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Molecular Mechanism of Opioid Receptor Selection

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ABSTRACT: Preferred conformations, orientations, and accumulations of 26 opioid peptides on lipid membranes were estimated and compared with pharmacologic and selective binding data taken from the literature. Interaction with μ-receptors was governed by the net positive charge effective at the message domain of the agonist peptides z(eff) as the Boltzmann term $e^{z(\mathrm{eff})}$ that determines relative accumulation on anionic biologic membranes. Selection for δ-receptors was reduced by z(eff) and correlated with $e^{-z(\mathrm{eff})}$. Selection for κ-receptors was governed by the peptide amphiphilic moment $\vec{A}$. A pronounced scalar magnitude A and almost perpendicular orientation of the N-terminal message domain as an α-helix were favorable for κ-site selection. Potencies as κ-agonists and binding affinities correlated with $A \cdot e^{z(\mathrm{eff})}$. The classical site selectivity caused by the receptor requirements for a complementary fit of the agonist to the discriminator site is thus crucially supplemented by a selection mechanism based on peptide membrane interactions (membrane requirements). In the model presented here, the δ-site is exposed to the aqueous compartment surrounding the target cell at a distance comparable to or greater than the Debye–Hückel length and is in a cationic vicinity. The μ-site is exposed to the anionic fixed-charge compartment of the membrane in aqueous surroundings. The κ-site is buried in a more hydrophobic membrane compartment close to the fixed-charge compartment. The relative accumulation of the opioid message domains in these compartments is determined by the address domains and constitutes a major part of the site selection mechanism. The peptide amphiphilic moment, $\vec{A}$, emerged as a new, important parameter for predicting site selectivity and potency and determining peptide quantitative structure–activity relationships (QSAR).

Opioid peptides offer excellent examples for receptor selectivity (Kosterlitz & Paterson, 1985), an important phenomenon in nervous and endocrine regulation. Thus, [Leu]- and [Met]enkephalin prefer opioid δ-receptors, and β-endorphin reacts about equally well with μ- and δ-sites, while dynorphin A is a typical κ-agonist. Opioid peptides have a common N-terminal tetrapeptide sequence, Tyr-Gly-Gly-Phe-, which is the "message" segment triggering the receptor responses, but they have different C-terminal "address" segments, which are responsible for their receptor subtype preferences.

Although peptide structural features that determine site specificity ("receptor requirements") have been recognized in general (Schwyzer, 1963, 1977, 1980) and specific (Chavkin & Goldstein, 1981; Schiller, 1984, 1986; Schiller & DiMaio, 1982) terms, a unified molecular mechanism for site selection has not been advanced. Existing rules for predicting quantitative structure–activity relationships of drugs [QSAR;[1] see Hansch & Leo (1979)] are notoriously unsuccessful in the peptide field, especially for receptor selection.

The discovery of specific interactions between neuropeptides and lipid bilayer membranes (Schoch et al., 1979; Gysin & Schwyzer, 1983, 1984; Gremlich et al., 1983, 1984; Erne et al., 1985) offers a new approach to the problem. Whereas the concept of "receptor requirements" tacitly assumes free accessibility of the receptor recognition site from the aqueous phase surrounding the target cell, the membrane may play an important role as catalyst for peptide–receptor interactions by

[1] Abbreviations: dynorphin$_{1-n}$ and ACTH$_{1-n}$, dynorphin A and adrenocorticotropin peptides, respectively, comprising residues 1–n; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; QSAR, quantitative structure–activity relationships.

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serving as an antenna to capture peptides and as a device for the selection and proper orientation of the message segment, thus facilitating productive receptor interactions (Schwyzer et al., 1983; Schwyzer, 1985a,b; Sargent & Schwyzer, 1986).

The preferred conformation, orientation, and accumulation of dynorphin A-(1–13)-tridecapeptide (dynorphin$_{1-13}$) on the surface of neutral lipid membranes observed by Erne et al. (1985) can be estimated (Schwyzer, 1986) from the free energy of transfer of amino acid residues from an aqueous to a hydrophobic phase (Von Heijne & Blomberg, 1979; Von Heijne, 1981) on the premiss that the α-helix is the most stable conformation of peptide domains interacting with a membrane hydrophobic phase (Henderson, 1979). From such calculations, the peptide amphiphilic moment and the hydrophobic association constant emerged as the two parameters necessary and sufficient for describing the behavior of dynorphin$_{1-13}$ and ACTH$_{1-24}$ in contact with membranes prepared from 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC). In biological systems, a further concentrating effect will occur for positively charged peptides due to a Boltzmann distribution between the bulk aqueous phase and the negatively charged membrane surface (Sargent & Schwyzer, 1986).

Here, I estimated the interactions of 26 peptides with anionic lipid membranes and compared the parameters with pharmacologic and binding data taken from the literature (Chavkin & Goldstein, 1981; Kosterlitz & Paterson, 1985). The peptide amphiphilic moment (which measures the orientation on an aqueous–hydrophobic interphase boundary) and the Boltzmann accumulation on the membrane surface turned out to be the two principal determinants for opioid receptor selectivity and potency. The amphiphilic moment is a new, crucial parameter for peptide QSAR.

The results suggested a mechanism of receptor selection in which the selectivity caused by specific peptide–receptor binding through induced molecular complementarity ("receptor requirements") is supplemented by the selectivity caused by specific peptide–membrane interactions ("membrane requirements"). Such interactions lead to regioselective, conformation-selective, and orientation-selective partitioning of peptides into different membrane compartments.

A model is proposed in which the three receptor sites μ, δ, and κ are exposed to three different membrane compartments, into which the message domains of the corresponding agonists partition preferentially. The accumulation of the message domains in the three compartments is governed by the nature of the address domains through net charge contributions and influences on the amphiphilic character of the peptides.

