The EMBO Journal vol.6 no.8 pp.2255–2259, 1987

Membrane-assisted molecular mechanism of neurokinin receptor subtype selection

Robert Schwyzer

Department of Molecular Biology and Biophysics, Swiss Federal Institute of Technology (ETH), CH-8093 Zürich, Switzerland

Communicated by R.Schwyzer

Based on the observed membrane structures of substance P, physalaemin, and eledoisin, preferred conformations, orientations and accumulations of 13 mammalian neurokinins and non-mammalian tachykinins were estimated and compared with pharmacologic and selective binding data taken from the literature. Principal site affinities and relative affinities supported the view that neurokinins bind to three principal mammalian sites: the NK-1 (preferring substance P), the NK-2 (preferring neurokinin A), and the NK-3 site (preferring neurokinin B). Strong hydrophobic membrane interaction of the C-terminal message segment as a perpendicularly oriented α-helical domain correlated with NK-1 selection. Electrostatic accumulation of the peptide at the anionic fixed charge layer of the membrane without hydrophobic interaction through a helix correlated with NK-2 preference. Electrostatic repulsion by the anionic fixed charge layer correlated with NK-3 selection. Thus, neurokinin receptor selection is guided by the same principles as opioid receptor selection. Membrane catalysis of specific agonist–receptor interactions may prove to be a quite general phenomenon, and the membrane structure of a peptide more important for its structure–activity relationship than its crystal structure or its mixture of conformers in solution or *in vacuo*.

Key words: neurokinins/tachykinins/substance P/membrane structures/receptor subtype selection

Introduction

The three mammalian tachykinins or neurokinins – substance P, neurokinin A and neurokinin B (Table I) – are reported to bind selectively to three distinct tachykinin receptor subtypes called the NK-1, NK-2 and NK-3 sites (using the nomenclature of Buck and Burcher, 1986a). The principal site affinity (Table II) of substance P is for the NK-1 site, neurokinin A binds preferentially to the NK-2 site and neurokinin B is the preferred ligand for the NK-3 site (Buck and Burcher, 1986b; Laufer *et al.*, 1986; Regoli *et al.*, 1987).

Tachykinins from amphibians and molluscs – physalaemin, kassinin and eledoisin – react with the same three sites, although more weakly and less selectively. The eledoison-preferring SP-E receptor (Erspamer *et al.*, 1980; Lee *et al.*, 1982), is no longer considered to be a distinct neurokinin site (for review, see Regoli *et al.*, 1987). It may represent mixtures of binding sites, its special properties being the result of the low selectivity of eledoisin for mammalian receptors (Table II).

The observed, rather complicated pharmacological patterns of neurokinins and tachykinins may arise from a combined stimulation of receptors present on different cells in a tissue: NK-3 sites are reported to be located only on postganglionic cholinergic neurons, NK-1 sites are present on these neurons and on smooth muscle cells (and possibly on neuroendocrine cells) and NK-2 sites occur only on smooth muscle cells (Barthó and Holzer, 1985; Laufer *et al.*, 1985; Buck and Burcher, 1986b; Kilbinger *et al.*, 1986; Regoli *et al.*, 1987).

The rank order of tachykinin binding at the three sites (Table II) suggests a requirement of the NK-1 receptor for an aromatic amino acid residue at the fourth position from the C terminus, whereas the NK-2 and NK-3 receptors appear to require β-branched aliphatic residues at this position. However, Cascieri *et al.* (1986) exclude the exchange of Phe-8 in substance P for Ile-8 and Val-8 in eledoisin and neurokinin B as a necessary and sufficient cause for receptor selection. They find that the SP-E (NK-2/NK-3) site has a requirement for a folded conformation of the C-terminal pentapeptide segment that can be met to certain degrees by all tachykinins (see also Lavielle *et al.*, 1986). Thus, the selective interaction of tachykinins with the different sites remains unexplained and the influence of the different N-terminal segments on receptor selectivity must be considered. Cascieri *et al.* (1986) state that 'unknown determinants in the amino-terminal sequences of substance P must strongly contribute to carboxyl-terminal peptide selectivity and conformation'.

