Opioid Receptors and their Ligands

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Abstract: This review gives a historical perspective, summarizing approximately 25 years of research on opioids. The "typical" opioid peptides produced in the brain, "atypical" opioids encrypted in milk protein or hemoglobin sequences, and extremely potent and selective opioids of amphibian origin are described. The main focus is on the structure-activity relationship studies of peptide ligands for three main opioid receptor types (μ, δ, κ), their selectivities and pharmacological activities *in vitro*. Chemical modifications that led to obtaining potent and selective agonists and antagonists for these receptors are discussed.

Key Words: Opioid peptides, opioid agonists and antagonists, structure-activity relationships, opioid receptor selectivity, receptor binding assay.

I. INTRODUCTION

Opioid peptides and their multiple receptors are part of the neurohormonal system. Endogenous opioid peptides are small molecules that are naturally produced in the central nervous system (CNS) and in various glands throughout the body, such as the pituitary and adrenal glands. These peptides produce the same effects as the chemicals known as classic alkaloid opiates, which include morphine and heroine. Endogenous opioid peptides function both as hormones and as neuromodulators. Endogenous opioid peptides that serve as hormones are secreted into the circulation by the producing glands and are delivered to a variety of distant target tissues, where they induce a response. Endogenous opioid peptides that serve as neuromodulators are produced and secreted by nerve cells and act in the brain and spinal cord to modulate the actions of other neurotransmitters. Through these two mechanisms, endogenous opioid peptides produce a large spectrum of physiological effects, ranging from inducing pain relief to preventing diarrhea. The development of the synthetic opioid receptor-specific ligands, both peptide and non-peptide, is of great importance. If they can provide a description of the particular physiological characteristics of each receptor type it should be possible to design new drugs that may produce only selected physiological responses. The development of potent opioid agonists and antagonists with high specificity for each of the three main receptor classes continues to be of major concern in opioid pharmacology.

This review summarizes two decades of research on endogenous opioid system, in particular on the three main types of opioid receptors and their ligands. Structure-activity relationship studies of peptide agonist and antagonist analogs of endogenous and synthetic ligands that have biological activity and therapeutic potential are discussed. This article will by no means be exhaustive but it does aim to give the readers a view of current knowledge and research in this area.

II. OPIOID RECEPTORS

The opioids were among the earliest neuropeptides identified in the nervous system [1]. Opioid receptors are most abundant in the CNS [2], but have also been localized in many peripheral tissues of the mammalian organism [3]. It is now well established from work carried out in many laboratories over the last 20 years that there are three well-defined types of opioid receptors; the μ, δ and κ receptors [4-6]. Genes encoding for these receptors have been cloned [7-10]. The comparison of the amino acid sequences of three main opioid receptors revealed that they show extensive structural homology with each other. Physiological roles for each of the opioid receptors have not been clearly defined. Pain relief effects are mediated by all three receptor types, but in different degree. μ-Receptor mediates the most potent antinociceptive effects, accompanied however by the development of dependence. δ-Receptor has lower efficacy in mediating pain relief but also a reduced addictive potential. κ-Receptor mediates analgesic effects in peripheral tissues.

More recently, cDNA encoding an "orphan" (ORL) receptor was identified [11). This receptor, in contrast to μ, δ and κ receptors, does not mediate typical opioid, but rather anti-opioid effects. There is pharmacological evidence for subtypes of each receptor and other types of novel, less-characterized receptors have also been postulated [12].

Opioid receptors belong to the big family of G-protein-coupled receptors. Effector systems activated or blocked upon opioid receptor G-protein interaction are adenyl cyclase, Ca^{2+} channels, K^{+} channels, or phosphoinositol turnover [13,14].

III. OPIOID RECEPTOR LIGANDS

Two types of ligands at opioid receptors can be differentiated in view of their chemical structure: alkaloids and peptides.

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1. Alkaloids

Agonists. The first known opiate alkaloid was morphine, isolated from the poppy seeds in 1803 by Seturner. The structure of morphine was elucidated 120 years later [15], and its full systematic name is: 7,8-didehydro-4,5-epoxy-17-methyl-(5α,6α)-morphinan-3,6-diol. Morphine and other opiates are widely used in clinical practice for blockade of most severe pain syndromes or for anesthetic purposes. Morphine is primarily an agonist ligand for the μ receptor. Its affinities for δ and κ receptors are sufficiently low that it is used as a selective μ receptor ligand in pharmacological studies [16]. In the last century a number of morphine analogs have been synthesized as reviewed by Misicka [17] and proposed to be used as analgesics, but morphine is still used in clinical practice.

Antagonists. Opioid antagonists most frequently used by pharmacologists are synthetic alkaloids such as naloxone and naltrexone [18]. Naloxone, which was the first pharmacologically pure antagonist identified, is considered to be a "universal", non-selective opioid antagonist. The action of an agonist is characterized as opioid only if its effects are "naloxone-reversible" [19]. Although naloxone and its analog naltrexone bind to all three opioid receptors, they have the highest affinity for the μ receptor [20]. There are several other synthetic opioid antagonists of alcaloid structure, which are not used as yet at the clinical level, but they are not the subject of this review.

2. Peptides

2.1. "Typical" and "Atypical" Opioid Peptides

Peptide opioid receptor ligands have been classified into two groups; "typical" and "atypical" opioid peptides [21].

