190. Membrane Structure of Substance P

I. Prediction of Preferred Conformation, Orientation, and Accumulation of Substance P on Lipid Membranes[1])

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Preferred conformation, orientation, and accumulation of substance P on a neutral hydrophilic-hydrophobic interface was estimated and extrapolated to interactions with neutral and anionic lipid bilayer membranes according to our general procedure. Nine residues at the C-terminus were predicted to be transferred to the hydrophobic phase as an α-helical domain, oriented quite perpendicularly on the membrane surface. The N-terminal residues remained in the aqueous phase with their charges exposed to H_2O. The molecular amphiphilic moment vector was strong (338 arbitrary units) and pointed its hydrophilic end towards the N-terminus, only 15° away from the helix axis. The molecular electric dipole moment vector was also strong (124 debye) and pointed its positive end towards the N-terminus, only 9° away from the helix axis. Thus, it reinforced the effect of the amphiphilic moment of a peptide intruding into the membrane dipole layer. The estimated dissociation constant for the equilibrium between membrane-bound and free substance P was $K_d \approx 46$ mM for neutral membranes, and $K_d \approx 0.43$ mM for anionic membranes with a *Gouy-Chapman* surface potential of −40 mV. Thus, substance P behaved similarly to dynorphin A and adrenocorticotropin peptides which insert their N-terminal message segments as perpendicularly oriented helical domains into membranes, whereas their C-terminal address segments remain in the aqueous phase as random coils. Substance P is the first instance of a neuropeptide which is expected to insert a C-terminal message into lipid membranes.

Introduction. – Substance P is the first peptide discovered to occur in both the gut and the central nervous system [1b]. Such common peptides are important for understanding psychosomatic communication (see ref. in [2]). Substance P is a putative neurotransmitter of afferent sensory nerves and is known to produce a plethora of other central-nervous and peripheral actions (recent review [3]). It belongs to a family of naturally occurring peptides with related pharmacologic properties, the tachykinins [4].

Substance P and the tachykinins express their actions through different subtypes of receptor [5]. Selection for opioid-receptor subtypes by members of the opioid-peptide family has been shown to involve a membrane-assisted molecular mechanism [6]. In this mechanism, accumulation of the receptor-triggering, N-terminal 'message' segment of the peptide with that membrane compartment into which the particular receptor site is exposed is determined by the C-terminal 'address' segment. Membrane interactions have been studied in detail for dynorphin-A and adrenocorticotropin peptides [7]. Experimentally observed preferential conformations, orientations, and accumulations of these peptides on membrane surfaces can be estimated to a high degree of accuracy by calculating hydrophobic association, helix length, molecular amphiphilic moment, molecular electric

[1]) Peptide nomenclature and abbreviations, see IUPAC-IUB JBCN Recommendations 1983 on 'Nomenclature and Symbolism for Amino Acids and Peptides' [1a].

dipole moment, and electrostatic membrane interactions [8]. However, the message segments of substance P and the tachykinins are not N-terminal, but C-terminal. As it seemed possible that selection for substance-P-receptor subtypes might follow the same pattern as for opioid receptors, we investigated the interaction of substance P with membranes. In this report, we used similar thermodynamic considerations as for other peptides [8] to predict the membrane structure of substance P. In subsequent reports, experimental evidence for or against the predictions will be presented [9].

Methods. – Four parameters are used for estimating conformation, orientation, and accumulation of peptides on neutral and charged aqueous-hydrophobic interfaces or lipid membrane surface [8]. They are briefly recapitulated in this section.

Hydrophobic Association. The *Gibbs* free energy of hydrophobic association, $\Delta G^\circ_{ass}(m)$, through m residues at the more hydrophobic end of a peptide chain is calculated from the free energy of transfer, $\Delta G^\circ_{tr}(i)$, of the individual residues from their random-coil conformation in H_2O to their helical conformation in a hydrophobic phase [10]. The relations are given in *Eqn. 1* and *2*,

$$\Delta G^\circ_{tr}(m) = \Sigma_{i=1}^{i=m} \Delta G^\circ_{tr}(i) + \Delta G^\circ_{tr}(\text{end}) \tag{1}$$

$$\Delta G^\circ_{ass}(m) = \Delta G^\circ_{tr}(m) + \Delta G^\circ_{t+r} \tag{2}$$

where $\Delta G^\circ_{tr}(\text{end})$ accounts for unsatisfied H bonds at the helix ends, and ΔG°_{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 bonded to the membrane [11]. The hydrophobic-association constant and the length of the helix, m, are determined from the position of the energy minimum [8].

