Structure-activity Relationship, Conformation and Pharmacology Studies of Morphiceptin Analogues - Selective μ-Opioid Receptor Ligands

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Abstract: Morphiceptin (Tyr-Pro-Phe-Pro-NH_2) is one of the most selective agonists for the μ-opioid receptor. In this review structure-activity relationships of morphiceptin analogues and studies resulting in defining low energy conformations are discussed. Finally, new developments in the control of tumour growth and cell proliferation by morphiceptin analogues are surveyed, which open future perspectives in the diagnosis and treatment of various cancers.

INTRODUCTION

A number of milk protein fragments has been shown to behave like opioid receptor ligands, able to address opioidergic systems in the adults' and in the neonates' organisms. These peptides are inactive within the sequence of the precursor milk proteins but can be released and thus activated by enzymatic proteolysis, for example during gastrointestinal digestion or during food processing. Activated opioid peptides are potential modulators of various regulatory processes in the body.

One class of such opioid peptides that show some preference for the μ-receptor [1, 2] is the group of β-casomorphins (β-CM), obtained from the milk protein, β-casein, by proteolytic fragmentation. Natural β-casomorphins include: β-CM-4, 5, 6 and 7, which are obtained by successive C-terminal amino acid cleavage of the 60-66 fragment (Tyr-Pro-Phe-Pro-Gly-Pro-Ile) in bovine β-casein and the 51-57 fragment (Tyr-Pro-Phe-Val-Glu-Pro-Ile) in human β-casein [3-5]. Human and bovine β-casomorphins-7 are identical except for two amino acids at positions 4 and 5 of the sequence. A tetrapeptide amide, Tyr-Pro-Phe-Pro-NH_2 (morphiceptin), was originally synthesised as an analogue possessing the N-terminal tetrapeptide fragment of β-casomorphin [6] and then was isolated from an enzymatic digest of bovine β-casein [7]. Morphiceptin is of particular interest, because it was found to have morphine-like physiological activity, to bind with fairly high affinity, and to be extremely selective for the μ-receptor [6]. This property is contrasted to that of enkephalins, which are selective for δ-receptor [8].

Despite the important role of morphiceptin as an extremely selective μ-receptor ligand, to the best of our knowledge, no review on synthesis and activity of morphiceptin analogues has been published so far.

In this review we tried to summarise structure-activity relationships of morphiceptin analogues, their conformation studies and receptor binding, as well as their physiological morphine-like and antiproliferative activities.

STRUCTURE-ACTIVITY RELATIONSHIP STUDIES

Linear Analogues

Since the discovery that morphiceptin is one of the most selective agonists for the μ-receptor the systematic studies of chemical modifications of this tetrapeptide have been carried on. These studies utilised structurally related amino acids and peptidomimetics to explore the importance of specific residues and to gain insight into the structural requirements for activity. The initial work on the structure-activity relationships of morphiceptin analogues was performed by Chang *et al.* [6, 9-11]. They modified morphiceptin by substitutions at the fourth, third and second amino acid residues (Table 1). It was found that replacement of Pro^4 with D-Pro (compound **2**) or other cyclic unnatural amino acids, such as thiazolidine-4-carboxylic acid (Thz) and thiazolidine-4-carboxylic acid sulfoxide [Thz(O)] (compounds **3** and **4,** respectively) yielded peptides with greater activity in the μ-receptor binding assay. The D-Pro substitution was the most effective. [$D\text{-}Pro^4$]-morphiceptin **2** was about 15 times more active in μ-receptor binding assay than morphiceptin. In the third position a methyl group was added to the nitrogen in Phe^3 to limit conformational freedom. It is known that the N-alkylation of an amide of an amino acid residue reduces allowed conformational space as compared to that for the corresponding unsubstituted residue [12]. Furthermore, the incorporation of an N-alkyl amino acid restricts conformations accessible to the C_α-C(O) bond of the preceding residue [12, 13]. The resulting analogue, Tyr-Pro-NMePhe-Pro-NH_2 **5**, was about twice as active as