Methods

Peptide Structure. Dynorphin$_{1-13}$, YGGFLRRIRPKLK, binds reversibly to artificial lecithin membranes from aqueous solutions with an apparent dissociation constant of 11 μM (10 mM KCl). The N-terminal nonapeptide segment folds into an α-helix, which is oriented perpendicularly on the membrane surface and contacts the hydrophobic layers. The C-terminal tripeptide segment remains in the aqueous phase as a random coil. The two domains are separated by the helix-breaking residue proline (Erne et al., 1985).

Three parameters are important for estimating conformation, orientation, and accumulation of peptides on a membrane surface (Schwyzer, 1986):

(*a*) *Hydrophobic Association.* The Gibbs free energy of hydrophobic association $\Delta G°_{ass}(m)$ through m residues at the more hydrophobic end of a peptide chain (here, the N-terminus) is calculated from the free energy of transfer $\Delta G°_{tr}(i)$ of the individual residues from their random-coil conformation

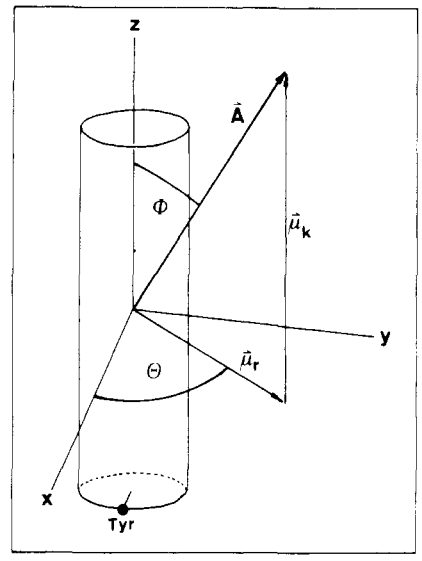

FIGURE 1: Definition of peptide amphiphilic moment, $\vec{A}$, and its component vectors perpendicular ($\vec{\mu}_r$) and parallel ($\vec{\mu}_k$) to the helix axis lying on the z axis of a right-handed spherical coordinate system. The N-terminal tyrosine has a negative z value, and its α-carbon atom points in the direction of the x axis. The helix is centered on the origin of the coordinate system.

in water to their helical conformation in a hydrophobic phase. The relations are

$$\Delta G°_{tr}(m) = \sum_{i=1}^{i=m} \Delta G°_{tr}(i) + \Delta G°_{tr}(\text{end}) \quad (1)$$

$$\Delta G°_{assoc}(m) = \Delta G°_{tr}(m) + \Delta G°_{t+r} \quad (2)$$

where $\Delta G°_{tr}$(end) accounts for unsatisfied H bonds at the helix ends and $\Delta G°_{t+r}$ is the free energy change caused by the loss of two degrees of rotational and one degree of translational freedom of the peptide bound to the membrane (Von Heijne & Blomberg, 1979). The hydrophobic association constant and the length of the helix, m, are determined from the position of the energy minimum obtained by plotting $\Delta G°_{assoc}(m)$ against m (Schwyzer, 1986).

(*b*) *Amphiphilic Moment.* Segregation of charged and uncharged amino acid residues into hydrophilic and hydrophobic domains endows peptides with an amphiphilic character. Such peptides will tend to accumulate on aqueous–hydrophobic interphase boundaries and orient themselves in the direction of minimum free energy. The segregation of hydrophobic and hydrophilic properties may be measured in analogy to the helical hydrophobic moment (Eisenberg et al., 1982) by the molecular amphiphilic moment (Schwyzer, 1986), Figure 1. In the helical hydrophobic moment, the direction vectors originate on the helix axis and are perpendicular to it, whereas in the amphiphilic moment the direction vectors originate from the center of the helix. The molecular amphiphilic moment is defined as

$$\vec{A} = \sum_{i=1}^{i=m} \Delta G°_{trh}(i)\vec{R}_i \quad (3)$$

where $\Delta G°_{trh}(i)$ is the signed numerical value of the Gibbs free energy change for the transfer of the ith residue in its helical conformation from water to a hydrophobic phase (Von Heijne 1981). End group effects (broken H bonds) were taken into account. However, helix dipole moments (that will tend to reduce A and increase Φ in the case of peptides with hydrophobic N-termini) have not yet been included in these calculations. $\vec{R}_i$ is the position vector from the helix center to the α-carbon of the ith residue measured in units of helix radius,

Table I: Association of Dynorphin A Peptides with Membranes[a]

peptide	C-terminal methyl esters			C-terminal free acids		
	$\Delta G°_{assoc}$	$\Delta G°_{Bol}$	K_d (M)	$\Delta G°_{assoc}$	$\Delta G°_{Bol}$	K_d (M)
YGGFLRRIRPKLK (**13**)	−28	−19	6 (−9)	−28	−15	3 (−8)
YGGFLRRIRPKL (**12**)	−28	−15	3 (−8)	−28	−12	1 (−7)
YGGFLRRIRPK (**11**)	−28	−15	3 (−8)	−28	−12	1 (−7)
YGGFLRRIRP (**10**)	−28	−12	1 (−7)	−28	−8	5 (−7)
YGGFLRRIR (**9**)	−28	−12	1 (−7)	−28	−8	5 (−7)
YGGFLRRI (**8**)	−12	−8	3 (−4)	−12	−4	2 (−3)
YGGFLRR (**7**)	7	−8	7 (−1)	7	−4	3 (0)
YGGFLR (**6**)	23	−4	2 (3)	23	0	1 (4)
YGGFL (**5**)	39	0	7 (6)	39	4	3 (7)

[a] $\Delta G°_{assoc}$ and $\Delta G°_{Bol}$ are the free energy changes in kJ/mol calculated for hydrophobic association (eq 2) and Boltzmann distribution (eq 4), respectively; for the molar dissociation constants, K_d, powers of 10 are shown in parentheses.

0.188 nm. Random-coil segments are assumed to exert their action at the helix end. The amphiphilic moment of a peptide located in a hydrophobic gradient produces a torque that tends to orient $\vec{A}$ perpendicular to the surfaces of equal hydrophobicity in the surrounding medium. The greater the scalar magnitude A, the less pronounced the thermal tumbling of the peptide molecules in the gradient. Procedures and considerations are given in detail for dynorphin$_{1-13}$ (Schwyzer, 1986).