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 [12] by the molecular amphiphilic moment $\vec{A}$ as defined in *Eqn. 3* [8]

$$\vec{A} = \Sigma_{i=1}^{i=m} \Delta G^\circ_{trh}(i) \vec{R}_i \tag{3}$$

where $\Delta G^\circ_{trh}(i)$ is the signed numerical value of the *Gibbs* free energy change for the transfer of the ith residue in its helical conformation from H_2O to a hydrophobic phase (values taken from *von Heijne* [10]). $\vec{R}_i$ is the position vector from the helix centre to the C(α) atom of the ith residue measured in units of helix radius, 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. Usually, a value of $A \geqslant 150$ arbitrary units is necessary to produce biologically relevant membrane associations [6] [8].

Electric-Dipole Moment. Alignment of peptide-bond dipoles 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 measured in analogy to the molecular amphiphilic moment (see *Eqn. 3* by *Eqn. 4*),

$$\vec{\mu} = \Sigma_{i=1}^{i=m} z_i \vec{R}_i \tag{4}$$

where z_i is the charge number (assumed to be an integer of e) of the ith residue or of the partial charges assigned to the helix ends (assumed to be $\pm 0.63\ e$ [13] located on the end residues, *i.e.* for $i = 1$ and $i = m$). $\vec{R}_i$ is the position vector from the helix centre to the C(α) atom of the ith residue, measured in units of 0.1 nm.

Net Charge. Charged peptides will be attracted or repulsed by the fixed charge layer of a membrane surface according to a *Boltzmann* distribution given in *Eqn. 5*,

$$c_b/(c_{b\,max} - c_b) = c_o \exp(-zFV_{gc}/RT) \tag{5}$$

where c_b is the surface concentration of the peptide (number of adsorbed molecules per unit area), $c_{b\,max}$ the surface concentration at saturation, c_o 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. Biologic membranes usually contain excess negatively charged lipid in such an amount that we may assume a characteristic $V_{gc} \approx -40$ mV for their lipid phase.

Results. – Inspection of the amino-acid sequence of substance P *(Fig. 1)* suggested a pronounced primary amphiphilicity and a favourable electric-dipole moment. Less hydrophilic amino-acid residues segregate at the C-terminal end of the sequence, and more hydrophilic, positively charged residues near the N-terminus. The scenario [8] for estimating helix length, hydrophobic-association constant, amphiphilic moment, and electric-dipole moment was thus as follows *(Fig. 1a)*: *a)* Simulation of progressive transfer of residues at the C-terminus into the hydrophobic phase accompanied by α-helix forma-

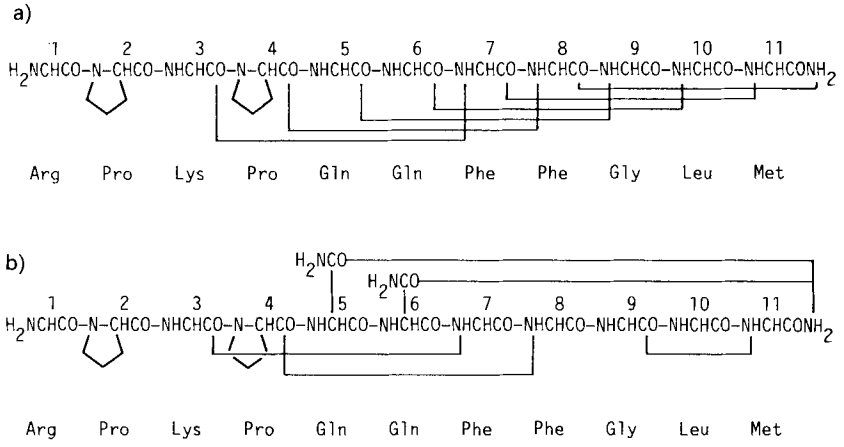

Fig. 1. *Schematic representation of proposed hydrogen-bonding systems for substance P. a)* The α-helix with $m = 9$ predicted here for lipid membrane interaction. *b)* The mixed α-helix/folded structure in MeOH as 'membrane mimicking' solvent proposed by *Chassaing et al.* [14].

