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# Conformation and orientation of regulatory peptides on lipid membranes

## Key to the molecular mechanism of receptor selection

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The reaction of regulatory peptides with their membrane-bound receptors often occurs via a membrane-associated state of the peptide. From infrared studies on thin lipid films, we have shown that several ligands of the opioid $\kappa$ receptor and the neurokinin NK-1 receptor insert their message segments as an $\alpha$-helix, more or less perpendicularly, into the membrane. The binding parameters for these membrane-associated states were determined from the capacitance minimization potential of lipid bilayers. A theory has been developed to account for the observed binding constants and the preferred conformation and orientation of these peptides. In contrast to the $\kappa$ and NK-1 receptors, ligands of the opioid $\mu$ and $\delta$, and the neurokinin NK-2 and NK-3 receptors, are predicted not to form the inserted $\alpha$-helical structure. A selection between the $\mu$ and $\delta$ (or NK-2 and NK-3) receptors appears to be made on the basis of an electrostatic gradient near the membrane surface. The molecular mechanism of receptor selection thus appears to be based to a large extent on the membrane-induced compartmentalization of ligands for the different receptors.

## 1. Introduction

Regulatory peptides are soluble molecules which induce or modify cellular responses. The details of the full mechanism by which such effects are elicited have yet to be unravelled, but the preliminary stages involve interactions with membrane-bound receptors. Thus, at first glance, the initial step – the binding of the regulatory peptide to its receptor – appears to represent a *heterogeneous reaction* between the soluble peptide and the membrane-bound receptor. However, since the pioneering work of Adam and Delbrück [1] and Berg and Purcell [2] on the influence of surfaces on heterogeneous reactions, it has become apparent that a *direct* reaction between free peptide and bound receptor is probably a rare event. Given the large area of the membrane as compared to the receptor itself, and the physics of two-dimensional as opposed to three-dimensional diffusion, there is an overwhelming likelihood that the peptide will encounter the membrane before it reacts with the receptor. In many instances, the peptide becomes adsorbed to the membrane/solution interface and this adsorbed form then interacts with the receptor.

We have been looking into these initial peptide-membrane interactions with the help of several model systems [3–5], and the work has revealed the importance of the lipid phase in various aspects of the overall peptide-receptor interaction. Based on our results with pure lipid membranes, it has become clear that the membrane phase acts to potentiate receptor binding and to increase reaction rates [6]. It also con-

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Abbreviations: $ACTH_{1-24}$, adrenocorticotropin-(1–24)-tetracosapeptide; $dynorphin_{1-13}$, dynorphin A-(1–13)-tridecapeptide.

tributes to the binding specificity of the peptides by inducing specific conformations and orientations, and by establishing functional compartmentalization in the membrane/solution interphase region [7,8]. Thus, in such systems, we view the lipid membrane as a codeterminant of the overall biological properties of the ligand-receptor system and the key to the molecular mechanism of receptor selection.

## 2. Methods

Our experimental results are based, to a large extent, on methods especially suitable for studying molecules at interfaces or in membranes. These include photolabelling from the lipid core of vesicle membranes with a hydrophobic label [4], infrared studies of thin films [8,9], and the measurement of the capacitance minimization potential of planar lipid bilayers. The infrared measurements on oriented lipid films provide detailed structural information. The capacitance minimization technique yields thermodynamic parameters, such as the dissociation constant and maximal binding density. As this method is less well-known, we shall introduce it quickly here.

### 2.1. Capacitance minimization potential

This method, developed in our laboratory in 1979 [10], is based on the change in surface potential of a planar lipid bilayer caused by the binding of a substance bearing either an electric charge or dipole moment. The change is monitored using the voltage dependence of the electrical capacitance of the bilayer, which is a minimum at zero transmembrane potential (fig. 1A). As this potential depends on both the externally applied potential and the surface potentials on the two sides of the bilayer, a change in surface potential caused by the binding of an electrically active substance can be compensated by the applied potential, hence the name "capacitance minimization potential'.

The capacitance minimization potential, $V_{Cmin}$, is given quantitatively by

$$-V_{Cmin} = \Delta V_{gc} + \Delta V_D, \quad (1)$$

where $\Delta V_{gc}$ denotes the difference of the fixed charge surface potentials (Gouy-Chapman potentials) on the two sides of the bilayer, and $\Delta V_D$ that in the corresponding dipole potentials. Each of these two components will now be discussed separately.