The "typical" opioid peptides, including enkephalins, dynorphins and β-endorphin are derived from three precursor molecules; pro-enkephalin, pro-dynorphin and pro-opiomelanocortin, all of which are expressed in the CNS, but their presence in peripheral tissues has also been confirmed [22]. Endogenous peptides derived from these precursors all have the tetrapeptide sequence Tyr-Gly-Gly-Phe at their N-terminus (Table **1**). These peptides vary in their affinity for μ, δ and κ receptors and have negligible affinity for ORL receptor, but none binds exclusively to one opioid receptor type. β-Endorphin is equiactive at μ and δ receptors with much lower affinity for κ receptor. [Met]- and [Leu]-enkephalin have high affinities for δ receptors, ten-fold lower affinities for μ receptors and negligible affinity for κ receptors. Dynorphin A and dynorphin B have high affinity for κ receptors, but also have significant affinity for μ and δ receptors. Nociceptin/orphanin FQ is the endogenous ligand for the ORL receptor and is derived from pro-nociceptin [23]. Nociceptin N-terminal amino acid sequence varies only at position 1 (Phe instead of Tyr) from the typical opioids mentioned above. Recently discovered endomorphin-1 and endomorphin-2 are putative products of an, as yet unidentified, precursor that have been proposed to be the endogenous ligands for the μ receptor, where they are highly selective [24]. The endomorphins are amidated tetrapeptides and are structurally unrelated to the typical opioid peptides (Table **1**).

The "atypical" opioid peptides originate from the variety of precursor proteins and carry various amino acid sequences at their N-terminal regions, only the N-terminal Tyr residue is conserved [25] (Table **2**). The N-terminal tetrapeptide of most atypical opioid peptides represents the minimum sequence for full opioid activity.

The first group of "atypical" opioid peptides, identified by Brantl *et al*. [26] in 1979 were milk protein derived β-casomorphins (β-CM), which are obtained by proteolytic fragmentation of β-casein.

Table 1. Mammalian Endogenous Opioid Peptides.

Precursor	Endogenous peptide	Amino acid sequence	Affinity for opioid receptors
Pro-enkephalin	[Met]enkephalin	Tyr-Gly-Gly-Phe-Met	δ, μ
	[Leu]enkephalin	Tyr-Gly-Gly-Phe-Leu	(δ>>μ)
		Tyr-Gly-Gly-Phe-Met-Arg-Phe	
		Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu	
	Metorphamide	Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val-NH_2	
Pro-opiomelanocortin	β-endorphin	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu	μ, δ (μ=δ)
Pro-dynorphin	Dynorphin A	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln	κ, μ, δ (κ>>μ and δ)
	Dynorphin A(1-8)	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile	
	Dynorphin B	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr	
	α-Neoendorphin	Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-Lys	
	β-Neoendorphin	Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro	
	[Leu]enkephalin	Tyr-Gly-Gly-Phe-Leu	
Pro-nociceptin	Nociceptin	Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln	ORL
Unknown	Endomorphin-1	Tyr-Pro-Trp-Phe-NH_2	μ
	Endomorphin-2	Tyr-Pro-Phe-Phe-NH_2	

Hemorphins are endogenous peptides generated by enzymatic hydrolysis of the blood protein, hemoglobin. In recent years hemorphin structures have been identified as naturally occurring peptides in brain, plasma and cerebrospinal fluid [27].

Two peptides recently isolated from brain tissue, Tyr-MIF-1 [28] and Tyr-W-MIF-1 [29, 30], which bind with high selectivity to μ compared with δ and κ receptors are another example of "atypical" opioid peptides.

A very important group of "atypical" opioid peptides are peptides isolated from amphibian skin, whose affinity to opioid receptors is orders of magnitude better than endogenous opioids (with the exception of endomorphins) [31, 32].

2.2. Bioactive Conformation of Opioid Peptides

The term "bioactive conformation" refers to a three-dimensional structure of the ligand molecule during receptor binding and appears to be one of the crucial features responsible for specificity of binding and consequently the selectivity of ligand action.

The structure of most naturally occurring peptide opioids can be divided into two components: the biologically important N-terminal tri- or tetrapeptide fragment ("message sequence") and the remaining C-terminal fragment ("address sequence") [33], as shown in Table **3**. The message sequence provides information for signal transduction that leads to the biological response, while the address domain primarily influences binding affinities and accommodates the elements of selectivity. The N-terminal message sequence is composed of two pharmacophoric amino acid residues, Tyr and Phe (only in the case of endomorphin-1 Phe is replaced by Trp), in which the amino and phenolic groups of Tyr and the aromatic ring of Phe (or Trp) are required for opioid receptor recognition. This sequence also includes a spacer residue(s), which join(s) the pharmacophoric residues of the message sequence.

However, three different opioid receptors, μ, δ, and κ require different bioactive conformations with specific arrays of the pharmacophoric groups within the N-terminal message sequence. It seems that the biological activity of opioid peptides is determined by the conformation of the N-terminal sequence. The C-terminal address sequence may stabilize a specific conformation accessible to the N-terminal message sequence. It would alter receptor selectivity, as suggested by the fact that dermorphin and deltorphins display the μ receptor and δ receptor selectivities, respectively, although they all possess the same N-terminal sequence Tyr-D-Ala-Phe [34].

2.3. Determination of Opioid Activity

The *in vitro* methods to determine the activity of opioid peptides include standard tests on isolated tissue preparations and the receptor binding studies on tissue homogenates.

The main focus in standard tests for opioid activity determination *in vitro* has been on μ and δ receptor interactions. These tests are based on inhibition of electrically evoked contractions of the guinea pig ileum (GPI) and the mouse vas deferens (MVD). The opioid effect in the GPI preparations is mainly mediated by μ receptors, whereas the predominant receptors of the MVD are of the δ type.

The receptor binding assays on tissue homogenates include saturation and competition studies. In the saturation binding studies, the affinity of different compounds to opioid receptors is characterized. The competition studies can be performed subsequently or independently to confirm these results.