Except for proline, only the backbone atoms are shown. Horizontal and vertical lines indicate H bonds between C=O and NH groups. The side-chain carboxamide groups of Gln-5 and Gln-6 are indicated in *b)* to show the interaction with the C-terminal Met amide group suggested by the French authors.

tion, leaving the untransferred residues in the aqueous phase as random coils (thermodynamic parameters for the transfer were taken from [10]). The point at which the total free energy of transfer reaches a minimum indicates the preferred helix length. *b)* The carbonyl O-atoms of three residues at the C-terminus lose H bonds to H_2O during transfer. The fourth C=O group becomes internally H-bonded to the C-terminal amide NH_2 group. *c)* The peptide NH groups of four residues at the N-terminal end of a helix lose H bonds to H_2O upon transfer to the hydrophobic phase. However, with an almost perpendicular orientation of the helix on the interface suggested by the amphiphilic and electric-dipole moments, molecular models show that one helix-terminal peptide NH may remain in contact with H_2O while the hydrophobic parts of all four residues interact with the hydrophobic phase. *d)* Side-chain carbamoyl and ammonium groups of Gln and Lys residues remain in contact with H_2O and, despite transfer of hydrophobic parts of the residues, are not transferred until they are two and four positions away from the helix end, respectively [10]. *e)* Molecules bonded to an interface were considered to have lost one degree of translational and two degrees of rotational freedom. The corresponding free-energy change was estimated according to *Janin* and *Chothia* [11] as approximately 49.5 kJ/mol. This value was used in the estimation of the free energy of hydrophobic association.

Table 1 lists contributions of individual residues and of C-terminal segments with $m = 1$ to 11 to the free energy of transfer of substance P. An energy minimum of −57.1 kJ/mol was reached at $m = 9$. A free-energy change of $\Delta G^\circ_{ass}(9) = -7.6$ kJ/mol was predicted from *Eqn. 2* for hydrophobic association with a neutral interface. For anionic interfaces with *Gouy-Chapman* potentials of −40 and −120 mV, additional free-energy

Table 1. *Estimated Free-Energy Difference* ΔG°_{tr} (m) [kJ/mol] *for the Transfer of Substance P from a Random-Coil Conformation in* H_2O *to a Partly Helical Structure on an Aqueous-Hydrophobic Interface through Increasing Numbers* m *of C-Terminal Residues in their* α*-Helical Conformation* (see *Eqn. 1*)

m	Residue	Hydrophobic contribution [kJ/mol]	H Bond [kJ/mol]	Charge [kJ/mol]	$\Delta G^\circ_{tr}(i)$ [kJ/mol]	m $\Sigma\Delta G^\circ_{tr}(i)$ i [kJ/mol]	ΔG°_{tr} (end) [kJ/mol]		$\Delta G^\circ_{tr}(m)$ [kJ/mol]
							C-End	N-End	
1	Met[b])	− 21.86	21.0	–	− 0.86	− 0.86	10.5	–	9.64
2	Leu	− 17.79	–	–	− 17.79	− 18.65	21.0	10.5	12.85
3	Gly	− 7.85	–	–	− 7.85	− 26.50	31.5	21.0	26.0
4	Phe	− 21.98	–	–	− 21.98	− 48.48	31.5	31.5	14.52
5	Phe	− 21.98	–	–	− 21.98	− 70.46	31.5	31.5	− 7.46
6	Gln	− 13.38[c])	–	–	− 13.38	− 83.84	31.5	31.5	− 20.84
7	Gln	− 13.38[c])	–	–	− 13.38	− 97.22	31.5	31.5	− 34.22
8	Pro	− 15.18	–	–	− 15.18	− 112.40	31.5	31.5	− 49.40
9	Lys^+	− 28.72[d])	21.0[d])	–	− 7.72	− 120.12	31.5	31.5	− 57.12
10	Pro	− 20.64[e])	42.0[e])	–	21.36	− 98.76	31.5	31.5	− 35.76
11	Arg^{2+}	− 16.2[f])	10.5	3.0	− 2.7	− 101.46	31.5	31.5	− 38.46