This postulate is compatible with observations that a C-terminal domain of the tachykinins (comprising their homologous C-terminal penta- to hexapeptide segments) is necessary for triggering neurokinin receptors, whereas the 'inactive' N-terminal domains serve to distinguish receptor subtypes and cause different biologic actions (Iversen, 1982, 1983; Oehme and Krivoy, 1983; Regoli *et al.*, 1984; Roske *et al.*, 1986; Treptow *et al.*, 1986). Such a subdivision into domains with different functions is quite generally found in flexible regulatory peptides. The triggering domains have been called 'message' segments and the domains endowing the peptide with receptor selectivity 'address' segments (Schwyzer, 1980).

The problem of receptor subtype selection is even more pronounced in opioid peptides, which have fully identical message

Table I. Amino acid sequences of tachykinins and analogs

Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
Neurokinin A	His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2
Neurokinin B	Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2
Physalaemin	Pyr-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH_2
Eledoisin	Pyr-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-NH_2
Kassinin	Asp-Val-Pro-Lys-Ser-Asp-Gln-Phe-Val-Gly-Leu-Met-NH_2
Septide	Pyr-Phe-Phe-Pro-Leu-Met-NH_2
(Gly-9)septide	Pyr-Phe-Phe-Gly-Leu-Met-NH_2
Senktide	Suc-Asp-Phe-MePhe-Gly-Leu-Met-NH_2
(C-3,6 Y-8)SP	Arg-Pro-Cys-Pro-Gln-Cys-Phe-Tyr-Gly-Leu-Met-NH_2
(C-2,5)NKB	Asp-Cys-His-Asp-Cys-Phe-Val-Gly-Leu-Met-NH_2
Antagonist A	Arg-pro-Lys-Pro-Gln-Gln-trp-Phe-trp-Leu-Met-NH_2
Antagonist B	Arg-Gln-trp-Phe-trp-Leu-Nle-NH_2

Suc is succinyl, SP is substance P, NKB is neurokinin B, and lower-case pro and trp indicate D-Pro and D-Trp respectively.

sequences. The molecular mechanism by which their address domains influence receptor subtype selectivity has been elucidated as a function of their interaction with lipid membranes (Schwyzer, 1986a). The address domains determine the accumulation, conformation and orientation of the message domains in one or other of three compartments of lipid bilayer membranes. This facilitates the interaction of the message with one or other of three different opioid receptor sites each located in one of these compartments. Opioid receptor selection is a special case of the selective catalysis of peptide–receptor interactions by the lipid phase of target cell membranes (Sargent and Schwyzer, 1986). Here, I provide evidence that tachykinin receptor selection is guided by the same principles as opioid receptor selection. The starting point was the similarity of the membrane-induced structure of substance P with that of dynorphin-(1-13)-tridecapeptide and adrenocorticotropin-(1-24)-tetracosapeptide in which the message segments assume a helical conformation that is in contact with hydrophobic membrane layers and is oriented perpendicularly on the membrane surface and in which the address segments are exposed to the bulk aqueous phase (Erne *et al.*, 1986; Rolka *et al.*, 1986; Schwyzer *et al.*, 1986).

Results

Neurokinin binding sites

Table II presents binding data of Buck *et al.* (1984), Burcher and Buck (1986) and Laufer *et al.*, (1986) according to the

Table II. Binding characteristics of neurokinins and tachykinins (the terms of affinity and relative affinity are defined in Materials and methods)