### 2.2. Fixed charge surface potentials

A charged surface attracts ions of the opposite charge and repels ions of the same charge as the surface itself (fig. 1B). This asymmetric distribution of ions counteracts the bound surface charge and reduces the effective potential, an effect known as shielding (see, e.g., ref. 11). As one proceeds from the surface into the adjacent medium, the excess of counterions results in a decay of the potential until it reaches the bulk value (fig. 1C, middle). The electrical potential near a charged surface is usually described by the Gouy-Chapman theory, which relates the 'fixed charge surface potential' to the surface charge density and the ionic composition of the bathing solution. For a monovalent electrolyte the Gouy-Chapman potential, $V_{gc}$, is given by the set of equations:

$$V_{gc} = 50.8 \ln(s^2 + s + 1)$$
$$s = 1.36\sigma/c, \quad (2)$$

where $c$ is the concentration in mol/l and $\sigma$ the surface charge density in elementary charges per nm$^2$. Note that the potential at the surface decreases with increasing ionic strength. A further characteristic revealed by the full analysis is that multivalent ions are much more effective at shielding than monovalent ions.

The concentration of any ion, including charged ligands, in the layer adjacent to the charged surface can be calculated from the Boltzmann equation:

$$c_s = c_f \exp(-zV_{gc}F/RT) \quad (3)$$

where $c_s$ is the concentration in the surface layer, $c_f$ the free (bulk) concentration, $z$ the charge of the species, $F$ the Faraday constant, $R$ the gas constant and $T$ the absolute temperature.

### 2.3. Surface dipole potential

A second component of the surface potential is that resulting from surface dipoles. In contrast to