[a]) Hydrophobic contribution calculated from the accessible area [10].
[b]) C-Terminal methionine amide residue.
[c]) Approximate contribution of Gln without its carboxamide group (see text).
[d]) Contribution of Lys without its ammonium group, but including contributions of Gln ($m = 6$) carboxamide.
[e]) Including contributions of Gln ($m = 7$) carboxamide.
[f]) Contribution of Arg without its guanidinium group but with the N-terminal charge (see text).

changes of −11.6 and −34.8 kJ/mol were expected for electrostatic interaction. The estimated values for hydrophobic association of dynorphin$_{1-13}$ and ACTH$_{1-24}$ with a neutral interface correspond closely to the values measured with 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and egg yolk lecithin membranes [7] [8]. Furthermore, the estimated hydrophobic and electrostatic interactions of more than 20 opioid peptides with an anionic interface predict the potencies at opioid κ-, μ-, and δ-sites [6]. We may, therefore, expect dissociation constants K_d for the reversible interaction of substance P with neutral membranes of $4.6 \cdot 10^{-2}$ M and with anionic membranes ($V_{gc} = -40$ mV) of *ca.* $4.3 \cdot 10^{-4}$ M, using *Eqns. 2* and *5*. With artificial liposomes containing a higher surface potential ($V_{gc} = -120$ mV), $K_d = 3.6 \cdot 10^{-8}$ M was predicted. *Table 2* contains these values and those estimated for a more hydrophobic analogue ([Leu^9]substance P) in which Gly-9 is replaced by Leu-9, and for a shorter, less charged analogue (des-Arg^1-substance P) in which Arg-1 is missing.

The substance-P molecule in its estimated state of lowest energy on an aqueous-hydrophobic interface is shown in *Fig. 1a*. The peptide N-atoms of residues 4–6, the peptide O-atoms of residues 9–11, and the side-chain carbamoyl group of residue 6 were assumed

Table 2. *Molar Dissociation Constants K_d for the Interaction of Substance P, [Leu^9]Substance P, and Des-Arg^1-substance P with Membranes as Estimated from* Eqns. 2 *and* 5

Peptide	Charge	K_d			
		Membrane V_{gc}	0 mV	− 40 mV	− 120 mV
Substance P	3 +		$4.6 \cdot 10^{-2}$	$4.3 \cdot 10^{-4}$	$3.6 \cdot 10^{-8}$
[Leu^9] Substance P	3 +		$8.4 \cdot 10^{-4}$	$7.8 \cdot 10^{-6}$	$6.7 \cdot 10^{-10}$
Des-Arg^1-substance P	2 +		$4.6 \cdot 10^{-2}$	$2.0 \cdot 10^{-3}$	$4.0 \cdot 10^{-6}$

Table 3. *Quantities Used for Estimating the Amphiphilic and Electric-Dipole Moments of Substance P*