(*c*) *Net Charge.* Charged peptides will be attracted or repulsed by the fixed-charge layer of the membrane surface according to a Boltzmann distribution:

$$c_x = c_o \exp[-zFV_{gc}/(RT)] \quad (4)$$

where c_x and c_o are the peptide concentrations on the surface and in the bulk phase, respectively, z is the peptide net charge, V_{gc} is the Gouy–Chapman fixed-charge potential, F is the Faraday constant, R is the universal gas constant, and T is the absolute temperature.

Biologic membranes usually contain excess negatively charged lipid [for review, see Robinson (1975)] in such an amount that we may assume a characteristic $V_{gc} = -40$ mV. The surface charge is located on the distal surface of the head group layer (distal with respect to the membrane core).

Positively charged peptides will accumulate in an aqueous compartment close to the fixed-charge layer. Negatively charged peptides will accumulate in the bulk aqueous compartment, whereas neutral peptides will be indifferent. The two compartments caused by the fixed-charge layer are characterized by the Debye–Hückel length:

$$l_D = [\epsilon_0 \epsilon RT/(2c_e)]^{1/2}/F \quad (5)$$

which is the distance x from the fixed-charge layer at which the electric potential $\phi(x)$ has decreased from its maximum ϕ_0 to ϕ_0/e. It depends on the dielectric constant ϵ of the phase (ϵ_0 is the electric field constant) and the electrolyte concentration c_e. At physiologic conditions (simulated by c_e about 0.1 M of a 1:1 electrolyte), l_D is about 1 nm or roughly 10 diameters of a water molecule, or the length of an α-helix composed of six to seven residues.

Comparison of Data. Relative values of individual parameters (V_i(rel) were obtained by comparing the individual value V_i with the value of a standard, V_{st}:

$$V_i(\text{rel}) = V_i/V_{st} \quad (6)$$

The results were in most cases equivalent with or not significantly different from those obtained by taking the lowest value in a series $V_{i=1}$ as a second reference:

$$V'_i(\text{rel}) = (V_i - V_{i=1})/(V_{st} - V_{i=1}) \quad (7)$$

Receptor Selectivity. Dynorphin receptor (κ-site) selectivity, Figures 2 and 3, was determined pharmacologically with the myenteric plexus-longitudinal muscle preparation of the guinea pig ileum (GPI) by Chavkin and Goldstein (1981). It is the reciprocal value of the antilogarithm of the mean of the differences in log IC_{50} in the presence and absence of 100 nM naloxone (reciprocal "potency shift" or "naloxone sensitivity").

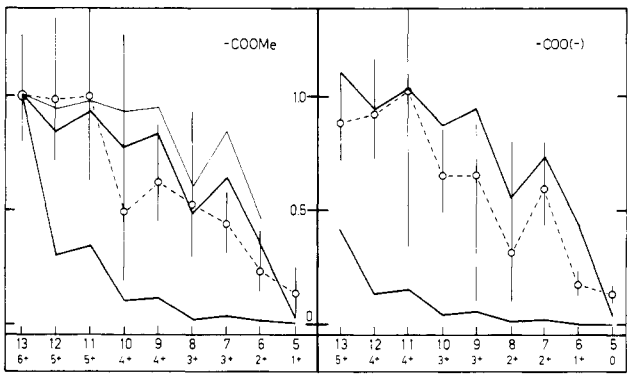

FIGURE 2: Amphiphilic moment ($\vec{A}_n$) and receptor selectivity in a series of N-terminal dynorphin fragments as COOH-terminal methyl esters (left panel) or free acids (right panel). (Abscissa) Number of amino acid residues (n) and net charge (z) of nine individual peptides. (Ordinate) Relative values of parameters normalized to dynorphin$_{1-13}$ methyl ester (n = 13, left panel). Reciprocal values of the potency shift caused by 100 nM naloxone in the guinea pig ileum myenteric plexus-longitudinal muscle bioassay (O, dashed line) with 95% confidence interval as determined by Chavkin and Goldstein (1981). Estimated relative values of A_n (upper heavy line) and $A_n e^z$ (lower heavy line). The uppermost light line (left panel) connects points indicating relative values of (cos Φ_n − cos 45°).

Relative binding affinities at the μ-, δ-, and κ-sites of guinea pig brain homogenates, Figures 4–6, are

$$K_i^{-1}(\text{for } \mu, \delta, \text{ or } \kappa)/[K_i^{-1}(\mu) + K_i^{-1}(\delta) + K_i^{-1}(\kappa)] \quad (8)$$

as defined by Kosterlitz and Paterson (1985). Site selectivity is measured by comparing the value of the relative binding affinity of the ith agonist for a given site with that of a reference agonist.

Results and Discussion

In their fundamental study of the structural features of dynorphin A that are responsible for high potency and pronounced receptor selectivity in the GPI assay, Chavkin and Goldstein (1981) used the potency shift caused by naloxone (naloxone sensitivity) to distinguish actions on the dynorphin receptor (κ-site: small shift) from those on other receptors (notably μ-sites: large shift). They examined two series of peptides (methyl esters and free carboxylates) obtained by sequential removal of C-terminal residues from dynorphin$_{1-13}$ down to dynorphin$_{1-5}$ ([Leu]enkephalin). The first part of this paper is concerned with the same 18 peptides. The results are shown in Tables I and II and Figures 2 and 3.