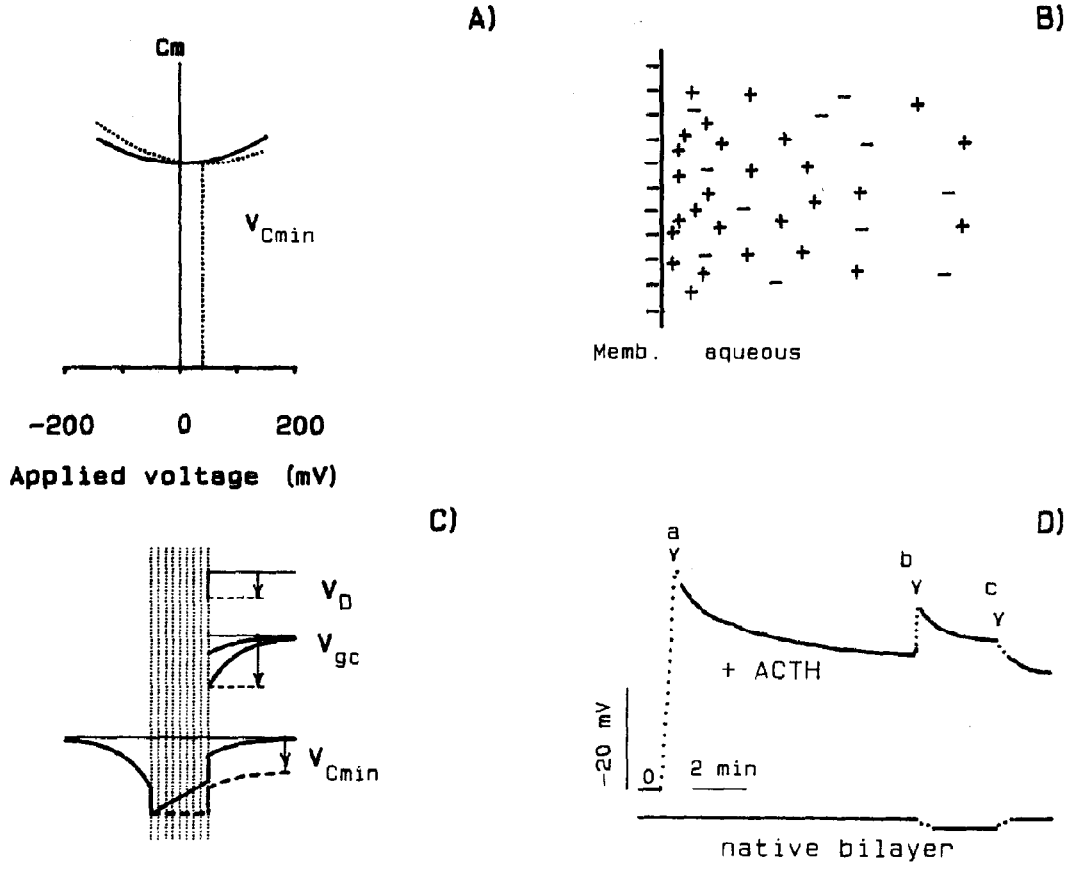


Fig. 1. (A) Voltage dependence of membrane capacitance. Under symmetric conditions, the minimal capacitance occurs at zero transmembrane potential, corresponding to zero applied potential (continuous curve). Any asymmetry, such as the binding of a charged molecule to one side of the membrane, or unequal ionic compositions of the two aqueous phases in the presence of a surface charge, shifts the voltage at which the minimum occurs ($V_{Cmin}$). The shift is equal to the difference in the surface potentials on the two sides of the membrane. (B) Qualitative representation of the distribution of coions and counterions near a charged interface. The counterions accumulate with respect to the bulk phase, thus 'screening' or 'shielding' the bound charges and reducing the surface potential. (C) Surface potentials, transmembrane potential and $V_{Cmin}$. The dipole potential, $V_D$, is localized in the plane of the dipoles, shown here at the edge of the membrane (dotted field). The Gouy-Chapman potential, $V_{gc}$, extends out into the solution for a distance comparable to the inverse Debye-Hückel length, e.g., about 1 nm for a 100 mM solution of a monovalent electrolyte. At a given surface charge density, the magnitude of $V_{gc}$ decreases with increasing ionic strength. The total surface potential on each side of the membrane is the sum of $V_D$ plus $V_{gc}$ on that side. To achieve a transmembrane potential of zero (the condition for minimal capacitance), the applied voltage must compensate the inequality of the surface potentials (dashed line, lower drawing). (D) Changes of $V_{Cmin}$ on a neutral lecithin membrane in the presence of $ACTH_{1-24}$. At time *a*, $ACTH_{1-24}$ is added to a final concentration of 0.16 mM on one side of the bilayer ('cis'), resulting in a rapid rise of $V_{Cmin}$ followed by a decay to the equilibrium value of −28 mV. Following addition of KCl on the *trans* side to a final concentration of 90 mM (time *b*), a further rise of $V_{Cmin}$ was observed, indicating the presence of a surface charge potential on the *trans* side, i.e., the side opposite to that to which the ACTH had been added. At time *c*, the ionic strength on the *cis* side was also raised to 90 mM; the change reflects the shielding of the bound $ACTH_{1-24}$, as would be expected. The lower curve (offset on the *Y*-axis) shows the response of a pure lipid bilayer to the same changes in electrolyte concentration as shown in the upper curve. The small changes may represent either minor impurities in the lipid or nonideal salt bridges used to make electrical contact to the bathing solutions and must be taken as the baseline for the upper curve.

the fixed charge surface potential, the dipole field is limited to the dipole layer itself (fig. 1C, upper) and is not subject to shielding:

$$V_D = 3.77 \times 10^4 c_b \mu_{mol}/\epsilon_a, \quad (4)$$

where $\mu_{mol}$ is the molecular dipole moment, $\epsilon_a$ the dielectric constant of the adsorption region, and $c_b$ the number of molecules bound per nm$^2$ of membrane surface ($\sigma/z$).

The effects of these two components of the surface potential on the transmembrane potential and the relationship to $V_{Cmin}$ are shown in fig. 1C for an asymmetric membrane. The capacitance minimization potential is equal simply to the difference of the combined fixed charge and dipole potentials on the two sides of the bilayer, as stated in eq. 1. The fixed charge potential, and thus the surface charge density, can be determined separately by measuring the change in the total potential when the ionic strength is varied. From this, the surface concentration of bound molecules can be calculated using the Gouy-Chapman theory. An actual experimental trace is shown in fig. 1D.

### 2.4. Discrete charge effects

While the Gouy-Chapman theory has been found to give an adequate description of the fixed charge surface potential for many purposes, situations in which the discrete nature of the charge distribution is important may give deviating results. Two such situations must be considered in studies of the binding of charged species carried out with the capacitance minimization technique.

The first is applicable to all binding studies with charged molecules. In general, the binding of a charged species will result in a buildup of the surface potential. This in turn tends to suppress further binding, leading to a slower approach to saturation, as described by the Stern equation [11]. One must not forget, however, that there is a lateral as well as a perpendicular component to the shielding by counterions. In other words, the field induced by a charged molecule bound to a surface decays as one moves away from the locus of binding along the membrane surface. If the binding density is low, neighboring bound molecules do not sense each other's presence. Under these conditions, the binding curve can be expected to be the same as for uncharged molecules. In our experience the lateral decay of surface potential is such that a mutual repulsion can be ignored if the binding density is less than about 1 molecule/1000 A$^2$ at an ionic strength of 0.1 M, or 1 molecule/$10^4$ A$^2$ at 0.01 M [13].

The second point to be considered is the effect of such localized 'islands' of electric potential on the capacitance minimization potential. As the transmembrane potential varies from place to place, it is not immediately obvious how the surface average of the compressive forces will be affected. A general analysis (see appendix A) reveals that the condition for minimum capacitance is still given by eq. 1. The interpretation of $V_{Cmin}$ in terms of the Gouy-Chapman theory is therefore not affected.