The ligand affinity in the binding studies may be affected by the type of radionuclide used for labeling of the peptide. The most significant changes in binding affinities were observed for iodinated compounds. The iodination of Tyr decreases peptide affinity ten times. However, some of the examined peptides, including endomorphins, maintain their affinity toward receptor sites after iodination. That suggests the differences in the ligand-receptor interactions for different labeled peptides [35].

Table 2. "Atypical" Opioid Peptides.

Source	Opioid peptide	Amino acid sequence	Affinity for opioid receptors
β-casein	bovine β-casomorphin (1-7)	Tyr-Pro-Phe-Pro-Gly-Pro-Ile-OH	μ
	human β-casomorphin (1-7)	Tyr-Pro-Phe-Val-Glu-Pro-Ile-OH	μ
	morphiceptin	Tyr-Pro-Phe-Pro-NH_2	μ
	[Val^4]-morphiceptin	Tyr-Pro-Phe-Val-NH_2	μ
hemoglobin	hemorphin-4	Tyr-Pro-Trp-Thr-OH	μ
bovine hypothalamus human brain cortex	Tyr-MIF-1	Tyr-Pro-Leu-Gly-NH_2	μ
bovine hypothalamus human brain cortex	Tyr-W-MIF-1	Tyr-Pro-Trp-Gly-NH_2	μ
frog skin	Dermorphin	Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2	μ
	dermenkephalin	Tyr-D-Met-Phe-His-Leu-Met-AspNH_2	δ
	deltorphin I	Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2	δ
	deltorphin II	Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2	δ

Table 3. Structural Components of Peptide Opioids.

Opioid peptide	Message sequence			Address sequence
	Pharmacophoric residue	Spacer	Pharmacophoric residue	
Leu-enkephalin	Tyr	Gly-Gly	Phe	Leu-NH_2
Met-enkephalin	Tyr	Gly-Gly	Phe	Met-NH_2
dynorphin A(1-8)	Tyr	Gly-Gly	Phe	Leu-Arg-Arg-Ile
deltorphin I	Tyr	D-Ala	Phe	Asp-Val-Val-Gly-NH_2
deltorphin II	Tyr	D-Ala	Phe	Glu-Val-Val-Gly-NH_2
dermenkephalin	Tyr	D-Met	Phe	His-Leu-Met-Asp-NH_2
β-casomorphin	Tyr	Pro	Phe	Pro-Gly-Phe-Ile-OH
morphiceptin	Tyr	Pro	Phe	Pro-NH_2
endomorphin-1	Tyr	Pro	Trp	Phe-NH_2
endomorphin-2	Tyr	Pro	Phe	Phe-NH_2
dermorphin	Tyr	D-Ala	Phe	Gly-Tyr-Pro-Ser-NH_2

The interaction between a ligand and a receptor site may be altered by various factors. The variability in the binding data between various laboratories may be attributable to differences in the synaptosomal preparations, the concentration, type of radioligand used, and the method of reporting binding (IC_{50}, K_i). Only data obtained in the same laboratory can be compared.

2.4. δ *Receptor Ligands*

Agonists In 1972 at the International Congress of Pharmacology Collier suggested for the first time that endogenous ligands for the opiate receptor must exist [36]. Three years later the first endogenous opioid peptides, Met- and Leu-enkephalins, were isolated and characterized [37]. These natural peptides have only modest affinity and selectivity for the δ receptor and are quickly metabolized. The search for more stable and selective analogs has begun. Early studies focused on modification of the enkephalins using classical approach of amino acid substitutions, additions, and deletions. These attempts resulted in agonists showing enhanced δ receptor selectivity, such as DADLE, Tyr-D-Ala-Gly-Phe-D-Leu [38], DSLET, Tyr-D-Ser-Gly-Phe-Leu-Thr [39] and DTLET, Tyr-D-Thr-Gly-Phe-Leu-Thr [40]. DSLET and DTLET were extensively used to characterize δ opioid receptor, e.g. its binding properties [41], distribution in the rat [42] and human [43] brains, and physiological role [44]. Comparison of the preferential conformations of DTLET obtained by NMR spectroscopy [45] or from theoretical calculations [46] suggested, that increasing the size of the residue in position 2 and/or 6 might induce a partial inhibition of the μ opioid receptor recognition. Introduction of a bulky O-*tert*-butyl-Ser^2 residue was shown to increase affinity for the δ receptor [47]. Taking these features into account, new derivatives of DSLET and DTLET, such as BUBU, Tyr-D-Ser(O-tBu)-Gly-Phe-Leu-Thr(O-tBu), DSTBULET, Tyr-D-Ser(O-tBu)-Gly-Phe-Leu-Thr and DTTBULET, Tyr-D-Thr(O-tBu)-Gly-Phe-Leu-Thr were prepared [43] of which BUBU showed a significant affinity and selectivity for δ opioid receptors (Table **4**). Other changes in enkephalin structure, such as introducing retro-inverso modification at the Gly^3-Phe^4 amide bond [49] did not produce active analogs.

In another approach, receptor selectivity was achieved through conformational restriction of linear opioid peptides. Schiller and co-workers [50, 51] synthesized a series of cyclic Leu-enkephalin analogs by substitution of D-α,ω-diamino acids in position 2 of the Leu-enkephalin sequence and cyclization of the ω-amino group to the C-terminal carboxy group of leucine. Cyclic analogs containing D-α,β-diaminopropionic acid (A_2pr), D-α,γ-diaminobutyric acid (A_2bu), D-ornithine or D-lysine in position 2 showed, in general, high potency in the GPI assay and low potency in the MVD assay (Table **5**), indicating the preference of the cyclic analogs for μ over δ receptors.

