Journal of Computer-Aided Molecular Design, 8 (1994) 341–344
ESCOM

J-CAMD 259

Structural comparison of NK_2 receptor agonists and antagonists

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Received 3 February 1994
Accepted 2 May 1994

Key words: Tachykinins; NK_2; MEN-10627; Binding; Agonist; Antagonist

SUMMARY

The conformational space of two NK_2 receptor agonists and a new potent antagonist has been sampled by the simulated annealing technique. Low-energy conformers were obtained, which were compared with respect to their key residues, namely phenylalanine, leucine and methionine. The hypothesis is that they share part of the binding site on the receptor.

Mammalian tachykinins are peptide neurotransmitters which interact, with different affinities, with a class of G-protein-coupled receptors. The behaviour of the natural agonists differentiates these receptors as NK_1, to which Substance P (SP) shows the highest affinity, and NK_2 and NK_3, which preferentially bind Neurokinin A and Neurokinin B, respectively. These receptors, particularly NK_1 and NK_2, are considered to be involved in pain transmission and in the so-called 'neurogenic inflammation' [1]. The amino acid sequences of the tachykinins are depicted in Scheme 1.

Although more potent at their own 'preferred' receptor, natural TKs are, at different concentrations, equally effective in stimulating the three receptor types. For this reason a number of receptor-selective synthetic agonists have been developed, each stimulating exclusively only one of the three receptor types. [β-Ala^8]NKA(4–10) [2] and GR-64349 [3] are two of the most potent synthetic NK_2 receptor agonists available.

Recently, a new potent NK_2 receptor antagonist has been discovered, the polycyclic peptide derivative MEN-10627 [4]. This peptide shares a feature common among compounds that are active as agonists on the NK_2 receptor, i.e., the sequence leucine-methionine. With the purpose to gain some insight into the mechanism of action of this new class of peptides with respect to the NK_2 receptor agonists, we have performed a conformational comparison between MEN-10627 and the two agonists [β-Ala^8]NKA(4–10) and GR-64349.

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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
[β-Ala^8]NKA(4–10):	Asp-Ser-Phe-Val-βAla-Leu-Met-NMe_2
GR-64349:	Lys-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2
MEN-10627:	*cyclo*[(Asp-Trp-Phe-Dap-Leu-Met)*cyclo*(1β-4β)]

Scheme 1. Amino acid sequences of natural mammalian tachykinins and of two NK_2 receptor agonists and an antagonist.

Even if the actual binding site of MEN-10627 has not yet been identified, the size and the structure of the molecule lead us to suppose that it could at least in part overlap with that of the agonists. In the light of this hypothesis, and considering the extreme rigidity of the polycyclic system [4], together with its nanomolar affinity for the NK_2 receptor, we assumed the MEN-10627 backbone as a template carrying the bioactive conformation. We therefore searched the conformational space of the two agonists for energetically accessible conformers able to fit this template, in an 'active analogue approach' [5].

The lowest energy conformational space of the three compounds was explored by means of the Simulated Annealing technique [6–8]. This method allows a good sampling of the potential energy surface without assumptions on the starting conformation of the compound, because the compound itself is 'heated' to high temperature. Molecular dynamics is used by keeping a molecule at a maximum temperature for some time, then annealing through a ramped temperature decrease to low temperature, thus 'freezing' the obtained conformation. The resulting structure is then fully minimised. This cycle is repeated a number of times to yield a set of low-energy conformations. We used the SYBYL 6.0 package (Tripos Associates, Inc.), with the following steps for each annealing cycle: (1) heating to 500 K; (2) 5.4 ps molecular dynamics at this plateau temperature (1 fs steps); (3) cooling to 50 K in 1000 steps. This cycle was repeated 20 times to obtain 20 conformations, stored in a database [7,9]. Solvent was not explicitly included, but the effect was simulated by setting the dielectric function proportional to the distance [10]. Each

TABLE 1
ENERGIES OF THE LOW-ENERGY CONFORMATIONS OF THE COMPOUNDS UNDER INVESTIGATION

	Energy (kcal/mol)				Energy (kcal/mol)		
Conf. no.	MEN-10627	GR-64349	[β-Ala^8]NKA(4–10)	Conf. no.	MEN-10627	GR-64349	[β-Ala^8]NKA(4–10)
1	20.86	17.74	5.71	11	25.05	26.84	8.64
2	21.24	18.04	5.76	12	25.15	27.30	9.48
3	22.67	18.46	5.90	13	26.90	27.42	9.80
4	22.94	21.13	5.91	14	27.91	27.69	10.47
5	23.32	22.39	6.13	15	28.22	28.60	10.57
6	23.33	23.84	6.44	16	28.61	28.96	11.35
7	23.37	23.85	7.09	17	30.60	29.25	11.40
8	23.73	24.02	7.46	18	30.65	31.33	11.49
9	24.07	25.77	7.56	19	30.77	31.86	13.48
10	24.63	26.59	8.41	20	32.50	35.36	13.67

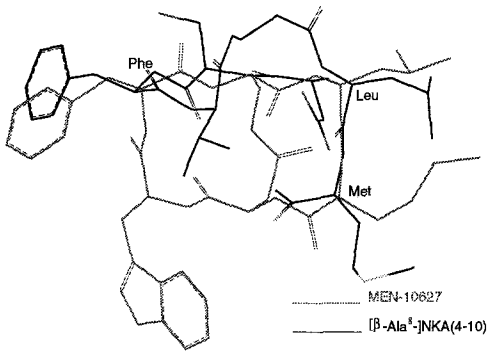

Fig. 1. Superposition of MEN-10627 (conformation 1) and [β-Ala^8]NKA(4–10) (conformation 1).