The Gouy-Chapman potential can be evaluated quantitatively to determine the amount of substance bound at a particular bulk concentration. This allows the determination of thermodynamic parameters for binding, such as the dissociation constant and the maximal binding density. Under certain circumstances, a limited amount of structural data is also available from a study of the capacitance minimization potential. For example, the surface dipole potential may allow conclusions to be drawn as to secondary structure and orientation. The detection of changes in the surface potential on the *trans* side of the bilayer may indicate *transmembrane* incorporation [3].

## 3. Results and discussion

Regulatory peptides generally have random conformations in aqueous solution but may assume preferred conformations in special environments. For example, in 2,2,2-trifluoroethanol, a solvent that mimics the lipid environment [14], $ACTH_{1-24}$ [15], substance P [16] and $dynorphin_{1-13}$ (unpublished data) adopt a partially helical structure. Our interest has centered on the heterogeneous environment in which the peptide-

receptor interaction is presumed to take place, viz., the membrane/solution interface.

We have studied several groups of regulatory peptides and have found that the membrane guides the peptides to specific membrane compartments which we have designated 'hydrophobic', 'fixed charge' and 'aqueous'. This discrimination usually involves both electrostatic and hydrophobic components, and results in the induction of preferred conformations, orientations and accumulations of the peptides in the different compartments. Furthermore, it has been possible to predict membrane-bound structures which are in good agreement with the experimental findings for all these peptides. Various parameters or combinations of parameters of the predicted structures correlate with the physiological response and binding properties to different receptors, indicating that the membrane-bound species plays a major role in the molecular mechanism of receptor selection.

The first molecule we looked at was the hormone and neuropeptide adrenocorticotropin (ACTH), of the opiomelanocortin family. Using the $V_{Cmin}$ technique [3], we found that the $ACTH_{1-24}$ analogue bound to neutral lecithin bilayers with a $K_d$ of about $4 \times 10^{-5}$ M. By itself, such a binding affinity is already strong enough to switch the route by which the hormone reaches its receptor from a three-dimensional random diffusion in solution to a two-dimensional search over the surface of the bilayer [2], with the corresponding advantages of increased speed and greater likelihood of ultimately finding the receptor. In addition, the interaction of $ACTH_{1-24}$ with the membrane is enhanced by the electrostatic accumulation of the highly charged $ACTH_{1-24}$ molecule (5–6 positive charges at physiological pH) on the negatively charged membrane. For a typical fixed charge surface potential of $-40$ mV [17], the Boltzmann equation predicts that the surface concentration of $ACTH_{1-24}$ will be about $10^4$-times higher than the bulk concentration [3]. This factor also rationalizes the difference between the $K_d$ measured on neutral bilayers ($4 \times 10^{-5}$ M) and the physiological $EC_{50}$ values in the nanomolar range.

The $V_{Cmin}$ studies also provided strong indications of specific interactions of the peptide with the membrane, as the time course of the approach of the capacitance minimization potential to its equilibrium value was complex (fig. 1D). This stimulated us to undertake a more detailed structural investigation. Infrared studies on thin lipid films revealed a partially $\alpha$-helical structure (the N-terminal message region), penetrating more or less perpendicularly into the lipid core [5], while the C-terminal address remained in the aqueous phase (fig. 2). Two complementary fragments of $ACTH_{1-24}$, $ACTH_{1-10}$ and $ACTH_{11-24}$, representing the functional message and address, respectively, showed qualitatively and quantitatively different interactions with lipid membranes; the reaction was much weaker and, in the case of $ACTH_{1-10}$, the conformation was different from the corresponding section of the intact $ACTH_{1-24}$ molecule ($\beta$-sheet cf. $\alpha$-helix). These findings emphasize the necessity of well-defined *amphiphilicity* for the establishment of the very specific conformation and orientation of the parent molecule. We also looked at the closely related hormone $\alpha$-MSH, which consists of the first 13 amino acids of ACTH, but with both termini blocked by uncharged groups. It carries only a single net charge, and has a much less pronounced amphiphilic character. $\alpha$-MSH reacted weakly with lipid membranes, and the conformations and orientations typical of the ACTH analogues were not found.