Introducing penicillamine (Pen), which is β,β-dimethyl-cysteine, into the sequence of enkephalins resulted in obtaining two cyclic rigid analogs, DPDPE, Tyr-c(D-Pen-Gly-Phe-D-Pen) and DPLPE, Tyr-c(D-Pen-Gly-Phe-Pen) which were shown to display high selectivity for the β type of opioid receptors [53-55]. These bis-penicillamine-containing enkephalins are conformationally restricted because of the imposed cyclization through the side-chain sulfurs via a sulfide linkage and are further restricted because of the effect of the penicillamine gem dimethyl groups. Analogs with a single penicillamine residue were less selective, but more potent [56]. Conformationally constraint δ selective agonists were extensively reviewed by Hruby and Agnes [57].

Table 4. Structure, Inhibitory Potencies and Receptor Binding Affinity of Enkephalins and their Linear Analogues.

Peptide	IC_{50} [nM]				IC_{50} (or K_i) [nM]		IC_{50} (or K_i) ratio	
	GPI	MVD	GPI/MVD	Ref.	μ	δ	μ/δ	Ref.
Tyr-Gly-Gly-Phe-Met (Met-enkephalin)	200	13	15.4	39	25.2	18.3	1.38	39
Tyr-Gly-Gly-Phe-Leu (Leu-enkephalin)	455	8.82	51.6	39	27.7	8.05	3.44	49
Tyr-D-Ala-Gly-Phe-D-Leu (DADLE)	47.8	0.54	88.5	39	11.4	2.31	4.93	48
Tyr-D-Ser-Gly-Phe-Leu-Thr (DSLET)	360	0.58	620	39	31	3.8	8.2	43
Tyr-D-Thr-Gly-Phe-Leu-Thr (DTLET)				39	25.3	1.61	16	43
Tyr-D-Ser(O-*t*Bu)-Gly-Phe-Leu-Thr(O-*t*Bu) (BUBU)	2800	0.6	4700	47	480	1.69	280	43
Tyr-D-Ser(O-*t*Bu)-Gly-Phe-Leu-Thr (DSTBULET)					374	2.81	130	43
Tyr-D-Thr(O-*t*Bu)-Gly-Phe-Leu-Thr (DTTBULET)					4500	1150	3.9	43

Table 5. Structure, Inhibitory Potencies and Receptor Binding Affinities of Cyclic Enkephalin Analogues.

Peptide	IC_{50} [nM]			IC_{50} (or K_i) [nM]		IC_{50} (or K_i) ratio	
	GPI	MVD	GPI/MVD	μ	δ	μ/δ	Ref.
Leu-enkephalin	246	11.4	21.6				51
Tyr-c(D-A_2pr-Gly-Phe-Leu)	23.4	73.1	0.32				51
Tyr-c(D-A_2bu-Gly-Phe-Leu)	14.1	81.4	0.17				51
Tyr-c(D-Orn-Gly-Phe-Leu)	48	475	.10				51
Tyr-c(D-Lys-Gly-Phe-Leu)	4.8	141	0.03				51
Tyr-c(D-Pen-Gly-Phe-D-Pen) (DPDPE)	2698	4.74	569	421	2.15	196	52
Tyr-c(D-Pen-Gly-Phe-Pen) (DPLPE)	5800	1.89	3068	3710	10.02	370	52
Tyr-c[D-Cys-Phe-D-Pen]				1210	1.9	637	53

More recently, deltorphin I, Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2, deltorphin II, Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2 and dermenkephalin, Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2 have been isolated from the amphibian skin [48, 58]. These peptides and μ selective dermorphin [59] are the first animal endogenous peptides, which possess a D-amino acid in the sequence. This phenomenon is a result of the post-translational enzymatic conversion of chirality [60]. Interestingly, the D-amino acid residue is always located in position 2 of the sequence [61]. The D-amino acid and the anionic residues, either Glu or Asp, as well as the unique amino acid composition are responsible for the remarkable biostability, high δ opioid receptor affinity, and bioactivity of deltorphins and dermenkephalin. Their structures and binding properties are reported in Table **6**. Hundreds of analogs of deltorphins and dermetenkephalin have been synthesized to establish their structure-activity relationships, as reviewed by Lazarus *et al*. [32].

Table 6. Structure-activity Relationship Studies of δ Selective Peptides Isolated from the Amphibian Skin.

Sequence	K_i or IC_{50} [nM]			Ref.
	μ	δ	μ /δ	
Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2 (dermenkephalin)	344	0.41	839	62
Tyr-**D-Ala**-Phe-His-Leu-Met-Asp-NH_2	43.7	0.36	121	62
Tyr-**D-Nle**-Phe-His-Leu-Met-Asp-NH_2	32.6	0.12	272	63
Tyr-D-Met-Phe-**D-His**-Leu-Met-Asp-NH_2	492	2.77	178	62
Tyr-D-Met-Phe-**His(1-Me)**-Leu-Met-Asp-NH_2	551	0.47	1184	64
Tyr-D-Met-Phe-His-**Nle**-Met-Asp-NH_2	203	0.39	521	65
Tyr-D-Met-Phe-His-Leu-**D-Met**-Asp-NH_2	146	0.96	152	62
Tyr-D-Met-Phe-His-Leu-**Ala**-Asp-NH_2	218	0.37	589	66
Tyr-D-Met-Phe-His-Leu-**Phe**-Asp-NH_2	134	0.22	611	66
Tyr-D-Met-Phe-His-Leu-**Tyr**-Asp-NH_2	96.8	0.38	255	66
Tyr-D-Met-Phe-His-Leu-**nBuG**-Asp-NH_2	820	0.14	18231	67
Tyr-D-Met-Phe-His-Leu-**Asp-Met**-NH_2	122	0.97	126	66
Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2 (deltorphin I)	387	0.21	1844	72
Tyr-**Aib**-Phe-Asp-Val-Val-Gly-NH_2	869	0.12	7242	68
Tyr-D-Ala-**Aib**-Asp-Val-Val-Gly-NH_2	4544	0.8	5680	
Tyr-D-Ala-**Ac_6c**-Asp-Val-Val-Gly-NH_2	6822	15.4	443	68
Tyr-D-Ala-**pFPhe**-Asp-Val-Val-Gly-NH_2	554	0.51	1086	69
Tyr-D-Ala-Phe-**D-Asp**-Val-Val-Gly-NH_2	110	0.69	159	70
				71
Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2 (deltorphin II)	1280	0.41	3122	63
Dmt-D-Ala-**Aib**-Glu-Val-Val-Gly-NH_2	108	0.13	832	32
Tyr-D-Ala-Phe-**Gln**-Val-Val-Gly-NH_2	158	0.20	768	72
Tyr-D-Ala-Phe-Glu-**Nle-Nle**-Gly-NH_2	275	0.085	3256	73
Tyr-D-Ala-Phe-Glu-**Ile-Ile**-Gly-NH_2	251	0.067	3760	73
Tyr-D-Ala-Phe-Glu-**Leu-Leu**-Gly-NH_2	250	0.227	1105	73