Table I shows the membrane association calculated on the premise that peptide segments associated with membranes

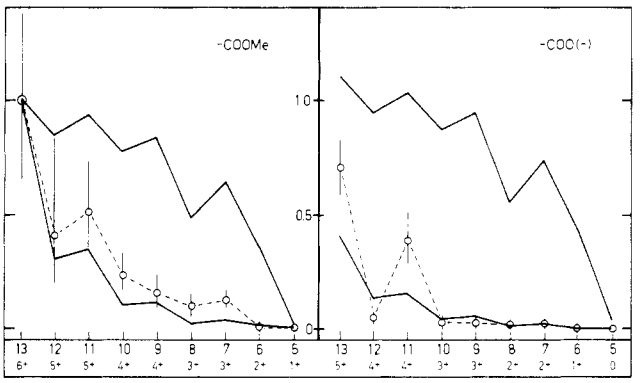

FIGURE 3: Amphiphilic moment ($\vec{A}_n$) and biologic potency in a series of N-terminal dynorphin A fragments as COOH-terminal methyl esters (left panel) or free acids (right panel). (Abscissa) Number of amino acid residues (n) and net charge (z) of nine individual peptides. (Ordinate) Relative values of parameters normalized to dynorphin$_{1-13}$ methyl ester (n = 13, left panel). Biologic potency in the guinea pig ileum myenteric plexus-longitudinal muscle bioassay (O, dashed line) with 95% confidence interval as determined by Chavkin and Goldstein (1981). Estimated relative values of A_n (upper heavy line) and $A_n e^z$ (lower heavy line).

Table II: Amphiphilic Moments of Dynorphin A Peptides

	C-terminal methyl esters			C-terminal free acids		
peptide	θ (deg)	A	Φ (deg)	θ (deg)	A	Φ (deg)
YGGFLRRIRPKLK (**13**)	144	332	14	132	400	13
YGGFLRRIRPKL (**12**)	144	278	17	132	344	15
YGGFLRRIRPK (**11**)	144	309	15	132	375	14
YGGFLRRIRP (**10**)	144	256	18	132	321	16
YGGFLRRIR (**9**)	144	277	17	132	343	16
YGGFLRRI (**8**)	188	158	30	198	204	18
YGGFLRR (**7**)	186	214	22	196	265	21
YGGFLR (**6**)	138	115	34	138	160	32
YGGFL (**5**)	136	8	159	48	32	39

assume helical structures (Henderson, 1979). Hydrophobic association through N-terminal segments increased from the pentapeptides to the nonapeptides, where the energy minimum and the maximal length of the helix nine residues) was reached (Schwyzer, 1986). The calculated energy minimum corresponded to that observed experimentally for the association between dynorphin$_{1-13}$ and neutral POPC membranes (−27.6 kJ/mol; Erne et al., 1985). When entering the membrane, the N-terminal amino group must lose its positive charge by deprotonation (Von Heijne & Blomberg, 1979). This was taken into account in the estimation of the Boltzmann distribution. The stepwise increase in effective net charge of peptides **5–13** determined the stepwise K_d decrease between peptides **9–13** and appreciably modified that between peptides **6–9**. Thus, the relative membrane affinities are dominated by the Coulomb term e^z of a linear combination of eq 1 and 3.

Scalar magnitudes A_n and angles Φ_n of the peptide amphiphilic moments (Table II) did not change monotonously with chain length (n) but were influenced by the nature of the C-terminal residues. Thus, C-terminal free carboxy groups increased A and decreased Φ more than methyl esters because of their greater hydrophilicity. Hydrophobic residues reduced A and increased Φ. Hence, the peptides **8**, **10**, and **12** had smaller A and greater Φ values than the preceding and following members. In this series of peptides, large values of A implied strong orientation of the helix axis almost normal (perpendicular) to the membrane surface as observed for dynorphin$_{1-13}$ (Erne et al., 1985).

The results of Tables I and II indicate that the pentapeptides do not accumulate and cannot become oriented on an anionic interface to adopt helical structures. This agrees with the weak enkephalin–membrane association observed by NMR and labeling methods (Bleich et al., 1976; Jarrell et al., 1980; Gysin & Schwyzer, 1983; Deber & Behnam, 1984, 1985). Interaction of the hexapeptide **6** and heptapeptide **7** free carboxylates with liposomes was shown by Dr. D. Erne in my laboratory to be weak but increasingly stronger than that of [Leu]enkephalin, as predicted here (unpublished results). It appears that A values of more than 100–150 arbitrary units and Φ values less than 30° to 35° are necessary to suppress molecular tumbling and to allow the development of significant hydrophobic membrane interactions. Methyl esters **7** and **8** and free acid **8** may associate strongly enough with membranes to convert three-dimensional receptor search to a two-dimensional process (Adam & Delbrück, 1968; Berg & Purcell, 1977). According to the adopted model of estimation, association of the longer peptides with biologic membranes may become very strong and approach that of observed peptide–receptor interactions to within a few orders of magnitude [Kosterlitz & Paterson, 1985; see Sargent & Schwyzer (1986) for a discussion in terms of membrane catalysis].

Figures 2 and 3 compare A and Ae^z with reciprocal naloxone sensitivity and with potency. All values are relative to dynorphin$_{1-13}$ methyl ester. A rather good general correlation of receptor selectivity with A and cos Φ and of potency with Ae^z was observed. The use of dynorphin$_{1-13}$ methyl ester as reference was arbitrary and perhaps not the best choice because of its large 95% confidence interval in both bioassays and relatively great uncertainties in the estimation of A_{13}. I did not attempt to optimize the fit, however, as it was sufficiently clear that the variations of A (low values of A_8, A_{10}, and A_{12}) and of Φ were reflected in the pharmacologic data. Moreover, receptor selectivity and A values both show a convex profile, whereas the profiles of the potency data and Ae^z are exponentially concave. Receptor selectivity did not follow exp(A), nor did potency follow e^z alone.

These findings suggested that κ-receptor selection is determined by the tendency of dynorphin peptide message domains to assume perpendicularly oriented ($\Phi \leq 30°$) α-helical structures on the membrane. This view is supported by the fact that replacement of Gly-2 by D-Ala-2, which impairs helix formation, increases naloxone sensitivity in dynorphin$_{1-13}$ methyl ester, dynorphin$_{1-11}$, and dynorphin$_{1-7}$ (Chavkin & Goldstein, 1981). It was not astonishing to find relative potency to correlate with Ae^z. Pharmacologic potency depends on agonist concentration in the vicinity of the receptor binding sites, and relative values of e^z determine the relative concentrations on the anionic membrane surface of peptide molecules exhibiting κ-selectivity, a function of A.