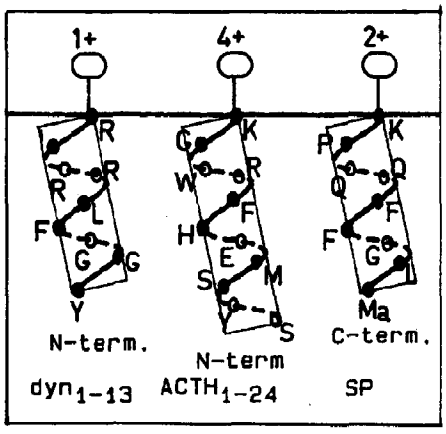


Fig. 2. Schematic representation of the predicted membrane-bound structures of dynorphin$_{1-13}$, $ACTH_{1-24}$ and substance P. The message segments insert as almost perpendicular $\alpha$-helices. The helix length, binding affinity and orientation can be predicted by the method given in the text.

The observations on the opiomelanocortins revealed the possible role of the lipid bilayer in *capturing* and *accumulating* molecules, and *selecting* or *inducing preferred conformations* in or near the membrane/solution interface.

Studies which we then undertook with a series of dynorphin analogues confirmed the applicability of these concepts to another family of peptides. Ultimately, they led to the realization that membrane interactions could guide the same message sequence to different receptors, depending on the physical chemistry of the address segment. This was a new concept, as previously the address had tacitly been thought to react with specific – presumably proteinaceous – sites, and the lipid phase was generally considered to be little more than a nonspecific matrix in which the receptor was embedded. Indeed, the specificity of the lipid environment is low, but by adding heterogeneity to the system (e.g., hydrophobic/hydrophilic character, electric field), it can serve to switch the site of action of a message sequence between one of several receptors.

Our experimental work with dynorphin $A_{1-13}$ on neutral lipid membranes [8] revealed a picture very similar to that found for $ACTH_{1-24}$. The N-terminal, more hydrophobic message region inserted perpendicularly as an $\alpha$-helix into the lipid membrane, while the address remained in a random conformation in an aqueous environment. A $K_d$ of $2\text{–}4 \times 10^{-5}$ M was measured on neutral bilayers.

The opioid system is interesting as at least three receptor subtypes are known [18], each responding to the identical message segment (Tyr-Gly-Gly-Phe-Leu). To explain the varying receptor site selectivity of different dynorphin analogues, Schwyzer developed a simple theoretical method to estimate the preferred conformation, orientation and accumulation of peptides on a membrane [19]. Four parameters have been found to be important: the Gibbs free energy of hydrophobic interaction, which determines the strength of binding and length of the inserted $\alpha$-helical segment; the amphiphilic moment and electric dipole moment, which influence the orientation of the inserted segment, and the net charge, which, in conjunction with the local electric field, determines the Boltzmann accumulation (or depletion) in the corresponding environment. (The exact definitions of the parameters are in given in appendix B.) The method yields values in excellent agreement with the number of residues found in the hydrophobic phase, the hydrophobic association constant, and the orientation on the membrane surface as determined experimentally for peptides such as $ACTH_{1-24}$ and dynorphin $A_{1-13}$.

Table 1

Opioid receptor selectivity versus theoretical membrane interaction parameters

Qualitative summary of data from ref. 7. Identification of compounds: (A) H-$\beta$-endorphin (N-terminal sequence YGGFMTSEKSQTP...); (B) YGGFMRF; (C) YGGFMRRV-amide; (D) YGGFMRGL; (E) [Met]-enkephalin, YGGFM; (F) [Leu]-enkephalin, YGGFL; (G) YGGFLRRI; (H) YGGFLRRIR; (I) dynorphin A, YGGFLRRIRPKLKWDNQ; (J) dynorphin B, YGGFLRRQFKVVT; (K) $\alpha$-neoendorphin; (L) dynorphin A, YGGFLRRIRPKLK.

| Receptor | Amphiphilic moment ($A$) [a] | Charge | |
|---|---|---|---|
| | | $\geq 2$ | $\leq 1$ |
| $\kappa$ [b] | G, H, I<br>J, K, L | G, H, I<br>J, K, L | |
| $\mu$ [c] | | C | |
| $\delta$ [b] | | | B, D, E, F<br>A [d] |

[a] Only ligands having both an amphiphilic moment of amplitude >150 units [7] and significant hydrophobic binding ($K_d < 10^{-3}$ M) are noted. The amphiphilic moment is based on an $\alpha$-helical model of the inserted peptide.
[b] Receptor selectivity.
[c] Binding affinity.
[d] Effective charge on message segment.