	Principal site affinity				Relative affinity[a]			
	NK-1[b]	NK-2[c]	NK-3[d]	E[e]	NK-1[b]	NK-2[c]	NK-3[d]	E[e]
SP	10.5	–	–	–	0.998	0.001	0.001	
					0.997	0.001	0.001	0.001
Phys	3.51	–	–	–	0.990	0.001	0.009	
					0.997	0.001	0.010	0.013
NKA	–	1.09	–	–	0.186	0.781	0.032	
					0.183	0.767	0.032	0.019
NKB	–	–	3.34	–	0.006	0.008	0.986	
					0.005	0.007	0.855	0.133
Eled	–	–	–	0.26	0.391	0.375	0.234	
					0.278	0.267	0.167	0.289
Kass	–	0.55	–	–	0.242	0.579	0.179	
					0.190	0.455	0.141	0.215

[a]Calculated for three and four sites.
[b]Displacement of 70 pM ^{125}I-Bolton–Hunter-labeled substance P from guinea-pig urinary bladder membranes (P sites), K_d = 120 pM (Burcher and Buck, 1986).
[c]Displacement of 70 pM ^{125}I-Bolton–Hunter-labeled neurokinin A from hamster urinary bladder membranes (K sites), K_d = 740 pM (Burcher and Buck, 1986).
[d]Displacement of 400–800 pm ^{125}I-Bolton–Hunter-labeled (Asp-5,6, *N*-methyl-Phe-8)substance P (5–11) heptapeptide ([^{125}I]BH-NH-Senktide) from rat cerebral cortex membranes (N sites), K_d = 900 pM (Laufer *et al.*, 1986). [L] was assumed to be 600 pM in all experiments, which gives possible errors of ~ ±15% in K_d.
[e]Displacement of 100 pM ^{125}I-Bolton–Hunter-labeled eledoison from rat cerebral cortex membranes (E sites, Buck *et al.*, 1984); K_d was assumed to be approximately equal to IC_{50} of eledoisin.

Table III. Estimated parameters of tachykinin–membrane interaction

Tachykinin	Association					Orientation	
	z	*m*	$\Delta G°_a$ (kJ/mol)	K_d	K_d(Bol)	*A*	Φ (degrees)
Substance P	3+	9	−20.5	2.5(−4)	9.3(−3)	338	165
Physalaemin	0	8	−15.3	2.1(−3)	1	271	169
Eledoisin	0	7	5.1	7.8	1	340	155
Kassinin	0	7	5.8	10.4	1	504	157
Neurokinin A	2+	7	17.7	1270	5.4(−2)	381	156
Neurokinin B	1−	7	−2.8	0.3	5	374	154
Septide	0	6	2.0	2.2	1	38	124
(Gly-9)septide	0	6	10.0	57	1	42	116
Senktide	2−	6	−5.0	16	22.5	175	145
(C-3,6,Y-8)SP	2+	9	−24.7	4.6(−5)	5.4(−2)	253	163
(C-2,5)NKB	1−	7	4.6	6.4	1	258	163
Antagonist A	3+	9	−23.1	9.1(−5)	9.3(−3)	329	175
Antagonist B	2+	7	−11.6	2.8(−3)	5.4(−2)	174	160

Estimation: see Materials and methods; *z* is the net charge, *m* the number of residues in the helix at the energy minimum (counted from the C terminus), $\Delta G°_a$ the free energy of association with a neutral aqueous/hydrophobic interface. K_d is the molar equilibrium constant calculated from the hydrophobic interaction, K_d(Bol) that resulting from electrostatic interaction of the peptide with the membrane (powers of ten are shown in brackets). *A* is the scalar magnitude of the amphiphilic moment in arbitrary units, Φ the angle of this vector with the helix axis.

scheme of Kosterlitz and Paterson (1985) for opioid receptors. It summarizes the affinities and selectivities of mammalian neurokinins and non-mammalian tachykinins for mammalian binding sites. The strongest affinity is that of the NK-1 site for substance P, followed by that for physalaemin. The affinities of neurokinin A and neurokinin B for their respective sites, NK-2 and NK-3, are weaker. The relative affinity (site selectivity) of substance P, physalaemin and neurokinin B for their principal sites is great; that of neurokinin A is definitely weaker, as this peptide slightly interacts with the NK-1 site. The principal site affinities of eledoisin and kassinin for mammalian receptors are weak, and their selectivities much less pronounced. The E site prefers the ligands eledoisin, kassinin and neurokinin B over neurokinin A, physalaemin and substance P. This supports the idea that the E site may be a mixture of NK-3 and NK-2 sites (Buck *et al.*, 1984; Laufer *et al.*, 1985; Wormser *et al.*, 1986; Regoli *et al.*, 1987). I shall therefore discuss the selection mechanisms for NK-1, NK-2 and NK-3 sites, only.