Dermenkephalin was the first δ selective opioid whose structure-activity relationships were systematically studied. The obtained results can be summarized as follows:

- The L-configuration of Tyr^1 and the D-isomer of Met^2 are essential
- Dermenkephalin requires an unmodified Phe residue at position 3 as similarly found with Phe^4 of enkephalins [74]
- Substitution of positively charged His^4, considered to be a crucial residue in dermenkephalin [64], by a variety of amino acids is generally detrimental
- An aliphatic side-chain and L-isomer at position 5 appear critical for activity
- The C-terminal residues (positions 6 and 7) can generally be replaced by other amino acids with only marginal effects, which suggests, that they participate to a lesser degree in the ligand – receptor interaction.

The structural change that sets deltorphin I apart from deltorphin II is the presence of Asp^4 instead of Glu^4. Some of the most interesting properties of deltorphin analogs are as follows:

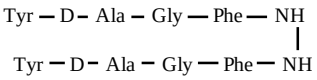
- Achiral α-aminoisobutyric acid (Aib) can replace either the D-Ala^2 or Phe^3, but not both simultaneously [68], other amino acid substitutions in position 2 are not well tolerated [75].
- The importance of the hydroxyl group of Tyr^1 for opioid binding activity was demonstrated by the loss of activity when replaced by Phe [75]. The similar observation was made with enkephalin analogs [74].
- Elimination of the negative charge in position 4 results in the lack of selectivity [68, 69, 71].
- The aromatic center at Phe^3 could be replaced by several heterocyclic aromatic residues, as well as other non-polar amino acids. Of particular interest is the ability of 1-aminocyclohexane carboxylic acid (Ac_6c) [68, 69, 76, 77] and Aib [68, 77] to substitute for Phe due to the enormal differences in the steric constraints between Phe and these unnatural amino acids. The conformations of deltorphin analogs containing Aib^3 and Ac_6c^3 are comparable, since the space occupied by the methyl groups of Aib is similar to that for aminocyclohexyl. These data suggest that the backbone conformation of the N-terminal domain is either compatible to that with Phe or the hydrophobic groups are spatially oriented in the same position as the aromatic ring of Phe [77].
- The hydrophobic nature of the residues at positions 5 and 6 is crucial in maintaining extraordinary affinity

of deltorphin II, evidenced in peptide analogs, in which aliphatic nature of the side-chain was enhanced [73, 78-80]. Crescenzi *et al*. [77] concluded that the backbone conformation of deltorphin is defined not only by the N-terminal sequence, but also by Val^5 and Val^6 residues, which contribute to the address domain.

Deltophin II has the highest δ opioid selectivity among amphibian opioids. The rank order of selectivity (K_i μ/K_i δ) is deltorphin II > deltorphin I > dermenkephalin (Table **6**). Opioid receptor binding of dermenkephalin and deltorphins is apparently facilitated by a common sequence in the message domain tripeptide [81, 82], which participates in an N-terminal β-turn [83, 84]. The entire C-terminal tetrapeptide address sequences are responsible for their high δ receptor binding affinities and high selectivities [85-87]. An anionic group in the address domain does not appear essential for high δ affinity, but is required for the remarquable δ selectivities of deltorphins and dermenek-phalin. The at least an order of magnitude higher affinities and remarkable selectivities of dermenkephalin and deltorphins for δ receptor type when compared with enkephalins and their analogs make them very useful models for studying the interaction of δ ligands in a variety of biochemical tests.

A different kind of modification leading to very potent analogs of opioid peptides is the "bivalent" ligand approach [88]. Bivalent ligands are molecules that contain two pharmacophores linked by a "spacer", whose constitution plays an important role in modulating selectivity and potency. A key feature of this model is that each type of opioid receptor is located on the dimeric or oligomeric subunits, whose supramolecular organization contains a unique array of recognition sites.

Bivalent ligands of opioid analogs containing two active elements in one molecule were synthesized [89-93]. Among the dimeric compounds biphalin, first synthesized by Lipkowski *et al*. [89], was found to be of the greatest interest. Biphalin is a dimeric enkephalin analog, in which the C-terminal amino acid is replaced by a second tertapeptide active fragment of the enkephalin analog and the two fragments are connected "tail to tail" by a hydrazine bridge:



Biphalin

The high probability of successful interaction of the molecule with two active sites on the receptor, and possible unique interactions with proteolytic systems, were the original rationals for synthesis of biphalin. Biphalin expresses high, nearly equal affinity for all three opioid receptor types (μ, δ and κ) [90, 94] and is an extremely potent analgesic in *in vivo* tests [95, 96]. Unique pharmacological and therapeutic potencies make biphalin a good candidate for further development as analgesic drug [97-99].

