turn often reveal the aspartic acid side-chain hydrogen bonded to the amide proton of amino acid after the proline (Wilmot and Thornton, 1988). This hydrogen bonding is identical to what we observe through the pH titration data, with large downfield shifts of the amide protons of the phenylalanine after the proline.

Models that are consistent with the NMR data for both the DP and YP peptides are shown in Figure 4. The most populated conformation in the DP peptides is a turn, and the YP peptides do not have any well-defined structure. In addition, we see no well-defined structure in the entire “LRF-NH₂” C-terminal region in any of the peptides. The conserved C terminus is directly involved in receptor binding and must be able to fit into an active site, and our data suggest that this fit requires a flexible group.

**DISCUSSION**

**Receptor binding studies**

The peptides for this study were chosen because of previously published detailed physiological and ligand displacement studies of the same peptides on membrane preparations from the brains of the snail *Helix aspersa* (Payza, 1987; Payza et al., 1989). The receptor binding results most relevant to this study are shown in Table 2.

Table 2 shows that the peptides with the sequence XDPFLRFa (X = S, N, pQ) need between 3 and 20 times the concentration to displace a reporter molecule than FLRFa, PFLRFa, or DPFLRFa. These effects are not simply a result of a peptide that is too long, as illustrated by the high receptor affinity of pQYPFLRFa (almost 10 times greater than FLRFa). Nor can these results be easily understood in terms of unfavorable interactions with the receptor because, for example, the pQ at the N terminus of DPFLRFa lowers the receptor affinity by a factor of three, whereas pQ at the N terminus of daYPFLRFa has no effect on binding.

**Table 2. Summary of receptor binding studies in *Helix* brain**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>IC (_{50}) (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMRFa</td>
<td>0.53</td>
</tr>
<tr>
<td>FLRFa</td>
<td>1.3</td>
</tr>
<tr>
<td>PFLRFa</td>
<td>0.86</td>
</tr>
<tr>
<td>DPFLRFa</td>
<td>3.4</td>
</tr>
<tr>
<td>NDPFLRFa</td>
<td>9</td>
</tr>
<tr>
<td>SDPFLRFa</td>
<td>15</td>
</tr>
<tr>
<td>pQDPFLRFa</td>
<td>10</td>
</tr>
<tr>
<td>pQYPFLRFa</td>
<td>0.22</td>
</tr>
<tr>
<td>daYPFLRFa</td>
<td>0.20</td>
</tr>
</tbody>
</table>

*This table is composed from data in Table 2 of Payza et al. (1989).*  
*Abbreviations include “a” = NH₂, “pQ” = pyroglutamic acid, “da” = des-amo, “nL” = norleucine.  
*Concentration needed to displace 50% ¹²⁵I-da YPFLRFa from a *Helix* brain membrane preparation.*
Comparison of NMR and receptor binding experimental conditions

The receptor binding experiments were performed with assay buffer of 80 mM piperazine-n,N′-bis(2-hydroxypropanesulfonic acid), pH 7.9, 1% BSA (Payza, 1987; Payza et al., 1989). To minimize the degradation of peptides, the receptor binding assays were performed on ice at 0°C.

NMR measurements have several experimental constraints. First, for solution studies in aqueous samples, the temperature must be above freezing. We used 4°C for most of the NMR results presented in this report. Second, amide protons exchange too rapidly for observation around pH 8. The pH titrations shown above clearly demonstrate that at around pH 5.5, the aspartic acid side-chains are fully deprotonated; no further change in these or any other side-chains will take place as the pH is increased. The N-terminal amino group is the only part of the peptides that would still be affected by differences in pH between the binding and NMR data. These relationships are currently under investigation in modeling studies.

We present a number of IC50 predictions below to further test our model.

Comparison of NMR and receptor binding results

Figure 5 is a plot of the measured NMR parameters that are sensitive to turn population (\(I_{\text{N}}/I_{\text{NN}}\), and pH shifts from Table 1) versus the IC50 values measured for the same peptides (Payza, 1987; Payza et al., 1989). Both NMR parameters increase with increasing values of IC50. The simplest interpretation of the NMR data is a rapid two-state equilibrium between a reverse turn and fully extended structure. For the DP peptides, the equilibrium is shifted in varying amounts toward the turn. For the YP peptides and PFLRF-NH2, the equilibrium is nearly 100% in favor of the extended structure.

A simple two-state model will lead to a linear relationship between the percentage of turn and the magnitude of both \(I_{\text{N}}/I_{\text{NN}}\) and the pH shifts. Thus, we used a linear relationship to fit the NMR parameters to the IC50 data. The correlation coefficients (\(r^2\)) for the fits were quite good at 0.93 and 0.82 for the \(I_{\text{N}}/I_{\text{NN}}\) and pH data, respectively. Deviations from linearity could result from either more than two predominant conformations in the unbound state or from additional (or different) factors involved in the binding data, such as direct interactions (favorable or unfavorable) between the ligands and receptor. The effects of multiple conformations are currently under investigation in modeling studies.