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morphiceptin in the μ-receptor binding assay. Simultaneous substitution of $NMePhe^3$ and $D\text{-}Pro^4$ produced an analogue Tyr-Pro-NMePhe-D-Pro-NH_2 **6,** which was 12 times more active than morphiceptin in the binding assay. Changes in position 2 of morphiceptin were not very successful. Replacement of Pro with its D-isomer produced an analogue with no bioactivity (compound **9**). However, Pro^2 in Tyr-Pro-NMePhe-D-Pro-NH_2 could be replaced by some unnatural amino acids, like Thz, Thz(O), pipecolinic acid (Pip) or 3,4-dehydroproline (ΔPro) (compounds **10-13,** respectively) with no loss of affinity, while other amino acids, like 4-hydroxyproline (Hyp) (compound **14**) substantially decreased affinity. Changes at the C-terminal, like removal of the amide group, replacement of the amide with a hydrazine group or removal of Pro^4 markedly reduced activity (peptides **15-17**). Activity was retained when the C-terminal amide was changed to the carbinol or when Gly-NH_2 was added as a fifth residue (peptides **18** and **19,** respectively).

The μ-site selectivity of the best morphiceptin analogues **2** and **6** is superior to that of other μ-agonists, such as morphine, fentanyl, and FK 33824 [14]. All peptide analogues of morphiceptin mentioned above exhibit extremely low affinity toward δ-binding sites. The IC_{50} values determined in the binding assay against the binding of ^{125}I-labeled δ-ligand, DADLE, to δ-sites were greater than 10 μM (Table 1).

Morphiceptin and its agonists have opiate-like properties in isolated tissue preparations. For example they inhibit the electrically stimulated smooth muscle contractions of the guinea-pig ileum (GPI) and mouse vas deferens (MVD). The GPI assay is usually considered as being representative for μ-receptor interactions [15], even though the ileum also contains κ-receptors. κ-Receptor interactions in the GPI assay are indicated by relatively high K_e values for naloxone as antagonist (20-30 mM) [16], in contrast to the low K_e values (1-3 nM) observed with μ-receptor ligands [8]. In the

Table 1. Binding Affinities of Morphiceptin Analogues with Substitutions at the Fourth, Third and Second Amino Acid Residue

Comp. No	Sequence	μ-Receptor affinity [a] (IC_{50}, nM)	Relative _-receptor affinity[b] (%)	_-Receptor affinity[c] (IC_{50}, nM)	Ref.
1	Tyr-Pro-Phe-Pro-NH_2 (morphiceptin)	63	100	30000	10
2	Tyr-Pro-Phe-*D-Pro*-NH_2	4,3	1470	20000	10
3	Tyr-Pro-Phe-*Thz*-NH_2	12	530	10000	10
4	Tyr-Pro-Phe-*Thz(O)*-NH_2	40	160	NT[d]	10
5	Tyr-Pro-*NMePhe*-Pro-NH_2	37	170	NT	10
6	Tyr-Pro-*NMePhe-D-Pro*-NH_2	5,3	1190	10000	10
7	Tyr-Pro-*D-Phe-D-Pro*-NH_2	200	32	NT	11
8	Tyr-Pro-*Trp-D-Pro*-NH_2	100	63	NT	11
9	Tyr-*D-Pro*-Phe-Pro-NH_2	>10000	-	NT	9
10	Tyr-*Thz-NMePhe-D-Pro*-NH_2	25	250	NT	11
11	Tyr-*Pip-NMePhe-D-Pro*-NH_2	16	390	NT	11
12	Tyr-*ΔPro-NMePhe-D-Pro*-NH_2	21	300	NT	11
13	Tyr-*Thz(O)-NMePhe-D-Pro*-NH_2	80	79	NT	11
14	Tyr-*Hyp-NMePhe-D-Pro*-NH_2	800	8	NT	10
15	Tyr-*Pro-NMePhe-D-Pro-OH*	186	34	20000	10
16	Tyr-Pro-*NMePhe-D-Pro-NHNH*$_2$	2000	3	20000	10
17	Tyr-Pro-*NMePhe*-NH_2	1500	4	80000	10
18	Tyr-Pro-*NMePhe-D-Pro-ol*	5	1260	20000	10
19	Tyr-Pro-*NMePhe-D-Pro-Gly-NH*$_2$	8,7	725	20000	10
	Morphine	0,4	15750	36	10
	DADLE	4	1575	1,6	10