Estimated parameters of tachykinin–membrane interaction, Table III

Substance P binds reversibly to artificial anionic lecithin membranes from aqueous solutions (Rolka *et al.*, 1986). The C-terminal nonapeptide segment folds into an α-helix which is oriented perpendicularly on the membrane surface (Erne *et al.*, 1986). The N-terminal charged segment remains in the aqueous phase. The change of the membrane surface dipole moment (20 D per molecule) agrees with this orientation of a short helical segment. The molar equilibrium dissociation constant corrected for the electrostatic Boltzmann accumulation of the positively charged peptide on the anionic membrane surface is found to be K_d = 0.235 mM (unpublished data). The estimated values of Table III agree with these experimental results. The energy minimum for hydrophobic interaction, corresponding to K_d = 0.25 mM, is reached with a helix length of m = 9 amino acid residues. The estimated amphiphilic moment has a scalar magnitude ($A > 150$), sufficient to ensure a stable orientation on an interface (Schwyzer, 1986a). Its direction (165°) points the C terminus of the helical domain towards the hydrophobic layers of the membrane, only 15° away from a perpendicular orientation on the surface. The electric dipole moment of an α-helix with nine residues (not shown in Table I) is ~30 D (Wada, 1976; Hol, 1985); it points in the proper direction to reduce the membrane surface dipole moment as observed.

The estimated parameters of Table III indicate the probability that a peptide will assume a membrane structure similar to that of substance P. This is only possible with an appreciable hydrophobic interaction and a proper magnitude and orientation of the amphiphilic moment vector. In addition, K_d(Bol) is a measure for the electrostatic accumulation of a peptide in the fixed charge layer.

Hydrophobic association and NK-1 selection

All peptides of Table III that are predicted to assume a membrane structure similar to that of substance P show selective interaction with the NK-1 site. Although the K_d values are higher than the physiological peptide concentrations, they are expected to be sufficient to catalyze interactions with membrane-bound receptors (Sargent and Schwyzer, 1986). As this type of catalysis influences not only the rate, but also the equilibrium constant of the reaction, membrane binding offers a plausible explanation for the particularly strong affinity of substance P for NK-1.

Physalaemin is expected to have a membrane structure similar to that of substance P, although it binds more weakly to the interface. This was confirmed experimentally for its interaction with artificial planar lecithin bilayers (unpublished data), and agrees with its NK-1 affinity and preference (Table II).

(Cys-3,6, Tyr-8)substance P is reported to bind ~1.5 times more strongly to NK-1 sites than substance P (Lavielle *et al.*, 1986). This is reflected in its predicted strong hydrophobic binding and the almost perpendicular orientation of the helix on an aqueous–hydrophobic interface. According to CPK models of the helical peptide, a disulfide bond with its preferred dihedral angle of 90° is easily formed between Cys-3 and Cys-6.

The two antagonists A and B (of a type pioneered by Folkers *et al.*, 1986) are also expected to show appreciable amounts of substance P-like membrane structures provided the message domain can assume a helical structure despite its content of D-Trp beside L-amino acids. Models show that this is easily possible without constraint by hard-sphere repulsions. I expect the stability of helices containing D- and L-amino acids to be less impaired in hydrophobic than in aqueous media, where optimal hydrophobic contacts between side-chains are important. This was confirmed by studies with substance P analogs containing D- or L-leucine in position 9 (unpublished data).