GPI potency, however, results from the combined stimulation of κ- and μ-receptors present in the preparation (Kosterlitz & Paterson, 1985). While κ-sites prefer the N-terminal message segment in their α-helical, oriented conformation, μ-sites must prefer another structure (β-turn? Schiller, 1984, 1986) without membrane interaction. This view is supported by the observations of Chavkin & Goldstein (1981) that replacement of Gly-2 by D-Ala-2 (which impairs κ-selectivity) leaves the potencies of dynorphin$_{1-10}$ and dynorphin$_{1-7}$ unchanged while increasing the potencies of dynorphin$_{1-6}$ and [Leu]enkephalin to about the same level as the former. Substitution by D-Ala-2 apparently shifts the site specificity of the deca- and heptapeptides from $\kappa > \mu$ to $\kappa < \mu$ by changing

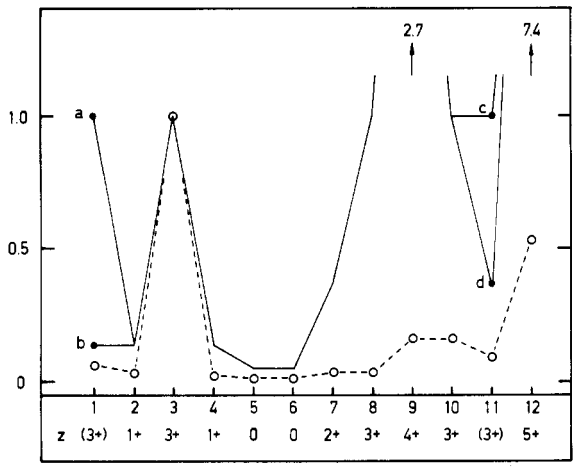

FIGURE 4: Net charge (z) and μ-site binding affinity of 12 peptides with different principal site affinities. (Abscissa) (**1**) H-β-endorphin (N-terminal sequence is YGGFMTSEKSQTP...); (**2**) YGGFMRF; (**3**) YGGFMRRV-amide; (**4**) YGGFMRGL; (**5**) [Met]enkephalin, YGGFM; (**6**) [Leu]enkephalin, YGGFL; (**7**) YGGFLRRI; (**8**) YGGFLRRIR; (**9**) dynorphin A, YGGFLRRIRPKLKWDNQ; (**10**) dynorphin B, YGGFLRRQFKVVT; (**11**) α-neoendorphin, YGGFLRKYPK; (**12**) dynorphin A-(1–13)-tridecapeptide, YGGFLRRIRPKLK. (Numbers **7**, **8**, and **12** correspond to **8**, **9**, and **13**, respectively, on the right panels of Figures 2 and 3.) (Ordinate) Binding affinities at the μ-site in homogenates of guinea pig brain at 0 °C (Kosterlitz & Paterson, 1985; dashed line) and values of e^z (solid line), both relative to metorphamide (**3**). Points a and b for peptide **1** (H-β-endorphin) are for z = 3+ and z(eff) = 1+; points c and d for peptide **11** (α-neoendorphin) are for z = 3+ and 2+, respectively (explanation see text).

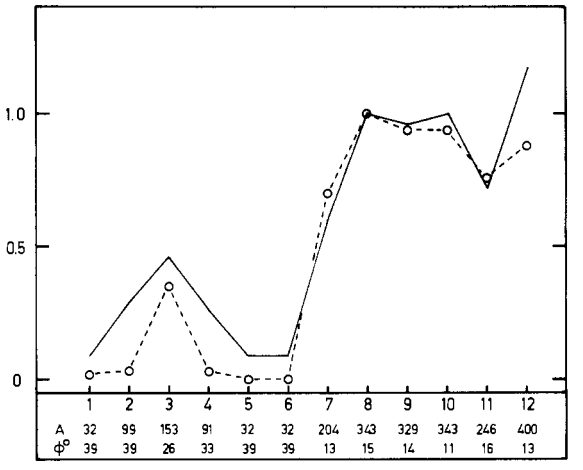

FIGURE 5: Amphiphilic moment ($\bar{A}$) and κ-site binding selectivity of 12 peptides with different principal site affinities. (Abscissa) See legend to Figure 4. (Ordinate) Relative binding affinities (eq 8) at the κ-site in homogenates of guinea pig brain at 0 °C (Kosterlitz & Paterson, 1985; dashed line) and values of A (solid line), both relative to dynorphin A-(1–9)-nonapeptide (**8**).

their preferred conformation from α-helix to β-turn. The potency increase in the shorter peptides was expected from earlier work with [D-Ala^2]enkephalins (Coy et al., 1976; Pert & Pert, 1976) and with cyclic analogues (Schiller et al., 1985).

These views were confirmed and extended to δ-site by correlations between net charge, amphiphilic moment, and receptor binding. In their fundamental studies of opioid receptor subtypes, Kosterlitz and Paterson (1985) developed methods for measuring quantitatively the specific binding of peptides and drugs to opioid μ-, δ-, and κ-receptors in guinea pig brain homogenates. They report the relative binding affinities, eq 8, for a number of agonists and antagonists. I examined the 12 peptides consisting of natural amino acids listed by these authors.