[a] IC_{50} values were determined by the concentration which inhibited the binding of ^{125}J-FK 33824 to μ-binding sites of rat brain membranes by 50%.

[b] The relative μ-receptor affinity is expressed as percent of morphiceptin (100%).

[c] IC_{50} values were determined by the concentration that decreased the binding of ^{125}J-DADLE by 50%.

[d] NT – not tested.

MVD assay opioid effects are primarily mediated by δ-receptors, however μ- and κ-receptors also exist in this tissue [17, 18]. The potency ratio in MVD and GPI assays is often used to assess the μ- and δ-receptor selectivity [19, 20].

In vitro GPI and MVD activities of morphicetin and some analogues are summarised in Table 2. [NMePhe^3, D-Pro^4]-morphiceptin **6** was more potent than morphine in all *in vitro* studies, though it had a lower μ-receptor affinity, suggesting a difference in the way in which peptide and alkaloid activate opioid receptors.

Table 2. ***In Vitro*** **Activity of Morphiceptin Analogues**

Comp. No	Sequence	*In vitro* activity (ED_{50}, nM) [a]	
		GPI	MVD
1	Tyr-Pro-Phe-Pro-NH_2 (morphiceptin)	318	4800
2	Tyr-Pro-Phe-*D-Pro*-NH_2	81	196
3	Tyr-Pro-Phe-*Thz*-NH_2	206	929
5	Tyr-Pro-*NMePhe*-Pro-NH_2	225	2300
6	Tyr-Pro-*NMePhe-D-Pro*-NH_2	34	240
15	Tyr-Pro-*NMePhe-D-Pro-OH*	255	2700
17	Tyr-Pro-*NMePhe*-NH_2	>10000	>10000
19	Tyr-Pro-*NMePhe-D-Pro-Gly-*NH_2	31	303
	Morphine	134	1300
	DADLE	26	0.62

[a] Data from ref. 10

E_{D50} – The concentration of an agonist which produces 50% inhibition of contractions.

Nelson *et al.* [21] proposed for morphiceptin a μ-receptor pharmacophore based on the structural homology with enkephalins and some non-peptide opioids. In order to test the pharmacophore hypothesis, morphiceptin analogues were synthesised with potentially important chemical groups modified or eliminated (Table 3). The smallest fully active fragment of morphiceptin was found to be Tyr-Pro-NH(phenethyl) **20**, suggesting that the C-terminal Pro-NH_2 is not required for activity. This indicates that pharmacophoric groups are limited to the first three amino acids of morphiceptin. The N-terminal amino group of Tyr is very important for the activity. N-Acetylation of the nitrogen produces an inactive compound **21**. Also the elimination or methylation of the phenolic hydroxyl (compounds **22** and **23,** respectively) results in a dramatic loss of activity. The effects of these modifications suggest that the phenolic hydroxyl could interact with the receptor via a hydrogen bond or ionic interaction. The Phe^3 phenyl group of morphiceptin is also significant for activity. Reduction of the phenyl group to a cyclohexyl derivative (compound **24**) results in a dramatic loss in activity, suggesting an important role for a flat hydrophobic and aromatic group. Introduction of a nitro group into the phenyl ring also drastically reduces activity (compound **25**). All these data show that there is a strict requirement for the Tyr^1 amino group, Tyr^1 phenolic group and Phe^3 phenyl group, supporting a three-group pharmacophore hypothesis (Fig. **1**).