The hybridization of some structural elements necessary for bioactivity from two different opioid peptides was explored as a way to correlate their topography [100]. The group of Hruby tried to modulate the activity of DPDPE through its hybridization with the C-terminal fragments of dermenkephalin [100] or deltorphins [97] which are responsible for the receptor selectivity. Hybridized analogs of DPDPE showed a significantly decreased potency. More successful was correlation of DPLPE with C-terminal dipeptide of deltorphins [97] (Table **7**). These studies demonstrated that a major structural feature determining the high potency of a hybrid analog is the chirality of the amino acid residue in position 5.

Antagonists. Receptor selective opioid antagonists are of interest both, as pharmacological tools and as potential therapeutic agents.

The enkephalin analog $(CH_2{=}CH\text{-}CH_2)_2$-Tyr-Aib-Phe-Leu (ICI 174864) was the first antagonist with substantial selectivity and moderate affinity for the δ receptor [101]. This compound was for several years the only antagonist used in pharmacological studies of δ receptor. In 1992 Schiller *et al*. [102] reported the discovery of a new class of opioid peptide derived δ antagonists that contain a 1,2,3,4-tetrahydroizoquinoline-3-carboxylic acid (Tic) in position 2 of the peptide sequence. The two prototype antagonists were the tetrapeptides Tyr-Tic-Phe-Phe (TIPP) and Tyr-Tic-Phe-Phe-NH_2 (TIPP-NH_2). The synthesis of TIPP has provided

Table 7. Binding Affinities of Biphalin and DPDPE-Deltorphin Chimeric Peptides.

Peptide	IC_{50} (or K_i) [nM]			
	μ	δ	μ /δ	Ref.
$(Tyr\text{-}D\text{-}Ala\text{-}Gly\text{-}Phe\text{-}NH)_2$ (biphalin)	1.4	2.16	1.85	94
Tyr-c(D-Pen-Gly-Phe-D-Pen) (DPDPE)	2840	16.2	175	97
Tyr-c(D-Pen-Gly-Phe-Pen) (DPLPE)	3710	10	371	97
Tyr- D -Ala-Phe-Asp-Val-Val-Gly-NH_2 (deltorphin I)	2140	0.60	3567	97
Tyr-c(D-Pen-Gly-Phe- D-Pen)-Val-Gly-NH_2	3950	59	70	97
Tyr-c(D-Pen-Gly-Phe-Pen)-Val-Gly-NH_2	2290	4.3	533	97
Tyr-c(D-Pen-Gly-Phe-Cys)-Val-Gly-NH_2	200	0.80	250	97
Tyr-c(D-Pen-Gly-Phe-Pen)-Nle-Gly-NH_2	3360	4.0	840	97

significant progress in the search for selective peptide-derived δ antagonists. TIPP itself has nanomolar affinity and extraordinary selectivity for the δ receptor [102]. The tetrapeptide amide TIPP-NH_2 was the first mixed μ agonist/δ antagonist known [103]. Such mixed μ agonists/δ antagonists are believed to have therapeutic potential as analgesics that do not produce tolerance and dependence. To eliminate the possibility of Tyr-Tic-diketopiperazine formation, the corresponding peptides containing reduced peptide bond between the Tic^2 and Phe^3 residues were synthesized [104]. The pseudopeptide analogs, Tyr-Ticψ[CH_2NH]-Phe-Phe (TIPP[ψ]) and Tyr-Ticψ[CH_2NH]-Phe (TIP[ψ]) showed increased δ-antagonist potency, higher δ receptor affinity and further improved δ selectivity (over 10000-fold δ versus μ) (Table **8**).

The properties of peptides containing Tic in the second position suggest that the message domain of opioid peptides can be composed of only two residues [105].

Tancredi *et al*. [106] have converted Leu-enkephalin and dermorphin to δ-selective antagonists by single substitution of the amino acids in position 2 by Tic. *cis* Tyr-Tic moiety is typical for all known δ selective antagonists and is suggested to be responsible for antagonist activity of all these peptides.

2.5. μ *Receptor Ligands*

Agonists. Early on modifications of the enkephalins, using the classical approach of amino acid substitutions, resulted in agonists showing substantial preference for μ receptors. Of many synthesized analogs Tyr-D-Ala-Gly-MePhe-Gly-ol (DAMGO) [38] was probably the most selective one.

At the same time the search for naturally occurring μ receptor ligands was going on. A number of milk protein fragments has been shown to behave like opioid receptor ligands able to address opioidergic systems. The bioactivities of opioid peptides encrypted in major milk proteins are latent until released and activated by enzymatic proteolysis, e.g. during gastrointestinal digestion. It is evident from many studies that opioid peptide fragments originating from milk proteins are potential regulatory compounds and modulators of various regulatory processes in the body [107, 108]. They all turned out to belong to the group of "atypical" opioid peptides and to be selective for μ opioid receptor.

The best known class of milk protein peptides is the group of β-casomorphins (β-CM), obtained from the milk protein, β-casein, by proteolytic fragmentation [26, 109]. 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 [110-112]. 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 synthesized as an analog possessing the N-terminal tetrapeptide fragment of β-casomorphin [113] and then was isolated from an enzymatic digest of bovine β-casein [114]. 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 [113]. The structure-activity relationship studies of morphiceptin analogs were reviewed by Janecka *et al*. [115]. The most potent analog, Tyr-Pro-NMePhe-D-Pro-NH_2 (PL017) [116] had an IC_{50} value in a low nanomolar range and showed virtually no activity at δ receptor binding sites.