i	Residue	Hydrophobic contribution[a]) [kJ/mol]	H Bond [a]) [kJ/mol]	Charge [a]) [kJ/mol]	End groups[a]) [kJ/mol]	$\Delta G^\circ_{trh}(i)$ [b]) [kJ/mol]	θ_i [°]	$\vec{k}_i$ [c])	$\Delta G^\circ_{trh}(i)\vec{k}_i$
1	Arg^{2+}	− 15.5	31.5	34.3[d])	21.0	71.3	0	− 3.19	− 227.5
2	Pro	− 7.1	–	–	21.0	13.9	0	− 3.19	− 44.34
3	Lys^+	− 13.0	10.5	–	10.5	8.0	0	− 3.19	− 25.52
4	Pro	− 7.1	–	–	10.5	3.4	100	− 2.39	− 8.13
5	Gln	− 10.9	21.0	–	10.5	20.6	200	− 1.60	− 32.96
6	Gln	− 10.9	21.0	–	10.5	20.6	300	− 0.8	− 16.48
7	Phe[e])	− 14.2	–	–	–	− 14.2	400	0	0
8	Phe	− 14.2	–	–	–	− 14.2	500	0.80	− 11.36
9	Gly	0.0	–	–	10.5	10.5	600	1.60	16.80
10	Leu	− 10.1	–	–	10.5	0.4	700	2.39	0.96
11	Met[f])	− 13.8	10.5	–	10.5	7.2	800	3.19	22.97

[a]) Energy contributions of changes in accessible area (hydrophobic contributions), H bonds, charges, and end groups.

[b]) $\Delta G^\circ_{trh}(i)$ is the *Gibbs* free energy of transfer of the ith residue from its α-helical conformation in H_2O to its α-helical conformation in a hydrophobic phase [10].

[c]) k_i relative to r_i measured in units of helix radius.

[e]) Helix center (*) at residue 7 ($x = y = z = 0$).

[d]) Including the contribution of 2.9 kJ/mol of the N-terminal charge, $pK \approx 6.5$ [17].

[f]) C-Terminal methionine amide.

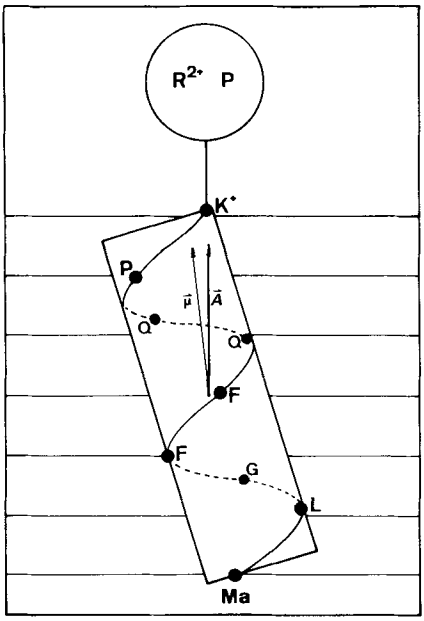

Fig. 2. *Predicted conformation and orientation of substance P in a hypothetic hydrophobic gradient or on a lipid membrane* (side view). The gradient is thought to increase from top to bottom and the horizontal lines indicate surfaces of equal hydrophobicity. The helix axis lies in the plane of the paper. Large residue symbols are in front of the plane, smaller ones behind. The usual one-letter symbols for amino-acid residues are used; Ma is C-terminal methionine amide. The N-terminal random-coil dipeptide domain is shown in a circle and is thought to exert its pull towards the aqueous phase on the C(α) atom of Lys-3 (= K). The directions of the amphiphilic and electric dipole moment vectors are shown by arrows. The amphiphilic moment was arbitrarily assumed to cause the orientation (the hydrophobic gradient was assumed to have no electric-dipole moment of its own). According to the angle Θ, Lys-3 lies 28° in front of the axis.

to have lost H bonds upon transfer to the hydrophobic phase *(Table 1)*. The peptide N-atoms of N-terminal residues 1–3, and the side-chain carbamoyl of 5 were assumed to remain in contact with the aqueous phase. Estimations of the molecular amphiphilic and electric-dipole moments *(Eqns. 3* and *4, Table 3)* were based on this structure. The estimated amphiphilic moment was characterized by $A = 338$ arbitrary units, $\Phi = 165°$, and $\Theta = 332°$. This indicated a high probability of 'almost perpendicular' insertion of the C-terminal substance-P helix into the hydrophobic phase (see *Fig. 2*). The estimated molecular electric-dipole moment was characterized by $\mu = 124$ debye, $\Phi = 171°$, and $\Theta = 0°$ *(Fig. 2)*. Thus, the electric-dipole moment was expected to reinforce the action of the amphiphilic moment.