Table 3. ***In Vitro*** **Activity of Morphiceptin Analogues with Potentially Important Chemical Groups Modified or Eliminated[a]**

Comp. No	Sequence	*In vitro* activity (ED_{50}, nM) GPI
1	Tyr-Pro-Phe-Pro-NH_2 (morphiceptin)	136
20	Tyr-Pro-*NH-*CH_2*-*CH_2*-Ph*	156
21	*NAc*-Tyr-Pro-Phe-Pro-NH_2	>20400
22	*Phe*-Pro-Phe-Pro-NH_2	9280
23	*Tyr(OMe)*-Pro-Phe-Pro-NH_2	4690
24	Tyr-Pro-*Cha*-Pro-NH_2	>10000
25	Tyr-Pro-*Phe(pNO*$_2$*)*-Pro-NH_2	9280

[a] Data from ref. 21.

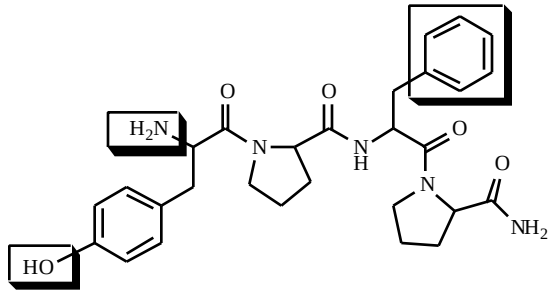

Fig. (1). A schematic representation of morphiceptin structure. Pharmacophoric groups are marked.

These results are consistent with the data for other opioids like enkephalins, endorphins or other β-casomorphins, whose physiological activity is determined by the conformation of the N-terminal sequence, so called message sequence [22]. In all peptide opioids message sequence is composed of two pharmacophoric amino acid residues, Tyr and Phe, which are required for the receptor recognition. This sequence also includes a spacer residue(s), which join(s) the pharmacophoric residues of the message sequence. In case of all casomorphins, including morphiceptin, Pro is a spacer residue. The remaining C-terminal fragment of opioid peptides is called address sequence. This sequence is supposed to stabilise the bioactive conformation, among various conformations accessible to the N-terminal message sequence (Table 4). Since receptor selectivity depends on the bioactive conformation of the peptide chain, address sequence is probably responsible for the selectivity of the peptide toward different receptor subtypes. Spacer residues play a significant role in orienting the biologically important Tyr and Phe residues in the correct array necessary for opioid activity.

Table 4. Structural Components of β-Casomorphins and Enkephalins

Opioid peptide	Message sequence			Address sequence
	Pharmacophoric residue	Spacer	Pharmacophoric residue	
bovine β-CM-7	Tyr	Pro	Phe	Pro-Gly-Pro-Ile-OH
bovine β-CM-6	Tyr	Pro	Phe	Pro-Gly-Pro-OH
bovine β-CM-5	Tyr	Pro	Phe	Pro-Gly-OH
bovine β-CM-4	Tyr	Pro	Phe	Pro-OH
human β-CM-7	Tyr	Pro	Phe	Val-Glu-Pro-Ile-OH
human β-CM-6	Tyr	Pro	Phe	Val-Glu-Pro-OH
human β-CM-5	Tyr	Pro	Phe	Val-Glu-OH
human β-CM-4	Tyr	Pro	Phe	Val-OH
morphiceptin	Tyr	Pro	Phe	Pro-NH_2
Leu-enkephalin	Tyr	Gly-Gly	Phe	Leu-OH
Met-enkephalin	Tyr	Gly-Gly	Phe	Met-OH