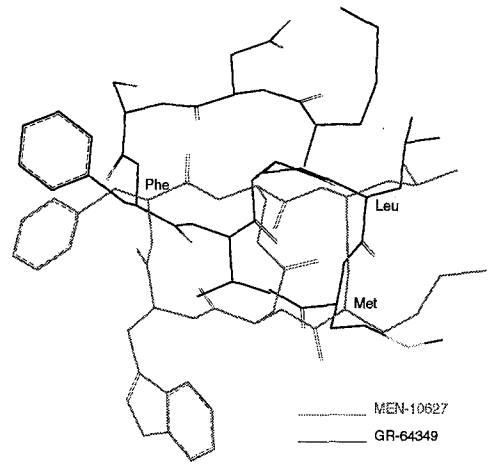

Fig. 2. Superposition of MEN-10627 (conformation 1) and GR-64349 (conformation 1).

structure was then minimised using the Tripos force field [11], with 500 steps of Powell minimisation [12], and then to an rms gradient of 0.05 kcal mol^{-1} Å^{-1} with the BFGS (Broyden, Fletcher, Goldfarb, Shanno) algorithm. Charges were calculated with MOPAC 5.0 [13] and the AM1 model Hamiltonian.

The polycyclic MEN-10627 peptide appeared to be very rigid: with the exception of some high-energy conformations, all conformations were well superposable on their backbone, mainly showing flipping of the amide bonds between the two trans forms of the bond. This is also confirmed by solution NMR data [4].

TABLE 2
RMS COMPARISON BETWEEN CONFORMATION 1 OF MEN-10627 AND AGONIST CONFORMERS

Conformation no.	rms (Å)	ΔC^{α} (Å)			ΔC^{β} (Å)	
		Phe	Leu	Met	Phe	Leu
[β-Ala^8]NKA(4–10)						
1	0.47	0.65	0.44	0.21	2.1	1.1
2	0.81	1.07	0.22	0.88	2.9	0.8
3	1.69	0.97	2.06	1.83	1.4	4.3
4	1.09	1.28	0.50	1.30	1.5	1.1
5	0.64	0.81	0.76	0.07	1.2	0.7
6	0.56	0.75	0.60	0.15	0.9	1.2
7	0.92	0.61	0.84	1.21	0.4	1.0
8	1.28	0.75	1.76	1.13	2.7	4.0
9	0.75	1.06	0.44	0.63	2.0	1.6
10	1.12	1.54	1.06	0.49	4.1	0.9
11	0.30	0.27	0.42	0.18	1.2	0.5
GR-64349						
1	0.53	0.46	0.72	0.34	2.4	0.7
2	0.89	0.53	1.10	0.95	0.6	0.9
3	1.18	0.82	0.99	1.59	1.0	0.8

Side chains clearly have more freedom, especially the benzyl moiety of phenylalanine. The lower energy conformation found by the method described above was taken as a reference. The 20 low-energy conformation sets obtained for the two agonists, [β-Ala^8]NKA(4–10) and GR-64349, are each ranked by potential energy, and values for the three molecules are listed in Table 1. The lowest energy conformers within a range of 3 kcal/mol are then compared by superposition to the chosen conformer of MEN-10627, matching the C^{α} of leucine, methionine and phenylalanine by rigid geometry fitting. Rms values of the C^{β} are also considered, since the direction of side chains is relevant for interaction with the receptor. Atom superposition requirements are, however, not very strict because of side-chain flexibility. Side chains are usually capable of some accommodation; this is especially true for the methionine side chain. Results are shown in Table 2, and the best fitting is illustrated in Figs. 1 and 2. The most interesting finding arising from this analysis is the close superposition of low-energy conformations of the two linear peptide agonists to MEN-10627 in the three key residues phenylalanine, leucine and methionine, which strongly supports the hypothesis of a consistent overlapping of their recognition sites on the NK_2 receptor protein. The superposition leads to the correspondence of amino acids among the three compounds as depicted below:

[β-Ala^8]NKA(4–10):	Asp-Ser-**Phe**-Val-βAla-**Leu-Met**-NMe_2
GR-64349:	Lys-Asp-Ser-**Phe**-Val-Gly-**Leu-Met**-NH_2
MEN-10627:	*cyclo*[(Asp-Trp-**Phe**-Dap-**Leu-Met**)*cyclo*(1β-4β)]

The single residue Dap (diaminopropionic acid) in the cyclic MEN-10627 compound substitutes the longer fragments Val-βAla and Val-Gly in the two linear peptides.

Results from site-directed mutagenesis experiments are expected to precisely identify the receptor residues relevant for binding of MEN-10627.

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