Pharmacologic analysis of the action of the two antagonists A and B on the guinea-pig ileum myenteric plexus–longitudinal muscle preparation indicates a preference for NK-1 over NK-3 (E) sites (Kilbinger *et al.*, 1986). These findings are slightly at variance with those of Jacoby *et al.* (1986) who find equal competition of the actions of substance P and of neurokinin B by antagonist A. This demonstrates the difficulty of unequivocally assigned pharmacologic effects to specific receptor subtype interactions. Regoli *et al.* (1987) conclude from their studies with site-specific assay systems that this type of antagonist appears 'to be adequate perhaps only for studying NK-P receptors'. In sum, I conclude from the literature that antagonists A and B interact considerably with NK-1 sites, which agrees with the prediction.

Electrostatic membrane interactions and the selection of NK-2 and NK-3 sites

The NK-2 and NK-3 sites do not require the message domain of the peptides as a perpendicularly oriented helix in contact with a relatively hydrophobic membrane compartment. This follows from the unfavorable hydrophobic interaction energies estimated for neurokinin A and neurokinin B in substance P-type membrane structures. However, the positively charged neurokinin A is predicted to accumulate in the (aqueous) anionic fixed charge layer of the membrane, whereas neurokinin B which carries excess negative charge (caused by the low pK value of the His imidazolium group) will be repulsed. It may interact with a receptor site protruding into the aqueous phase at a distance comparable to the Debye–Hückel length and situated in a positively charged environment, whereas neurokinin A would preferably interact with a site located in a negatively charged environment. This situation resembles that observed for opioid μ and δ site selection (Schwyzer, 1986a).

Eledoisin was shown by i.r. attenuated total reflection spectroscopy not to interact with lipid membranes in the presence of water. When forced into contact with phosphatidylcholine membranes, it assumed a β-structure, perhaps the result of peptide aggregation (unpublished data). These findings agree with the predicted membrane and site interaction. As a neutral peptide without appreciable substance P-like membrane structure, it has access to the NK-3 and NK-2 sites, but its access to the NK-1 sites is less favorable.

Kassinin is predicted to behave similarly to eledoisin when in contact with membranes. In bioassays, kassinin has been found to interact with E-type receptors in much the same manner as eledoisin (Erspamer *et al.*, 1980; Iversen, 1982). This agrees with the binding data, Table II.

Septide and (Gly-9)septide, derivatives of the substance P-(6-11) message segment, are predicted not to interact with lipid membranes and NK-1 sites as substance P does. Their estimated hydrophobic membrane interactions and amphiphilic moments are unfavorable. Electrostatic accumulation or repulsion at the fixed charge layer is not expected. This suggests a strong preference of NK-2 and NK-3 over NK-1 sites, which is supported by reports from other authors. Thus, (Gly-9)septide is ~40 times less potent than substance P in displacing [^{125}]-Bolton–Hunter-substance P from rat brain cortex and parotid cells, but about as efficient as eledoisin in displacing [^{135}I]-Bolton–Hunter-eledoisin (Cascieri *et al.*, 1986). In the guinea-pig ileum bioassay, it is ~10 times more potent on the 'muscular receptor' (NK-1 plus NK-2) than on the 'neuronal receptor' (NK-1 plus NK-3) as shown by atropine sensitivity (Wormser *et al.*, 1986). Extremely weak binding at NK-1 sites (Cascieri *et al.*, 1986) suggests that its atropine-insensitive action is at NK-2 sites on smooth muscle cells, and that the atropine-sensitive action is at nerve cell NK-3 sites, without appreciable action at NK-1 sites.