The morphiceptin and its analogues with a Pro at the second position show *cis/trans* isomerization about the Tyr-Pro amide linkage. The coexistence of the *trans* and *cis* forms makes it difficult to gain a consistent explanation for the biological activity of these analogues at the receptor. In order to eliminate the coexistence of the *cis* and *trans* forms Goodman and co-workers [23, 24] synthesised morphiceptin analogues of the sequence: Tyr-βAc^5c-Phe-Xaa-NH_2 (Xaa = Pro, Val or D-Val), in which Pro^2 was substituted with stereoisomeric 2-aminocyclopentane carboxylic acids (βAc^5c). The βAc^5c residue is a β-amino acid with two chiral centres resulting in four possible stereoisomers: two enantiomeric *cis* forms, (1R,2S and 1S,2R) and two enantiomeric *trans* forms, (1R,2R and 1S,2S). The βAc^5c-analogues can only adopt a *trans* amide bond structure about Tyr-βAc^5c linkage. It was found that the chirality of the βAc^5c residue played a large role in the configurational preferences and biological activity of the morphiceptin analogues. Only the analogues containing *cis*-(1S,2R)-βAc^5c were active in both, GPI and MVD assays (Table 5, compounds **26, 31,** and **34**). By use of the conformational analysis utilising ^{1}H-NMR, molecular dynamics and mechanics calculations it was found that the separation of aromatic rings of the Tyr and Phe residues, as expressed by the centre-to-centre distance, is 10.1-12.7 Å for the preferred conformation of the bioactive analogues containing the *cis*-(1S,2R)-βAc^5c residue, while a range of 4.8-7.0 Å was observed for the preferred conformations of the inactive analogues with the *cis*-(1R,2S)-βAc^5c residues [24]. These findings led to the conclusion that only the analogues

Table 5. *In Vitro* Activity of Morphiceptin Analogues with 2-Aminocyclopentane Carboxylic Acid (βAc^5c) at the Second Position

Comp. No	Sequence	*In vitro* activity (ED_{50}, nM)		MVD/GPI ED_{50} ratio	Ref
		GPI	MVD		
1	Tyr-Pro-Phe-Pro-NH_2 (morphiceptin)	318	4800	15,1	10
26	Tyr-*cis(1S, 2R)*- βAc^5*c*-Phe-Pro-NH_2	54	278	5,1	23
27	Tyr-*cis(1R, 2S)*- βAc^5*c*-Phe-Pro-NH_2	6650	>11400	-	23
28	Tyr-*trans*-βAc^5*c*–Phe-Pro-NH_2 (diastereotropic mixture)	1200	>11400	-	23
29	Tyr-Pro-Phe-*Val*-NH_2	66	3800	57,5	24
30	Tyr-*cis(1R, 2S)*- βAc^5*c*-Phe-*Val*-NH_2	>24600	>11400	-	24
31	Tyr-*cis(1S, 2R)*- βAc^5*c*-Phe-*Val*-NH_2	90	>11400	-	24
32	Tyr-Pro-Phe-*D-Val*-NH_2	60	9900	165	24
33	Tyr-*cis(1R, 2S)*- βAc^5*c*-Phe-*D-Val*-NH_2	>24600	>11400	-	24
34	Tyr-*cis(1S, 2R)*- βAc^5*c*-Phe-*D-Val*-NH_2	1040	>11400	-	24

incorporating the (1S,2R)-βAc^{5}c residue display the relatively large separation of the Tyr and Phe side chains which is required for the μ-opioid receptor activity of morphiceptin analogues. It was found that one of the low energy conformations calculated for morphiceptin with the *cis* form about the Tyr-Pro amide bond has considerable structural topology with the preferred conformation of the bioactive analogues containing the *cis*-(1S,2R)-Ac^{5}c residue. Although the βAc^{5}c analogues can only adopt a *trans* configuration about Tyr-βAc^{5}c amide bond, the bioactive analogues Tyr-(1S,2R)-βAc^{5}c-Phe-Xaa-NH_2 are topochemically similar to morphiceptin with the Tyr-Pro amide bond in a *cis* configuration [25]. These findings indicate that the *cis* configuration about the Tyr-Pro amide linkage is likely required for the biological activity of the morphiceptin and related analogues with Pro^2.