Using this method, the preferred conformations, orientations, and accumulations of 28 opioid peptides on lipid membranes have been estimated and compared with pharmacologic and selective binding data taken from the literature [7,20] (table 1). Selections for $\kappa$ receptors were 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 $\alpha$-helix were favorable for $\kappa$ site selection. Potencies as $\kappa$ agonists and binding affinities correlated with $Ae^z$. Interaction with $\mu$ receptors was influenced by the net positive charge effective at the message domain of the agonist peptides, $z$(eff),

following the Boltzmann term, $e^{z(\mathrm{eff})}$. Selection for $\delta$ receptors was reduced by $z$(eff) and correlated with $e^{-z(\mathrm{eff})}$. 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). We have proposed a model (fig. 3) in which the $\delta$ 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 $\mu$ site is exposed to the anionic fixed-charge compartment of the membrane in aqueous surroundings. The $\kappa$ 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. The same method has also been applied to neurokinin receptor subtype selection [21]. In this group of regulatory peptides, the hydrophobic message segment is located at the C-terminus rather than the N-terminus. Notwithstanding this difference, data on 13 mammalian neurokinins and nonmammalian tachykinins support the view that the neurokinins bind to three principal mammalian sites. Receptor selection is guided by the same principles as for opioid receptor selection;

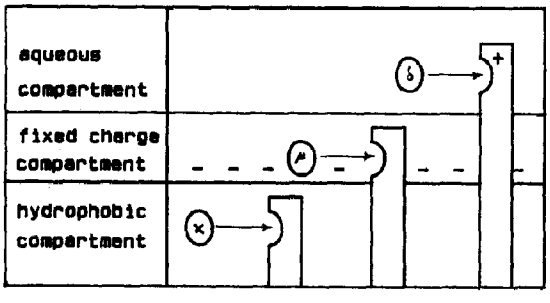


Fig. 3. Schematic representation of the functional compartmentalization for the access of opioid ligands to the $\kappa$, $\mu$ and $\delta$ receptors.

Table 2

Neurokinin receptor selectivity versus theoretical membrane interaction parameters

Qualitative summary of data from ref. 21. Assignment to the receptor classes is based on the observed relative receptor affinities [17]. Identification of compounds: (M) substance P; (N) physalaemin; (O) eledoisin; (P) kassinin; (Q) neurokinin A; (R) neurokinin B; (S) septide; (T) (Gly-9)septide; (U) senktide; (V) (C-3,6,Y-8)substance P; (W) (C-2,5)neurokinin B; (X) antagonist A; (Y) antagonist B.

| | Amphiphilic moment ($A$) [a] | Charge | | |
|---|---|---|---|---|
| | | + | 0 | − |
| NK-1 | M, N, V, X | M, V, X, Y | N | |
| NK-2 [b] | | Q | (O, P, S, T) [c] | |
| NK-3 | | | (O, S, T) [c] | RUW |

[a] Only ligands having both an amphiphilic moment of amplitude > 150 units [7] and significant hydrophobic binding ($K_d < 10^{-3}$ M) are noted. The amphiphilic moment is based on an $\alpha$-helical model of the inserted peptide.

[b] The most selective NK-2 ligand (NKA) is positively charged.

[c] These ligands have a low selectivity between NK-2 and NK-3 sites.

the NK-1 (preferring substance P), the NK-2 (preferring neurokinin A) and the NK-3 site (preferring neurokinin B) (table 2). A strong hydrophobic membrane interaction of the C-terminal message segment as a perpendicularly oriented $\alpha$-helix domain correlates with NK-1 selection. Electrostatic accumulation of the peptide at the anionic fixed-charge layer of the membrane without hydrophobic interactions through a helix correlates with NK-2 preference. Electrostatic repulsion by the anionic fixed-charge layer correlates with NK-3 selection.

## 4. Conclusion

If a segment of a peptide is sufficiently hydrophobic in character, then a spontaneous association with the membrane phase can be expected. The hydrophobicity of a segment will depend on both the primary and secondary structure. We have developed model calculations to predict the strength of the membrane association of $\alpha$-helical structures. For spontaneously inserting $\alpha$-helical

segments, the amphiphilic and electric dipole character will tend to orient the segment on aqueous/hydrophobic interface boundaries. On this basis, we can predict preferred conformations, orientations and accumulation of peptides on lipid bilayer membranes. The regulatory peptides thus far examined appear to have no unique three-dimensional structure in solution, but many adopt well-defined conformations upon interaction with the membrane. A general feature of the opioid $\kappa$ and neurokinin NK-1 ligands, for example, is an $\alpha$-helical segment, corresponding to the 'message' of the peptide, which inserts more or less perpendicularly into the hydrophobic phase, leaving the hydrophilic 'address' in the aqueous environment. Such a configuration results in the message being sequestered in a hydrophobic environment at a well-defined depth and orientation, which presumably corresponds to optimized conditions for reaction with a particular receptor. Other regulatory peptides do not insert and are thereby restricted to react with receptors directly accessible from the aqueous phase. Here again, however, selection between receptor types can be effected through the mechanism of electrostatic forces by making use of the ubiquitous electric surface potential associated with biological membranes. Examples are the selection between the opioid $\mu$ and $\delta$ receptors, and the neurokinin NK-2 and NK-3 receptors. Thus, it is evident that through these seemingly simple concepts, these ligands are functionally restricted to distinct compartments through the mediation of the membrane. This adds a crucial contribution to the ultimate reaction between ligand and receptor: the membrane requirements complement the classical receptor requirements for receptor selectivity.