Conformation constraints affect the site selection of (Gly-9)septide

A two-carbon bridge between the α-carbon atom of Gly-9 and the amino group of Leu-10 decreases the potency against the substance P radioligand, but leaves that against the eledoisin radioligand unchanged (Cascieri *et al.*, 1986). Pro-9 in septide does not influence biologic potency at the 'muscular receptor' (NK-2), but reduces that at the 'neuronal receptor' (NK-3) by two orders of magnitude. Introduction of Me-Phe-8 into (Gly-9)septide enhances the action on the 'neuronal receptor' (NK-3) ~10-fold, but decreases that at the 'muscular receptor' (NK-2) by the same amount (Wormser *et al.*, 1986). As hydrophobic and electrostatic membrane interactions are expected to be similar in all these cases, the observed activities may suggest that conformational constraints change receptor selectivity by causing the peptides to meet different conformational receptor requirements. Thus, the two-carbon bridge is tolerated by NK-2 and/or NK-3 sites, Pro-9 in septide is tolerated by the NK-2, but not the NK-3 site, and Me-Phe-8 does not meet the NK-2 site requirements, but facilitates interaction with NK-3 sites. Similar conformational receptor requirements are well documented for opioid μ and δ sites (Schiller and DiMaio, 1982; Schiller *et al.*, 1985a,b).

Senktide is a selective NK-3 (SP-N) agonist (Wormser *et al.*, 1986) and an excellent ligand for the NK-3 site (Laufer *et al.*, 1986; see Table II). This agrees with its predicted inability to assume a substance P-like membrane structure, and with its repulsion from the anionic fixed charge layer (Table III). Replacement of its negative by positive charges as in (Arg-6, Me-Phe-8)substance P-(6-11)-hexapeptide strongly impairs NK-3 site selection (Wormser *et al.*, 1986), again in agreement with the prediction. Introduction of D-Pro and D-Trp into substance P sequences (Folkers *et al.*, 1986, and references cited therein) invariably leads to compounds with appreciable NK-1 interaction (e.g. antagonists A and B, Table III). However, in the guinea-pig ileum bioassay, (D-Pro-2, D-Trp-6,8, Nle-10)neurokinin B is a strong competitive inhibitor of the action of neurokinin B, but not of substance P and neurokinin A (Jacoby *et al.*, 1986). Apparently, the net negative charge inhibits its approach to the anionic fixed charge layer and, hence, its hydrophobic interaction with the membrane, and directs the peptide to the positive environment of the NK-3 site. (Cys-2,5)neurokinin B, finally, is a selective ligand for the [^{3}H]neurokinin B specific binding site on rat cortical synaptosomes (Lavielle *et al.*, 1986). This agrees with its predicted properties on a membrane: no hydrophobic interaction and repulsion because of its negative charge.

Discussion

This study suggests that selection of NK-1, NK-2 and NK-3 sites by neurokinins and tachykinins is guided by the same principles as the selection of κ, μ and δ sites by opioid peptides (Schwyzer, 1986a). NK-1 and κ sites require hydrophobic membrane association of the peptide message segments as perpendicularly oriented α-helical domains, whereas the hydrophilic address segments remain in contact with the aqueous phase. Electrostatic accumulation of the peptide message segment in the anionic fixed charge layer of the membrane, which is located between the bulk aqueous phase and the hydrophobic compartment, facilitates interaction with NK-2 and μ sites. It attenuates the interaction with NK-3 and δ sites which require peptides with zero or negative charges. These membrane requirements for productive receptor interaction are the basis for catalysis of specific peptide–receptor interactions by the lipid phase of the target cell membrane (Schwyzer, 1985; Sargent and Schwyzer, 1986). The membrane requirements complement the classical receptor requirements for receptor selection. This study therefore suggests that the membrane structure of a peptide (its accumulation, conformation and orientation on an aqueous–hydrophobic interface or membrane surface) may be more important for the study of structure–conformation–activity relationships (Schwyzer, 1963, 1970) than its conformation in a crystal, in solution or *in vacuo*.

Materials and methods

The parameters used for estimating conformation, orientation and accumulation of peptides on neutral and charged aqueous–hydrophobic interfaces or lipid membrane surfaces are described by Schwyzer (1986b,c) and Schwyzer *et al.* (1986).