Cyclic Analogues

High degree of flexibility of the linear opioid peptides is believed to be the likely reason of their lack of receptor selectivity toward the different opioid receptor types (μ, δ, κ) [26], since conformational adaptation to the various receptor topographies is possible. Conformational constraints play an important role in rational design of peptides that can overcome this problem [27-30]. Conformational restriction can be achieved through cyclization of linear sequences using the functional groups of the N- and C-terminus as well as these in the side chains of the amino acid residues. A series of cyclic β-casomorphin–5 (Tyr-Pro-Phe-Pro-Gly-OH) analogues was synthesised by substitution of the Pro^2 with various diaminocarboxylic acids followed by cyclization of the ω-amino group to the C-terminal carboxylic group of glycine, in analogy to previously described cyclic enkephalin analogues [31, 32]. These compounds showed high potency in opioid receptor binding studies and bioassays *in vitro* as well as high antinociceptive activity [33, 34].

Based on these results Vogel *et al.* [35] tried to increase selectivity of morphiceptin analogues synthesising a series of cyclic analogues. They used the tetrapeptide sequence Tyr-D-Xaa-Phe-Yaa-OH (Xaa = Lys, Orn, A_2bu, Yaa =Pro in L- or D-configuration) to obtain cyclic analogues with a peptide bond between the ω-amino group of Xaa and the C-terminal carboxyl group (Table 6). In none of the reactions performed with L-proline as a Yaa amino acid could a cyclic monomer be detected, and the cyclodimer (**41-43**) was the exclusive product in each case. Cyclodimerization was also the favoured reaction in the attempted formation of the 11-membered ring of the cyclic [D-A_2bu^2, D-Pro^4]-morphiceptin analogue **46.** In the case of both the [D-Lys^2, D-Pro^4]-analogue **44** and [D-Orn^2, D-Pro^4]-analogue **45,** the cyclomonomer was the major product. The cyclic monomers **43** and **44** showed high opioid activity in GPI (ED_{50} = 2-5 nM) and MVD (ED_{50} = 50-60 nM) assays, whereas the potency of the cyclodimers was 2-3 orders of magnitude lower in both *in vitro* bioassays.

Mixed μ-Agonist/σ-Antagonist Analogues

Analgesic effects of morphine are mediated through the μ-receptors, while the tolerance and dependance are mediated through σ-receptors. The fact that μ- and σ-opioid receptors differ in their conformation requirements [27] opens the door for designing analogues with mixed opioid

Table 6. Comparison of the Cyclization Reactions and *in Vitro* Activity of Morphiceptin Analogues

Comp. No	Sequence	Ring size	Monomer (%)	*In vitro* activity (ED_{50}, nM) [a]		MVD/GPI ED_{50} ratio
				GPI	MVD	
1	Tyr-Pro-Phe-Pro-NH_2 (morphiceptin)			318	4800	15,1
35	Tyr-*D-Lys*-Phe-Pro-OH			2975	10000	-
36	Tyr-*D-Orn*-Phe-Pro-OH			5750	10000	-
37	Tyr-*D-A$_2$bu*-Phe-Pro-OH			1560	8000	-
38	Tyr-*D-Lys*-Phe-*D-Pro*-OH			600	7750	-
39	Tyr-*D-Orn*-Phe-*D-Pro*-OH			1270	10000	-
40	Tyr-*D-A$_2$bu*-Phe-*D-Pro*-OH			1410	3390	-
41	(Tyr-c[*D-Lys*-Phe-Pro])$_2$	26	<5	10000	92,6	-
42	(Tyr-c[*D-Orn*-Phe-Pro])$_2$	24	<5	10000	10000	-
43	(Tyr-c[*D-A$_2$bu*-Phe-Pro])$_2$	22	<5	1200	1420	1,18
44	Tyr-c[*D-Lys*-Phe-*D-Pro*]	13	78	5,41	60,6	11,2
45	Tyr-c[*D-Orn*-Phe-*D-Pro*]	12	85	2,36	52,3	22,2
46	(Tyr-c[*D-A$_2$bu*-Phe-*D-Pro*])$_2$	22	<5	158	306	1,94

[a] Data from ref. 35.