Hemorphins are endogenous peptides also belonging to the family of "atypical" opioid peptides. Originally the hemorphins were isolated from enzymatically treated bovine blood, but recently have been identified as naturally occurring peptides in brain, plasma and cerebrospinal fluid. Similarly with β-casomorphins, the hemorphins contain the Tyr-Pro dipeptide at the N-terminus. The minimum sequence necessary for activity is the tetrapeptide Tyr-Pro-Trp-Thr. This peptide was the first of a number of hemoglobin-derived opioid peptides to be identified and was named hemorphin-4. All hemorphin structures tested in a binding assay exhibit affinity for μ opioid receptor with IC_{50} values in the μM range [27].

Dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2), a heptapeptide isolated from the skin of the South American frog *Phyllomedusa sauvagei* [31], was until recently among the most potent opioid peptides with high μ selectivity. In contrast to all mammalian opioid peptides, dermorphin contains a D-amino acid in position 2 (as in δ selective deltorphins and dermenkephalin) and is therefore relatively stable against enzymatic degradation [117]. Dermorphin was the first member of a peptide family that in the past twenty

Table 8. Binding Affinities of δ Antagonists at μ and δ Receptors in Rat Brain Homogenates.

Peptide	IC_{50} (or K_i) [nM]		IC_{50} (or K_i) ratio	
	μ	δ	μ /δ	Ref.
Tyr-Gly-Gly-Phe-Leu (Leu-enkephalin)	9.43	2.53	3.73	104
Tyr-Tic-Phe-Phe (TIPP)	1720	1.22	1410	104
Tyr-Tic-Phe-Phe-NH_2 (TIPP-NH_2)	78.8	3.00	26.2	104
Tyr-Ticψ[CH_2NH]-Phe-Phe (TIPP[ψ])	3228	0.308	10500	104
Tyr-Ticψ[CH_2NH]-Phe (TIP[ψ])	10800	1.94	5570	104

years has grown to reach a total of seven naturally occurring peptides and many synthetic analogs [118].

In 1986 Schwyzer [119] published his "membrane compartment concept" suggesting that in addition to ligand-receptor complementarity, specific interactions of opioid peptides with various membrane compartments might also contribute to their ability to interact selectively with a distinct receptor type. Elaboration of this concept for opioid peptide-receptor interactions had led to the assumption that μ and δ receptors are located in anionic and cationic membrane compartments, respectively, and to the prediction that positively charged opioid receptor ligands should display μ receptor selectivity. To assess the validity of this model Schiller *et al*. [120] synthesized a series of dermorphin analogs carrying a net positive charge from 1+ to 3+. The data obtained for some, but not all of the prepared compounds supported the concept. The most selective analog, Tyr-D-Arg-Phe-Phe-Lys-NH_2 (DALDA), showed extremely high preference for μ receptors over δ receptors, being more than ten times as μ selective as DAMGO.

The N-terminal tetrapeptide of dermorphin is known to be the minimum sequence required for opioid activity [121], although this fragment shows lower potency than that of the parent heptapeptide [122]. Several synthetic peptides derived from the dermorphin tetrapeptide, of general sequence Tyr-D-AA-Phe-AA have been reported to show a potent agonistic activity at the μ opioid receptor [123]. Substitution of D-Ala in position 2 with D-Arg has been widely applied since the studies on D-Arg-kyotorphin were reported [124] in order to increase the potency and the duration of action of the peptide. Sasaki *et al.* [125] recently found that N$^{\alpha}$-amidino-Tyr-D-Arg-Phe-Gly-OH (ADA-DER) is one of the most potent dermorphin tetrapeptides. An analog with N-methylation at position 4, Tyr-D-Arg-Phe-Sar (DAS-DER) shows selectivity for the μ receptor and has oral antinociceptive activity in rats without inducing respiratory depression [126]. Introducing a β amino acid at position 4 produced a selective μ opioid agonist, Tyr-D-Arg-Phe-β-Ala (TAPA) [127], with more potent and longer lasting antinociceptive effect than morphine. Based on these findings Ogawa *et al*. [128] designed a new analog, N$^{\alpha}$-amidino-Tyr-D-Arg-Phe-MeβAla (ADAMB) (Fig. **1**.) which has high affinity at μ receptor and exceptional antinociceptive activity.

First reported by Darlag *et al*. in 1991 [129], Tyr-c[D-Lys-Phe-Ala] (YKFA) is a non-selective superpotent agonist at both μ and δ opioid receptors. This molecule has been conformationally constrained by cyclization between the side chain ε amino group of the lysine in position 2 and the C-terminus, as well as by the removal of the flexible glycine spacer, present in the δ selective peptide, such as DPDPE. YKFA is capable of facile interconvertion between the two conformations required for bioactivity at the μ and δ opioid receptors [130], as evidenced by the fact that it exhibits subnanomolar potencies at both receptors. Several analogs of YKFA, in which the usual Tyr^1, Phe^3 combination found in opioid peptides was transposed, have been synthesized [131] in an effort to modulate the selectivity. However, the lack of the hydroxyl group in position 1 produced a significant decrease in both μ and δ opioid receptor affinity.

Schiller *et al*. [132] converted a selective δ antagonist, TIPP-NH_2 in a potent and selective μ agonist by introducing the enantiomeric D-Tic in position 2.