The Gibbs free energy of hydrophobic association, $\Delta G°_a(m)$, through m residues at the C-terminal end of tachykinins was calculated from the free energy of transfer, $\Delta G°_{tr}(i)$, of the individual residues from their random-coil conformation in H_2O to their helical conformation in a hydrophobic phase (Schwyzer *et al.*, 1986). However, it was assumed that only one hydrogen bond with water is broken by introducing the methionine primary amide nitrogen into the hydrophobic phase and that the energy needed to break hydrogen bonds of the methionine sulfur atom is proportional to its Pauling electronegativity value.

The amphiphilic moment describes the segregation of charged and uncharged amino acid residues into hydrophobic and hydrophilic domains with respect to the helix center. An angle $\Phi = 0°$ indicates that the more hydrophilic end of the amphiliphic moment vector points along the helix axis towards the C terminus. The vector is therefore a measure for the orientation of a helix in a hydrophobic gradient or on an interface.

The electric dipole moment was not explicitly calculated for all compounds, as it tends to reinforce the amphiphilic moment of the tachykinins (Schwyzer *et al.*, 1986).

The Boltzmann accumulation of charged peptides on the membrane surface was estimated for an assumed Gouy–Chapman surface potential of $V_{gc} = -40$ mV as a rough approximation of the charge on a biologic lipid membrane.

A presentation of binding data was chosen which is similar to that introduced by Kosterlitz and Paterson (1985). These authors define the inhibitory binding constants by $K_i = IC_{50}/(1 + [L]K_d)$, where [L] is the concentration of the labeled ligand and K_d its equilibrium dissociation constant (Cheng and Prusoff, 1973). The binding affinity constant $(K_i, nM)^{-1}$ is the reciprocal of the inhibition constant and was used to describe the affinity of a ligand to its principal site in Table II. To give an indication of the relative binding affinities at different sites 1, 2 and 3, Kosterlitz and Paterson (1985) use the following expression:

$$K_i^{-1}(1, 2 \text{ or } 3)/[Ki^{-1}(1) + K_i^{-1}(2) + K_i^{-1}(3)]$$

Space-filling molecular models were used throughout and were built with a CPK Precision Molecular Models Protein Set (The Ealing Corporation, South Natick, MA).

References

Barthó,L. and Holzer,P. (1985) *Neuroscience*, **16**, 1–32.

Buck,S.H. and Burcher,E. (1986a) *Trends Pharmacol. Sci.*, **7**, 437.

Buck,S.H. and Burcher,E. (1986b) *Trends Pharmacol. Sci.*, **7**, 65–68.

Buck,S.H., Burcher,E., Schults,C.W., Lovenberg,W. and O'Donohue,T.L. (1984) *Science*, **226**, 987–989.

Burcher,E. and Buck,S.H. (1986) *Eur. J. Pharmacol.*, **128**, 165–177.

Cascieri,M.A., Chicchi,G.G., Freidinger,R.M., Colton,C.D., Perlow,D.S., Williams,B., Curtis,N.R., McKnight,A.T., Maguire,J.J., Veber,D.F. and Liang,T. (1986) *Mol. Pharmacol.*, **29**, 34–38.

Cheng,Y.-C. and Prusoff,W.H. (1973) *Biochem. Pharmacol.*, **22**, 3099–3108.

Erne,D., Rolka,K. and Schwyzer,R. (1986) *Helv. Chim. Acta*, **69**, 1807–1816.

Erspamer,G.F., Erspamer,V. and Picinelli,D. (1980) *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **311**, 61–65.

Folkers,K., Rosell,S., Chu,J.-Y., Lu,L.-A., Tang,P.-F.L. and Ljungqvist,A. (1986) *Acta Chem. Scand.*, **B40**, 295–302.

Hol,W.G.J. (1985) *Prog. Biophys. Mol. Biol.*, **45**, 149–195.