We have talked exclusively about membrane effects in this paper. It is of course quite likely that similar phenomena will be found to operate more generally, such as at the boundaries of hydrophobic regions of macromolecules. The influence of micelles in the catalysis of chemical and biochemical reactions [22] is an example of a similar phenomenon. Whatever the source of the heterogeneity of a system, when studying reactions at interfaces it seems imperative to emphasize local rather than bulk conditions. It is to be hoped that more powerful techniques – both theoretical and experimental – will be developed to enable us to understand interfaces and interfacial reactions in more detail.

## Appendix A

### *A1. The influence of a discrete charge distribution on the capacitance minimization potential*

The classical treatment of surface potentials stems from Gouy [23] and Chapman [24] and considers the charge to be smeared uniformly over the membrane surface. The many assumptions made in this approach have been discussed at length elsewhere [12]. In many cases, the Gouy-Chapman theory has provided an adequate description of experimental results, especially where macroscopic properties have been investigated, and this has been rationalized as stemming from the mutual compensations of the assumptions involved. Where effects on the molecular scale are involved, however, significant deviations from the predictions of the Gouy-Chapman theory have been observed (e.g., refs. 25 and 26). In this section we shall consider the effect of a discrete charge distribution on the measured capacitance minimization potential and show that the basic interpretation using the Gouy-Chapman theory for quantitative evaluation is acceptable for low charge densities.

The voltage dependence of the membrane capacitance reflects electrostrictive forces acting on the fluid dielectric. With a non-uniform surface potential, these forces will be a function of position. Thus, the overall capacitance must be found by integrating over a suitable repeating unit. For the sake of simplicity, we will assume a radially symmetric potential distribution, $V_c(r)$, about each bound molecule. The membrane area associated with each bound molecule, i.e., the area over which averaging has to be performed, will be taken to be circular, with a radius $r_0$. The average surface potential is then given by

$$V_{av} = \frac{1}{\pi r_0^2} \int_0^{2\pi} \int_0^{r_0} V_c(r) r \, dr \, d\theta = \frac{2}{r_0^2} \int_0^{r_0} r V_c(r) \, dr. \tag{A1}$$

The specific capacity (capacity per unit area) is given approximately by

$$C' = C_0'\left(1 + k\Delta\psi(r)^2\right) = C_0'\left(1 + k\left(V_c(r) + V_{ext}\right)^2\right), \quad \text{(A2)}$$

where $C_0'$ is the specific capacitance at zero transmembrane potential, $k$ a constant, $\Delta\psi$ the local transmembrane potential and $V_{ext}$ the externally applied potential [10]. The total capacitance associated with the area per bound molecule is then

$$C_{tot} = \int_0^{2\pi}\int_0^{r_0} rC'(r)\,d\theta\,dr = 2\pi\int_0^{r_0} r\left\{C_0'\left(1 + k\left[V_c(r) + V_{ext}\right]^2\right)\right\}dr = \pi r_0^2 C_0' + 2\pi k C_0'\int_0^{r_0} r\left(V_c(r) + V_{ext}\right)^2 dr \quad \text{(A3)}$$

The potential for minimal capacitance is found by differentiating eq. A3:

$$dC_{tot}/dV_{ext} = 2\pi k C_0'\int_0^{r_0} 2r\left(V_c(r) + V_{ext}\right)dr = 4\pi k C_0'\left(\int_0^{r_0} rV_c(r)\,dr + \frac{r_0^2}{2}V_{ext}\right). \quad \text{(A4)}$$

Setting $dC_{tot}/dV_{ext} = 0$ in eq. 4, we obtain

$$V_{Cmin} = -\frac{2}{r_0^2}\int_0^{r_0} rV_c(r)\,dr \equiv -V_{av} \quad \text{(by eq. A1)}$$

It was shown earlier that $V_{gc}$ and an averaged potential analogous to $V_{av}$ as defined above are very similar [13]. Thus, even in the presence of a discrete charge distribution, the quantitative evaluation of $V_{Cmin}$ in terms of $V_{gc}$ is still valid.