These peptides are characterized by a large range of principal site affinities, K_i^{-1}, and binding site selectivities, eq 8. Thus, the octapeptide amide [Met]enkephalyl-Arg-Arg-Val-NH_2 (**3**; metorphamide; adrenorphin) is one of the most potent open-chain μ-agonists known. Its affinity at this site is about 33 times that of [Met]enkephalyl-Arg-Phe (**2**) or 18 times that of β-endorphin (**1**), two other agonists with principal μ-site affinity. The relative μ-site affinities of peptides **1**–**6**, Figure 4, correlate with e^z as expected from the discussion of Figure 3. β-Endorphin (**1**), however, only fits into the picture if we assume a single effective charge (1+) on the message domain (point b in Figure 4) instead of 3+ (point a). This is justified by the fact that the positive charges on Lys-19 and Lys-24 are separated from the message by 15–20 residues, including those of the flexible "hinge region", residues 6–13, that prevents helix formation and is so important for biologic activity (Kaiser & Kézdi, 1983). Thus, the only charge effective at the message is that on the amino group of Tyr-1, because the next in line, Lys-9, is neutralized by Glu-8.

Figure 4 also shows the μ-site affinities of peptides with principal δ-site affinity. The two enkephalins, **5** and **6**, are practically devoid of μ-affinity (1% of **3**, but peptide **4** ([Met]enkephalyl-Arg-Gly-Leu) is somewhat stronger (2% of **3**). Their μ-affinities correlate reasonably well with e^z.

The μ-affinities of the peptides with principal κ-site affinity, **7**–**12**, Figure 4, hardly correlate with e^z, although a common trend is indicated The relative μ-site affinities of dynorphin$_{1-8}$ (**7**), dynorphin$_{1-9}$ (**8**), dynorphin A (**9**), dynorphin B (**10**), α-neoendorphin (**11**), and dynorphin$_{1-13}$ (**12**) increase and decrease in line with e^z, but much less strongly. My explanation is based on the conclusions drawn from Figures 2 and 3: a strong amphiphilic moment (see Figure 5) shifts the affinity from μ to κ. Hence, although these peptides are accumulated on the membrane according to e^z, they react preferentially with κ-receptors because of their induced helicity and perpendicular orientation. Another, perhaps minor, factor that must be considered when explaining the particularly great differences between e^z and μ-site affinity of dynorphin A (**9**) and dynorphin$_{1-13}$ (**12**) may be the distance between the message domain and the positive charges near the C-terminus. These charges may not contribute fully to the accumulation of the message domains on the fixed-charge layer, as explained for β-endorphin. Furthermore, in the case of α-neoendorphin (**11**), the net charge of 3+ (point c) may be reduced by partial deprotonation of the Tyr OH group next to Lys-7. Point d indicates the effect of a maximal reduction to net charge 2+.

Figure 5 illustrates the correlation between κ-site binding selectivity and calculated values of A and Φ, the principal determinants of the amphiphilic moment (cos Φ not plotted; θ not relevant for receptor selection). The correlation may be considered as excellent if we take into account that A values smaller than about 100–150 arbitrary units (see above) are without significance. This would mean that the effective A values of β-endorphin (**1**), [Met]enkephalyl-Arg-Phe (**2**), [Met]enkephalyl-Arg-Gly-Leu (**4**), [Met]enkephalin (**5**), and [Leu]enkephalin (**6**) would be practically nil and coincide with observed κ-selectivity (0.02, 0.03, 0.03, 0, and 0, respectively). As mentioned for Figures 2 and 3, the A value of dynorphin$_{1-13}$ (**12**) may be slightly overestimated. Figure 5 strongly supports the view that the peptide amphiphilic moment is a decisive property determining κ-selection.

Figure 6, finally, illustrates the correlation between δ-site binding selectivity and the reciprocal value of e^z. Again, the correlation is excellent, provided the same two corrections are made for β-endorphin (**1**) and α-neoendorphin (**11**) as in Figure 4. Point b is e^{-z} for one effective charge on the N-

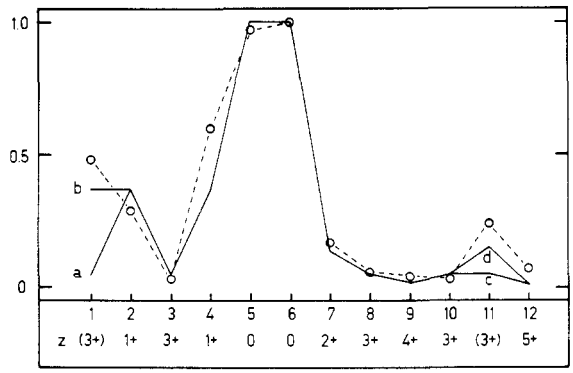

FIGURE 6: Net charge (z) and δ-site binding selectivity of 12 peptides with different principal site affinities. (Abscissa) See legend to Figure 4. (Ordinate) Relative binding affinities (eq 8) at the δ-site in guinea pig brain homogenates at 0 °C (Kosterlitz & Paterson, 1985; dashed line) and values of e^{-z} (solid line), both relative to [Leu]enkephalin (**6**). Points a and b for peptide **1** (H-β-endorphin) are for z = 3+ and z(eff) = 1+; points c and d for peptide **11** (α-neoendorphin) are for z = 3+ and 2+, respectively (for explanation, see text).

terminal message segment of β-endorphin, and point d is e^{-z} for two effective charges on α-neoendorphin (assuming deprotonation of the Tyr-8 OH group). The consistency of Figures 4 and 6 in this respect provides convincing evidence for the existence of an effective net charge that is smaller than the total net charge.

The observed correlation of δ-selectivity with $e^{-z(\text{eff})}$ and the lack of correlation with amphiphilicity indicated that the δ-receptor is exposed to an aqueous compartment that is less accessible to positively charged than to neutral agonists. This may be due to a local change in the Gouy–Chapman potential, eq 4, caused by positive charges connected with the δ-receptors. Such phenomena were postulated and observed for the vicinity of membrane-bound melittin (Schoch & Sargent, 1980). Effects of positive charges on an anionic membrane may be considerably enhanced if the charged site of the receptor extends into the aqueous phase to a distance comparable with or greater than the Debye–Hückel length, l_D, eq 5. This is not impossible, as the discriminator sites of the nicotinic acetylcholine receptor are known to be located at a distance of about 4 nm distal of the lipid head groups of the membrane (Klymkowsky & Stroud, 1979; Conti-Tronconi & Raftery, 1982). A somewhat shorter distance, about $3l_D$, may easily be bridged by β-endorphin in its conformation postulated by Kaiser and Kézdi (1983): its address positive charges could remain in contact with the fixed-charge compartment, and its message domain could reach the δ-site. An alternative δ-compartment would be the surface of a separate "δ-cell" with a positive Gouy–Chapman potential. This situation would imply that enkephalins with a net negative charge would be more selective and potent δ-agonists than the natural peptides with z = 0, which is amenable to experiment.