Iversen,L.L. (1982) *Br. Med. Bull.*, **38**, 277–282.

Iversen,L.L. (1983) *Annu. Rev. Pharmacol. Toxicol.*, **23**, 1–27.

Jacoby,H.I., Lopez,I., Wright,D. and Vaught,J.L. (1986) *Life Sci.*, **39**, 1995–2003.

Kilbinger,H., Stauss,P., Erlhof,I. and Holzer,P. (1986) *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **334**, 181–187.

Kosterlitz,H.W. and Paterson,S.J. (1985) *Phil. Trans. R. Soc.*, **B308**, 291–297.

Laufer,R., Wormser,U., Friedman,Z.Y., Gilon,C., Chorev,M. and Selinger,Z. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 7444–7448.

Laufer,R., Gilon,C., Chorev,M. and Selinger,Z. (1986) *J. Biol. Chem.*, **261**, 10257–10263.

Lavielle,S., Chassaing,G., Besseyre,J., Marquet,A., Bergström,L., Beaujouan, J.-C., Torrens,Y. and Glowinski,J. (1986) *Eur. J. Pharmacol.*, **128**, 283–285.

Lee,C.M., Iversen,L.L., Hanley,M.R. and Sandberg,B.E.B. (1982) *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **318**, 281–287.

Oehme,P. and Krivoy,W.A. (1983) *Trends Pharmacol. Sci.*, **4**, 521–523.

Lembeck,F., Amann,R. and Barthó,L. (1986) *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **333**, 290–293.

Regoli,D., Escher,E. and Mizrahi,J. (1984) *Pharmacology*, **28**, 301–302.

Regoli,D., Drapeau,G., Dion,S. and D'Orléans-Juste,P. (1987) *Life Sci.*, **40**, 109–117.

Rolka,K., Erne,D. and Schwyzer,R. (1986) *Helv. Chim. Acta*, **69**, 1798–1806.

Roske,I., Oehme,P., Hecht,K., Nieber,K., Hilse,H. and Wachtel,E. (1986) *Pharmazie*, **41**, 799–805.

Sargent,D.F. and Schwyzer,R. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 5774–5778.

Schiller,P.W. and DiMaio,J. (1982) *Nature*, **298**, 74–76.

Schiller,P.W., Nguyen,T.M.-D., Lemieux,C. and Maziak,L.A. (1985a) *J. Med. Chem.*, **28**, 1766–1771.

Schiller,P.W., Nguyen,T.M.-D., Maziak,L. and Lemieux,C. (1985b) *Biochem. Biophys. Res. Commun.*, **127**, 558–564.

Schwyzer,R. (1963) *Pure Appl. Chem.*, **6**, 265–295.

Schwyzer,R. (1970) In Bucher,H. (ed.), *Proc. IV International Congress on Pharmacology*. Schwabe, Basel, Vol. 5, pp. 196–209.

Schwyzer,R. (1980) *Proc. R. Soc.*, **B210**, 5–20.

Schwyzer,R. (1985) In Zalewski,R.I. and Skolik,J.J. (eds), *Natural Products Chemistry 1984*. Elsevier, Amsterdam, pp. 591–598.

Schwyzer,R. (1986a) *Biochemistry*, **25**, 6335–6342.

Schwyzer,R. (1986b) *Biochemistry*, **25**, 4281–4286.

Schwyzer,R. (1986c) *Helv. Chim. Acta*, **69**, 1685–1698.

Schwyzer,R., Erne,D. and Rolka,K. (1986) *Helv. Chim. Acta*, **69**, 1789–1797.

Treptow,K., Morgenstern,R., Oehme,P. and Bienert,M. (1986) *Pharmazie*, **41**, 727–729.

Wada,A. (1976) *Adv. Biophys.*, **9**, 1–63.

Wormser,U., Lauffer,R., Hart,Y., Chorev,M., Gilon,C. and Selinger,Z. (1986) *EMBO J.*, **5**, 2805–2808.

Received on April 8, 1987