We present a number of IC50 predictions below to further test our model.

Biological roles of conformational ensembles

The strong linear structure–function relationships in Figure 5 suggest that the observed IC50 data can best be explained by the conformation of the unbound peptides. Namely, peptides with high populations of turn displace radio-labeled ligands at higher concentrations, and the turns are preventing the peptides from interacting with the receptors in the binding assay. These rela-

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tionships suggest a simple modification to elementary ligand–receptor equations. In the simplest case of binding, a ligand \( L \) binds to a receptor \( R \) to form a ligand–receptor complex \( LR \) with an equilibrium dissociation constant \( K_{eq} \):

\[
L + R \rightleftharpoons LR.
\]

The fraction of bound receptors \( (P_B) \) for this simple equilibrium is:

\[
P_B = \frac{[L]}{[L] + K_{eq}}.
\]

The effect of our model is to reduce the active form of the ligand to \( L^A \), which in the current study represents peptides in extended or random conformations:

\[
L^A \rightleftharpoons L^R.
\]

The inactive form of the ligand, \( L^I \) (here representing molecules in a turn conformation), is not able to bind directly to the receptor, thus reducing the fraction of bound receptors:

\[
P_B = \frac{[L^A]}{[L^A] + K_{eq}}.
\]

It must be stressed that active or inactive forms of a ligand are defined with respect to a particular receptor.

The model and data described above apply to a single peptide ligand and a single receptor that binds the active ligand \( L^A \) conformation. However, receptor heterogeneity can play a large role in our model. As described above, the role of conformational ensembles of a single ligand interacting with a single receptor will lead to changes in the bound population of that receptor. If there is a second receptor that preferentially binds to the inactive form (defined with respect to the first receptor) of the same ligand, then more complicated signaling can occur. Depending on the degree of coupling between the two receptors, the resultant signals from the two different conformations of the peptide could be divergent or convergent, leading to increased “physiological flexibility” (Brezina et al., 1996).

Payza and coworkers found that the \textit{Helix} XDPFLRF-NH\(_2\) peptides have a complicated physiology. At concentrations <0.03 \( \mu \text{M} \) they are \( \sim 30–50 \) times more cardiostimulatory than FMRF-NH\(_2\), but at concentrations >0.03 \( \mu \text{M} \) they are cardioinhibitory (Payza, 1987). From these observations, Payza hypothesized that the XDPFLRF-NH\(_2\) peptides must interact with different or additional receptors. We showed above that the XDPFLRF-NH\(_2\) peptides have up to \( \sim 80\% \) reverse turn in solution. Therefore, if we assume that the XDPFLRF-NH\(_2\) peptides bind with the same affinity to both the stimulatory and inhibitory receptors, then our model would predict that stimulatory receptor preferentially binds the turn conformation (dominant conformation) and the inhibitory receptor preferentially binds the extended conformation (minor conformation). Clearly, more data are needed to prove this relationship.

There are currently two cloned FLP receptors: a peptide-gated sodium channel (Green et al., 1994; Linguegilla et al., 1995) and a G-protein-coupled receptor (Tensen et al., 1998). In addition to the two sequences, several other FLP receptors have been biochemically characterized (Payza, 1987; Payza et al., 1989; Chin et al., 1994; Wang et al., 1995a,b). All available evidence, therefore, points to the presence of multiple FLP receptors (Tensen et al., 1998). We hypothesize that the effect of conformational ensembles in differential binding to multiple receptors will be widespread.

Most of the focus in ligand–receptor interactions has naturally involved the ligand–receptor complex, and these direct contacts with receptors are obviously important. However, our data clearly show the importance of the unbound conformational state in ligand–receptor interactions. From our model we can make a number of predictions for future binding assays under the same conditions used by Payza and coworkers (1987, 1989). (1) On the basis of our NMR measurements (Table 1), GDPFLRF-NH\(_2\) and GYPFLRF-NH\(_2\) will have IC\(_{50}\) values of 13 \( \mu \text{M} \) and <1 \( \mu \text{M} \), respectively. (2) YDPFLRF-NH\(_2\) will have IC\(_{50}\) values near the other DP peptides (10–15 \( \mu \text{M} \)). (3) SDZPFLRF-NH\(_2\) (Z any amino acid except D, N, or P) will have IC\(_{50}\) values near the YP or tetrapeptides (<1 \( \mu \text{M} \)). (4) SNPFLRF-NH\(_2\) will have IC\(_{50}\) values near SDPFLRF-NH\(_2\) (15 \( \mu \text{M} \)), because asparagine (N) has hydrogen bonding properties and type I turn propensities (Wilmut and Thornton, 1988) similar to those of aspartic acid (D).

We have demonstrated that populations of reverse turns in a series of neuropeptides are inversely correlated with their receptor-binding affinities. This finding suggests that one of the roles for diverse N-terminal amino acid sequences in FLPs is to provide different unbound conformational ensembles and thus lead to different receptor binding affinities.

REFERENCES