Recently, *Chassaing et al.* [14] proposed a preferred conformation of substance P in MeOH that was supposed to be similar to the hypothetical conformation of substance P bounded to a lipid membrane. It comprises a flexible Arg-Pro-Lys- segment, an α-helical structure -Pro-Gln-Gln-Phe-Phe-, and a 'U-turn' in the molecule that allows the -Gly-Leu-Met-NH_2 segment to establish H bonds between the terminal amide N-atom (donor) and each of the side-chain carbamoyl groups (acceptors) of Gln-5 and Gln-6. The H-bonding system of this conformation is shown in *Fig. 1b*. Approximate estimations

gave considerably weaker amphiphilic and electric-dipole moments for the conformation in *Fig. 1b* ($A \approx 170$ arbitrary units and $\mu \approx 70$ debye) than for that shown in *Fig. 1a*. Also the estimated free energy of transfer is less favourable by *ca.* 31 kJ/mol, taking into account only the difference between the numbers of unsatisfied H bonds in the hydrophobic phase. This data excluded membrane interaction of the conformation shown in *Fig. 1b*.

Discussion. – To react with membranes, peptides must have a pronounced amphiphilic character [7]. Amphiphilicity is a consequence of the segregation of hydrophobic and hydrophilic properties in a peptide molecule in its membrane-bonded conformation. It may be measured by the 'helical' or 'structural' hydrophobic moments [12], or by the 'molecular amphiphilic moment' [8]. The estimated amphiphilic moment of substance P in the conformation shown in *Fig. 1a* was strong enough to reduce thermal tumbling of the molecule to such a degree, and had the proper direction to orient it in such a manner as to allow membrane interaction through the relatively more hydrophobic C-terminal segment [6] [8]. Studies with IR attenuated total reflection spectroscopy supported the predicted 'almost perpendicular' orientation of a substance P C-terminal α-helix in contact with flat lipid membranes [9].

Membranes usually possess a surface dipole moment that is oriented quite perpendicularly on the surface and points its negative end towards the aqueous phase. Its magnitude for phosphatidylcholine membranes has been estimated as *ca.* 14 debye per lipid molecule [15]; however, its exact location in the H belt and head-group layers is unknown. If a peptide penetrates this layer, another stabilizing or destabilizing effect will be produced by the interaction of the membrane surface dipole moment with the effective dipole moment of the peptide [8]. Should substance P penetrate into the membrane dipole layer, the effective dipole moment would reinforce the effect of the amphiphilic moment and stabilize the orientation of the substance-P helix on the membrane *(Fig. 2)*.

Substance P has a random conformation in H_2O [17], where it tends to form aggregates containing β-structures [18]. Contact of the C-terminal, relatively hydrophobic segment of substance P with relatively hydrophobic membrane layers was assumed to induce an α-helical conformation as is quite generally postulated for peptides and proteins [16] [10] (see [8] for a discussion of effects of the diffuse membrane-H_2O interphase). Helix induction on membranes was compatible with the CD experiments of *Wu et al.* [19] using solubilized lipids, and with our own CD experiments using liposomes [9]. The estimated helix length of nine residues was in excellent agreement with the results of an IR amid I band shape analysis of Substance-P peptides in the membrane-mimicking solvent CF_3CH_2OH [9].

The estimated hydrophobic and electrostatic membrane association of substance P suggested that interactions with neutral membranes will be seen only at excessively high concentrations, but that interactions with anionic membranes may be observed with IR and CD at concentrations of substance P of 0.1 to 1 mM. This agreed qualitatively with the spectroscopically observed induction of helical domains in substance P by solubilized phosphatidylserine and sodium dodecyl sulfate [19] [14], but not by phosphatidylcholine [19]. The predictions were also supported by the experiments of *Lembeck et al.* [20] who studied the partitioning of substance P and related peptides between buffers and solutions of lipid in a $MeOH/CHCl_3$ mixture. They found that the peptides partitioned into the

organic phase as a function of the positive charge on the peptide and the negative charge on the lipid.