agonist/antagonist properties that produce an agonist effect at μ-receptor and act as antagonists at σ-receptor. The first known example of a mixed μ-agonist/σ-antagonist is the tetrapeptide analogue Tyr-Tic-Phe-Phe-NH_2 (TIPP-NH_2), reported by Schiller *et al.* [36]. Replacement of Phe^3 in the cyclic casomorphin analogue Tyr-c[D-Orn-Phe-D(or L)-Pro-Gly] with amino acid residues containing more extended aromatic ring systems in the side chain (like Trp, 1-Nal or 2-Nal) resulted in the discovery of a new class of cyclic mixed μ-agonist/σ-antagonists [37]. The key compound was found to be Tyr-c[D-Orn-2-Nal-D-Pro-Gly] which was a potent μ-agonist in the GPI assay (ED_{50} = 384 nM) and a moderately potent σ-antagonist in the MVD assay [38, 39].

To the best of our knowledge no μ-agonist/σ-antagonist analogues of morphiceptin have been reported so far.

CONFORMATIONAL STUDIES

In the theoretical studies on the conformation of peptides the main hypothesis is that only analogues which can attain the proper conformation will have high affinity for the receptor. Comparing possible low energy conformations of morphiceptin analogues and receptor affinities may lead to the identification of a likely μ-binding conformer. The relative energies required to attain the common μ-interacting conformation, and the extent of overlap of the conformer with that of a high affinity analogue appear to be predictors of relative μ-receptor affinity.

Loew *et al.* [40] demonstrated that both, conformational and electronic properties of the morphiceptin analogues can alter the μ-receptor affinity. A candidate conformer for high affinity opioid peptide binding at the μ-receptor has been identified as one with a β_{II}-like turn. Since the same type of a conformer was earlier identified for μ-selective enkephalin analogues [41] it is very likely that all μ-selective opioid peptides share the same type of conformation, characterised by a β-turn.

Conformational studies of morphiceptin analogues have been carried out mainly by using theoretical energy calculations. Momany and Chuman [41] proposed requirement of a relatively compact conformation with a small distance between the two aromatic rings of the Tyr and Phe (or NMePhe) residues. Loew at al. [40] reported low energy conformations for the all *trans* forms of morphiceptin and related analogues, which are quite extended, with the large distance between the two aromatic side chains. Examining the conformational preferences of a series of highly constrained morphiceptin analogues Nelson *et al.* [21] reported distances of 9-12 Å between the aromatic rings for the all *trans* form and slightly smaller distances of 6-12 Å for the low energy conformers containing *cis* amide bond between the Tyr-Pro residues in the most active analogues. Goodman and Mierke [42] observed in their NMR studies of morphiceptin and [$NMePhe^3$, D-Pro^4]-morphiceptin that only approximately 60% of the molecules were in all *trans* configuration, with 25-35% of the population adopting a *cis* arrangement around the Tyr-Pro amide linkage. These data show that a *cis* configuration is less favoured than a *trans* configuration. However, the energy difference between these two states is within a range from 0.26 to 0.53 kcal/mol and is small enough to be paid for by ligand-receptor interactions.

The comparison between the μ- and σ- pharmacophore conformations of the series of cyclic β-casomorphins demonstrated not only differences in spatial orientation of both aromatic groups, but also in the backbone conformations of the ring part. Assuming that both the μ- and σ-pharmacophore conformations bind with the Tyr residue in the similar orientation at the same transmembrane domain of the receptors, the side chain of Phe^3, as a second binding site, has to dock with different domains [39].

MORPHICEPTIN ANALOGUES AS POTENTIAL PHARMACEUTICAL AGENTS

Morphiceptin and its analogues act as specific agonists of μ-opioid receptors and have both, central and peripheral opioid activity [6]. For example intracerebroventricular (i.c.v.) administration of morphiceptin induces dose-related analgesia in the cold or hot water tail-flick test in rats [43]. Morphiceptin was also reported to produce in rats a significant increase in the threshold for catecholamine induced ventricular arrhytmias [44]. As selective μ-receptor agonists, morphiceptin analogues microdialyzed into the POAH in rats were observed to produce hyperthermia [45]. Peripherally morphiceptin produces a naloxone-reversible bradycardia and fall of blood pressure [46].