The main influence of a discrete charge distribution is on the basal capacitance value. From eq. A3, one observes that for a uniform distribution the minimal capacitance is $\pi r^2 C_0'$, whereas otherwise, $C_{tot}$ will be larger due to a contribution from the integral term. By evaluating the second derivative of eq. A3 it is easily shown that the measured compressibility of the bilayer is not affected by the form of the charge distribution.

## Appendix B

### *B1. Definition of the parameters used to estimate the preferred conformation, orientation and accumulation of peptides at the membrane / water interface*

#### *B1.1. Hydrophobic association*

The Gibbs free energy of hydrophobic association, $\Delta G^0_{ass}(m)$, through $m$ residues at the more hydrophobic end of a peptide chain is calculated from the free energy of transfer, $\Delta G^0_{tr}(i)$, of the individual residues from their random-coil conformation in water to their helical conformation in a hydrophobic phase [27]. The relations are:

$$\Delta G^0_{tr}(m) = \sum_{i=1}^{m}\Delta G^0_{tr}(i) + \Delta G^0_{tr}(\text{end}) \quad \text{(B1)}$$

$$\Delta G^0_{ass}(m) = \Delta G^0_{tr}(m) + \Delta G^0_{t+r} \quad \text{(B2)}$$

where $\Delta G^0_{tr}$(end) accounts for unsatisfied hydrogen bonds at the helix ends, and $\Delta G^0_{t+r}$ 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 [28]. The hydrophobic association constant and the length of the helix, $m$, are determined from the position of the energy minimum.

#### *B1.2. 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 [29] by the molecular amphiphilic moment [30]. 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

$$\vec{A} = \sum_{i=1}^{m}\Delta G^0_{trh}(i)\vec{R}_i \quad \text{(B3)}$$

where $\Delta G^0_{trh}(i)$ is the signed numerical value of the Gibbs free energy change for the transfer of the $i$-th residue in its helical conformation from water to a hydrophobic phase (values taken from ref. 31). $\vec{R}_i$ is the position vector from the helix center to the $\alpha$-carbon of the $i$-th residue measured in units of helix radius, 0.188 nm. 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 will be the thermal tumbling of the peptide molecules. Usually, a value of $A$ of at least 150 arbitrary units is necessary to produce biologically relevant membrane associations [7].

*B1.3. Electric dipole moment*

Alignment of peptide bond dipoles [32] and asymmetric arrangement of charged amino acid residues endow peptide helices with a molecular dipole moment. Such helices will tend to orient themselves in the direction of minimum free energy within the surface dipole layer of membranes. The molecular dipole moment was estimated in analogy to the molecular amphiphilic moment (eq. B3) by

$$\vec{\mu} = \sum_{i=1}^{m} z_i \vec{R}_i \qquad \text{(B4)}$$

where $z_i$ is the charge number of the ionic side chain (assumed to be $\pm 1\, e_0$, $e_0$ being the elementary charge) of the $i$-th residue, or of the partial charges assigned to the helix ends (assumed to be $\pm 0.63 e_0$ [32] located on the end residues, $i = 1$ and $i = m$). $\vec{R}_i$ is the position vector from the helix center to the $\alpha$-carbon of the $i$-th residue, measured in units of 0.1 nm.

*B1.4. Net charge*

Charged peptides will be attracted or repelled by the fixed charge layer of a membrane surface according to a Boltzmann distribution:

$$c_s = c_0 \exp(-zFV_{gc}/RT) \qquad \text{(B5)}$$

where $c_s$ is the concentration of the peptide adjacent to the surface, $c_0$ the molar peptide concentration in the bulk phase, $z$ the net charge, $V_{gc}$ the Gouy-Chapman fixed-charge potential, $F$ the Faraday constant, $R$ the universal gas constant and $T$ the absolute temperature (in K). Biological membranes usually contain excess negatively charged lipid in such an amount that we may assume a characteristic $V_{gc}$ of at least $-40$ mV for the lipid phase [33].

Positively charged peptides will accumulate in an aqueous compartment close to the fixed-charge layer and negatively charged peptides will be depleted, whereas neutral peptides will be indifferent. These concentration shifts are effective over a distance characterized by the Debye-Hückel length:

$$\lambda_d = (\epsilon_0 \epsilon RT/2c_e)^{1/2}/F \qquad \text{(B6)}$$

which is the distance from the fixed charge layer at which the electric potential has decreased to $1/e$ of its maximum value. It depends on the dielectric constant ($\epsilon$) of the phase ($\epsilon_0$: permittivity of free space) and the electrolyte concentration ($c_e$). Under physiologic conditions (simulated by $c_e$ about 0.1 M of a 1 : 1 electrolyte), $\lambda_d$ is about 1 nm or roughly 10 diameters of a water molecule, or the length of an $\alpha$-helix with six to seven residues.

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