The predictions for membrane association of *Table 2* agreed with our own observations [9]. We studied the helix formation of the three peptides of *Table 2, i.e.* substance P, [Leu^9]substance P, and des-Arg^1-substance P, with CD at peptide concentrations of $1 \cdot 10^{-4}$ M in the presence of liposomes prepared from mixtures of phosphatidylserine and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine. In 10 mM KCl, a liposome preparation of pure phosphatidylserine may have a *Gouy-Chapman* surface potential of roughly −120 mV, a preparation with 10–20% phosphatidylserine a potential of *ca.* −40 mV (see [15]). The predictions from *Table 2* were compatible with the results: all three peptides did not interact with neutral liposomes, and all interacted strongly with liposomes containing only phosphatidylserine. However, the expected differences showed up with liposomes containing 10–20% phosphatidylserine: des-Arg^1-substance P showed no interaction, substance P interacted marginally, and the interaction of [Leu^9]substance P could not be distinguished from that with liposomes with 100% phosphatidylserine.

After completion of this manuscript, Dr. *C. M. Deber* kindly gave us a preprint of a paper then in press [21a] and personally discussed the matter with us [21b]. The authors contend that the influence of membrane lipids on substance P conformation may be critical to substance-P-receptor interactions. They characterized in detail the complexes formed between substance P and sodium dodecyl sulfate, lysophosphatidylglycerol, and lysophosphatidylcholine micelles. CD spectra displayed significant induced secondary structure upon addition of these lipids. Potentiometric titration data demonstrated increases of the pK_a of the peptide N-terminal and lysine side chain amino groups, suggesting direct interaction of the substance P N-terminus with the lipid head group region. Red shifts in UV spectra of the Phe rings in the membrane-bounded peptide suggested an increased hydrophobic environment for these substituents. High-resolution 1-D and 2-D COSY NMR spectra displayed differential chemical shift movements of Gln, Leu, and Met NH protons with added lipid, suggesting involvement of the C-terminal portion of the peptide in the induced secondary structure. Furthermore, the side-chain carbamoyl group of Gln-6 behaved differently from that of Gln-5: it showed chemical shifts expected for increasingly hydrophobic surroundings and for paramagnetic shielding by Phe side chains. This is in complete agreement with our predicted substance P membrane structure, particularly with respect to the proposed helix length.

Conclusions. – We predict that substance P interacts reversibly with lipid membranes from its aqueous solutions. With neutral phosphatidylcholine membranes, the molar dissociation constant is in the order of $5 \cdot 10^{-2}$ M, but with anionic lipid membranes having *Gouy-Chapman* surface potentials of −40 and −120 mV, the molar dissociation constant may be as low as $4 \cdot 10^{-4}$ M and $4 \cdot 10^{-8}$ M, respectively. During adsorption, the random-coil conformation of substance P in H_2O is changed to a highly ordered structure on the membrane. We propose the model of *Fig. 2* as representing the preferred membrane structure of substance P.

The C-terminal message-containing nonapeptide segment (residues 3–11) is quite perpendicularly oriented on the membrane as an α-helical domain, whereas the N-terminal segment, which is part of the address, remains in the aqueous phase as a random-coil domain. This structure is supported by experimental observations reported in the literature and recently made by ourselves (see the accompanying papers [9]).

The predicted membrane structure of substance P is analogous to those of adrenocorticotropin-(1–24)-tetracosapeptide and dynorphin-A-(1–13)-tridecapeptide [7] [8]. In all these cases, the receptor-triggering message domains are inserted into the membranes as quite perpendicularly oriented α-helices. The message is C-terminal in substance P, but N-terminal in the other two neuropeptides, and the hydrophobic and electrostatic association of substance P is much weaker. The membrane structures of opioid peptides (enkephalins, dynorphins, endorphins) are essential elements of the molecular mechanism of receptor subtype selection [6]. Whether or not the membrane structures of substance P and the tachykinins are relevant for receptor selection shall be discussed elsewhere.

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