Recent numerous synthetic, pharmacological and physicochemical studies have focused on opioid peptides, which are of interest as possible substitutes for alkaloid opiate drugs. The first examples of μ-agonist/σ-antagonist analogues mentioned above [36, 37] give basis for introducing in the future new non-opiate analgesic drugs, free of morphine-like side-effects [47, 48].

The observation that morphine inhibits the cell proliferation of certain cancer cells initiated a new trend in the studies of morphiceptin analogues [49, 50]. Hatzoglou *et al.* [51] found that morphine and morphiceptin decrease, in the dose-dependent manner, the cell growth of T47D human breast cancer cells, despite the lack of μ-opioid receptors in the cell membranes. They suggested a non-opioid mediated action of morphine and morphiceptin through somatostatin receptors, which belong to the same membrane protein superfamily [52]. The three opioid receptors have high similarity to somatostatin receptors, with approximately 40% amino acid sequence identical [53, 54]. It was found that μ-acting opioids interact with the sst2 receptor subtype [55, 56].

On the other hand morphiceptin and other β-casomorphins inhibited cell proliferation of human prostate cell lines, by mechanism partly involving opioid receptors. As opioid neurones can be found in the prostate gland, and casomorphin peptides might reach the gland through the general circulation, these findings indicate a putative role of opioids in prostate cancer cell growth [57].

Morphiceptin and [D-$Cl_2$$Phe^3$]-morphiceptin were found to have relatively high affinity for the mouse mammary

adenocarcinomas. Preliminary studies revealed that both labelled compounds, ^{131}I-Tyr-Pro-Phe-Pro-NH_2 and ^{131}I-Tyr-Pro-D-Cl_2Phe-Pro-NH_2 showed increased accumulation in the tumour [58].

Summing up, morphiceptin analogues and other opioids decrease cell proliferation in different systems, including breast and prostate, through a yet unidentified interaction with opioid as well as somatostatin receptor systems. They may be in the future used for the diagnosis and treatment of various cancers.

CONCLUSIONS

Morphiceptin, originally synthesised as an analogue possessing the N-terminal peptide fragment of β-casomorphin was than isolated from enzymatic digest of β-casein. Morphiceptin was found to have morphine-like physiological activity and to be one of the most selective agonists for the μ-receptor. The structure-activity relationships of the morphiceptin analogues indicate for a strict requirement for the Tyr^1 amino and phenolic groups, and Phe^3 phenyl group as pharmacophores. Tyr-Pro-NMePhe-D-Pro-NH_2 was found to be the most potent and selective morphiceptin analogue. Conformationally restricted cyclic analogues of morphiceptin were also synthesised and some of them were very active in *in vitro* assays. The *cis* amide bond between Tyr^1 and Pro^2 residues was found to be necessary for the activity. NMR studies revealed that only 30% of molecules adopt a *cis* arrangement around Tyr-Pro amide linkage. However, the energy difference between *cis* and *trans* forms is small enough to be paid for by ligand-receptor interactions.

Pharmacological properties of morphiceptin analogues, such as analgesic effects and inhibition of cancer cells proliferation, encourage further studies on this group of opioids.

ABBREVIATIONS OF UNNATURAL AMINO ACIDS

Thz = thiazolidine-4-carboxylic acid

Thz(O) = thiazolidine-4-carboxylic acid sulfoxide

Pip = pipecolinic acid

ΔPro = 3,4-dehydroproline

Hyp = 4-hydroxyproline

βAc^5c = 2-aminocyclopentane carboxylic acid

A_2bu = 2,4-diaminobutiric acid

Cl_2Phe = 3,4-dichlorophenylalanine

Tic = 1,2,3,4-tetrahydroizoquinoline-3-carboxylic acid

1-Nal = 1-naphthylanine

2-Nal = 2-naphthylalanine

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