Conclusions

According to this study, selective interaction of peptides with opioid μ-, δ-, and κ-sites is mediated by specific peptide–membrane interactions. Thus, the molecular mechanism of opioid receptor selection is based both on *receptor requirements* and on *membrane requirements*. Receptor requirements are defined by a list of peptide structural features necessary for productive (Franklin, 1980) binding to the discriminator site of a particular receptor. Membrane requirements are defined by a list of peptide structural features necessary for productive membrane binding. Productive membrane binding means a membrane interaction specifically facilitating productive

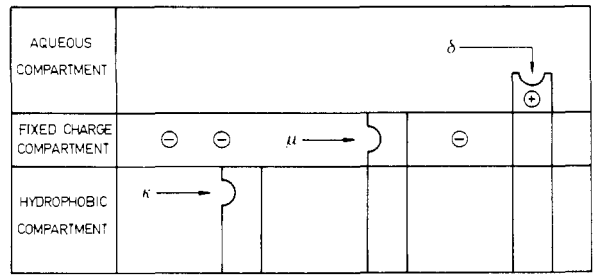

FIGURE 7: Membrane-assisted molecular mechanism of opioid receptor selection (membrane requirements).

binding of a peptide to a particular discriminator site (Schwyzer et al., 1983; Schwyzer, 1983, 1985).

Receptor requirements have been established over the past 10 years and extensively reviewed [e.g., Chavkin & Goldstein (1981), Kosterlitz & Paterson (1985), Mosberg et al. (1983), Schiller (1984, 1986), Schiller & DiMaio (1982), and Schiller et al. (1985)]. The requirement of μ-receptors for an opioid message domain that is able to adapt itself to the discriminator site in a folded conformation that may be loosely described as a β-turn is not contradicted by the present study. This applies also to the δ-site requirement for a quite different message conformation of the bound peptide. However, a hitherto unknown requirement of the κ-site for an α-helical structure of the message oriented perpendicularly on the membrane surface became apparent. This does not exclude additional requirements for basic residues, as postulated by Chavkin and Goldstein (1981).

According to the present study, *membrane requirements for opioid receptor selection* involve mainly effective charge and amphiphilic moment of the opioid peptides. They may be summarized as follows:

(1) Interaction with μ-receptors measured with pharmacologic potency, $1/IC_{50}$, and binding affinity, K_i^{-1}, is governed by the net positive charge effective at the message domain (effective charge). The charge function that correlates with μ-potency and affinity is $e^{z(\text{eff})}$.

(2) Selection for δ-receptors is reduced by the effective positive charge. Correlation is with $e^{-z(\text{eff})}$. (Whether introduction of $z < 0$ enhances δ-selection and potency remains to be seen.)

(3) Selection for κ-receptors, determined pharmacologically and with binding affinity, is governed by the peptide amphiphilic moment, $\vec{A}$. A pronounced scalar magnitude, A, and a small angle, Φ, are favorable for κ-site selection. Potency as κ-agonist and binding affinity to κ-sites, however, correlate with $A \cdot e^{z(\text{eff})}$.

With these membrane requirements in mind, a model for *membrane-assisted opioid receptor selection* may be constructed, Figure 7.

A peptide approaching a membrane surface by diffusion will be attracted or repulsed by the fixed-charge layer of the membrane according to a Boltzmann distribution, eq 4. Provided all other parameters are kept constant, the e^z term (z = net charge) measures the relative accumulation, c_x/c_o, of different peptides, and the $e^{z(\text{eff})}$ term measures the relative accumulation of the message domains, c'_x/c_o, at this layer. Any receptor that has its discriminator site exposed in a membrane compartment close to the fixed-charge layer ("fixed-charge compartment") will experience a local message concentration that is c'_x/c_o times that in the bulk aqueous phase. Hence, the potency, $1/IC_{50}$, and the binding affinity, K_i^{-1}, measured with respect to bulk concentration, c_o, will change by the same factor.

I conclude that the μ-sites are exposed to the anionic fixed-charge compartment on the target cell membranes, because their occupancy is enhanced by increasing effective charge of the peptides. This μ-compartment must be aqueous, because orientation of the message at the interphase boundary into the proximal, more hydrophobic compartments strongly reduces μ-potency. The productive conformation of the peptide must be imposed directly upon the peptide by the receptor.

κ-Sites experience the same electrostatic peptide accumulation as the μ-sites, but they are able to react only with a message inserted into the more hydrophobic compartment as a perpendicularly oriented α-helix. The orientation of the individual peptides in the κ-compartment, and their site selectivity, is determined by A and Φ. Relative numbers of oriented peptides and relative receptor occupancy are determined by $A \cdot e^{z(\mathrm{eff})}$. I conclude that κ-sites are exposed to a relatively hydrophobic compartment close to, and proximal of, the anionic fixed-charge layer of the target cell membrane.

δ-Sites are exposed to a positively charged, aqueous compartment. The change of the Gouy–Chapman potential from negative to positive values may be local, only in the vicinity of the discriminator sites. Such local changes have been postulated and demonstrated by Schoch and Sargent (1980). A plausible situation with local positive V_{gc} is shown in Figure 7. The δ-discriminator site is located on a slightly positively charged receptor protein at a distance from the fixed-charge compartment that is greater than the Debye–Hückel length, l_D. In such a situation, the β-endorphin molecule could simultaneously interact with the membrane through its address (Kaiser & Kézdi 1983) and with the δ-site through its message, according to the effective charge on the message.

In this model, the membrane screens peptides for their ability to interact with different membrane compartments by making use of their effective net charge and amphiphilic moment. After partitioning into the appropriate compartment(s), the peptide interacts with the receptor subsites exposed to this compartment. Thus, membrane requirements and receptor requirements combine to a very effective molecular mechanism of receptor selection. This mechanism explains inter alia why highly selective κ-agonists have not yet been found that do not display μ-activity. It offers a simple explanation for the well-known observation that enkephalin amides (1+ charge) are selective for μ-sites, whereas enkephalins with a free C-terminal carboxy group (neutral) show preference for δ-sites. It is quite possible that similar selection mechanisms apply to other regulatory peptides as well and that parameters for peptide–membrane interaction will provide a basis for prediction of receptor selectivity and potency.

Registry No. YGGFLRRIRPKLK (methyl ester), 76617-72-6; YGGFLRRIRPKLK, 72957-38-1; YGGFLRRIRPKL (methyl ester), 79985-33-4; YGGFLRRIRPKL, 79985-35-6; YGGFLRRIRPK (methyl ester), 79985-36-7; YGGFLRRIRPK, 79985-34-5; YGGFLRRIRP (methyl ester), 79985-37-8; YGGFLRRIRP, 79994-24-4; YGGFLRRIR (methyl ester), 79994-22-2; YGGFLRRIR, 77259-54-2; YGGFLRRI (methyl ester), 79985-38-9; YGGFLRRI, 75790-53-3; YGGFLRR (methyl ester), 79994-23-3; YGGFLRR, 77101-32-7; YGGFLR (methyl ester), 79985-39-0; YGGFLR, 75106-70-6; YGGFL (methyl ester), 63441-70-3; YGGFL, 58822-25-6; YGGFMRF, 73024-95-0; YGGFMRRV (amide), 88377-68-8; YGGFMRGL, 80501-44-6; YGGFLRRIRPKLK-WDNQ, 80448-90-4; YGGFLRRQFKVVT, 83335-41-5; YGGFLRKYPK, 77739-20-9; POPC, 26853-31-6; β-endorphin, 61214-51-5; [Met]enkephalin, 58569-55-4.

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Conformational Transitions in N-Linked Oligosaccharides†

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ABSTRACT: An assignment strategy involving ^1H–^1H correlated spectroscopy (COSY), relayed correlation spectroscopy (RECSY), nuclear Overhauser effect spectroscopy (NOESY), and triple quantum filtered correlated spectroscopy (TQCOSY) is described for six related N-linked oligosaccharides. These are of three "types", i.e., complex, bisected complex, and oligomannose. Using spin–spin coupling constant data derived from these assignments, together with semiempirical quantum mechanical energy calculations, we have examined the rotamer distributions at the Manα1–6Manβ– linkage in each structure, and additionally at the Manα1–6Manα– linkage in oligomannose oligosaccharides. We show that while several primary sequence differences are "passive", certain key residues modulate the orientation of the α1–6 arms. These residues may be proximal or distal to the site of the conformational change. There is no direct correlation between these perturbations and the oligosaccharide type. These data are discussed in terms of the proposed recognition function of oligosaccharides in biological systems.

The role of oligosaccharides as modulators of cell–cell recognition has been implicated in a variety of systems (Ivatt, 1984). Oligosaccharides in secreted and cell-surface glycoproteins (Ivatt, 1984; Fukuda & Fukuda, 1984) and cell-surface glycolipids (Feizi, 1981) are known to change during developmental and transformational events. This implies that the precise spatial and temporal disposition of a cell is communicated to the external milieu by oligosaccharide-dependent recognition phenomena. Studies on the conformational properties of oligosaccharides may lead to a better understanding of the molecular basis of these events. In principle, small differences in oligosaccharide primary sequence could lead to new conformational determinants, and two mechanisms can be envisaged whereby this might occur.

In the first mechanism, the addition of a monosaccharide residue generates a new determinant and masks others (or conversely, a deletion results in the loss of a determinant and the reexposure of masked residues). No significant conformational change need occur in the overall structure. This mechanism is known to exist in blood group determinants (Szulman, 1980) and in glycolipid differentiation antigens (Feizi, 1981). The second mechanism involves the addition or deletion of a monosaccharide which may lead to a conformational rearrangement of preexisting determinants either adjoining or distal to the added or deleted residue. This could create a totally new overall conformation which may form a new determinant not involving the added monosaccharide. The nascent ligand generated by the second mechanism would be difficult to predict from the primary sequence.

The determination of the three-dimensional structures of oligosaccharides with related but distinct primary sequence is a first step in studying the above events, thereby leading to a better understanding of the proposed roles of oligosaccharides not only as recognition signals but also as possible structural elements. At present, the only method with which to determine oligosaccharide solution conformations with accuracy is high-resolution NMR.[1] Our early one- and two-dimensional ^1H NMR studies (Homans et al., 1982, 1983a) showed that the biantennary *oligosaccharide* derived from human serotransferrin exists in solution with regions of defined secondary structure. Subsequently, Brisson and Carver have investigated the solution conformations of a variety of *glycopeptides* (Brisson & Carver, 1983a,b) and found that conformational variance was primarily associated with the Manα1–6Manβ1– linkage of the core. More specifically, the rotamer distributions about the C5–C6 bond of the Manβ1–residue were found to depend upon the oligosaccharide "class" (i.e., oligomannose, complex, bisected complex, and hybrid). An important consequence of such structural changes is that the *overall* solution

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[1] Abbreviations: NMR, nuclear magnetic resonance; COSY, ^1H–^1H correlated spectroscopy; TQCOSY, triple quantum filtered ^1H–^1H correlated spectroscopy; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; 1D, one dimensional; 2D, two dimensional; MNDO, modified neglect of diatomic differential overlap; RECSY, multiple-step relayed correlation spectroscopy.

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