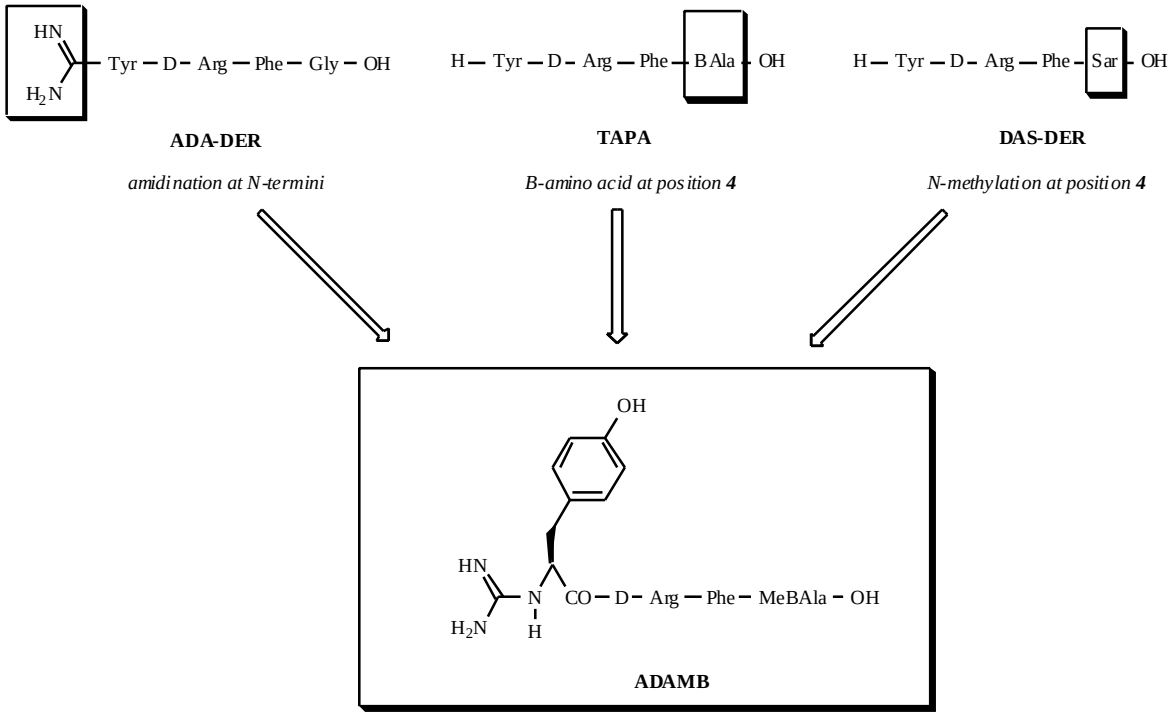


Fig. (1). Design of ADAMB based on the structure of several dermorphin tetrapeptide analogs.

For a long time no mammalian peptide has been identified that would show high μ selectivity. Two peptides isolated from the brain, Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) [28] and Tyr-W-MIF-1 (Tyr- Pro-Trp-Gly-NH_2) [29, 30] have been shown to display high selectivity for μ receptor sites, though their affinity was relatively low. Two cyclic analogs of these brain peptides Tyr-c[D-Lys-Trp-Gly] and Tyr-c[D-Orn-Trp-Gly] had greatly increased affinity [133]. Peptides containing all possible amino acid substitutions at position 4 of Tyr-W-MIF-1 were synthesized by Zadina et al. [24] and screened for opioid receptor binding. A high affinity, selective and biologically potent sequence, Tyr-Pro-Trp-Phe-NH_2, was discovered and then identified in the brain. A new peptide which was named endomorphin-1 showed remarkable affinity for the μ receptor (360 pM) and selectivity of 4000- and 15000-fold for the μ receptor over the δ and κ receptors, respectively. A second peptide, Tyr-Pro-Phe-Phe-NH_2, endomorphin-2, which differs by one amino acid was also isolated.

The identification of endomorphins opened a new era in the research of the μ opioid system. They are the first reported brain peptides that label μ receptor with high affinity and selectivity and therefore are proposed as the endogenous μ opioid receptor ligands. Although related to each other, the sequences of endomorphin-1 and endomorphin-2 are quite distinct from traditional opioids in which the first four amino acids are Tyr-Gly-Gly-Phe. Trp and Phe represent the only two natural amino acids that maintain binding and activity in position 3 of the opioid pharmacophore. Trp^3 was found in Tyr-W-MIF-1 and in hemorphin-4 (Tyr-Pro-Trp-Thr) derived from enzymatic digests of blood but affinity of these peptides was 1-2 orders of magnitude lower than that of endomorphin-1. β-Casomorphins contain Tyr-Pro-Phe sequence found in endomorphin-2, but contain a less active Pro in position 4 and are not found in neuronal tissue.

Podlogar et al [136] presented a structural and comparative study of edomorphin-1 to identify its bioactive conformation and attributes responsible for μ selectivity. They proposed a hypothesis of the existence of spatially distinct selectivity pockets among opioid ligands. NMR data have shown that Pro^2 provides necessary stereochemical requirements for activity of endomorphin-1 at the μ opioid receptor. The bioactive conformation is characterized by a structure in which the Tyr^1 and Trp^3 side chains have opposite orientations with respect to Pro^2 [137]. Synthesizing pseudoproline-containing analogs of endomorphin-2 , which are known to be quantitative inducers of the *cis* conformation Schiller and co-workers [138] provided evidence that the Tyr-Pro amide bond in the bioactive conformation is cis. Numerous structure-activity relationship studies have been performed recently to better understand ligand recognition and the selectivity of opioid receptors [139-143]. These studies revealed that very important features for μ selectivity and affinity are the presence of a Tyr^1, a Pro^2 or D-Ala^2, lipophilic residues at positions 3 and 4, and the amidation at the C-terminus.

The receptor binding data for all μ receptor ligands mentioned above are summarized in Table 9.

Table 9. Structure and Receptor Binding Affinities of Naturally Occuring and Synthetic μ Opioid Receptor Ligands.

Peptide	Sequence	IC_{50} (or K_i) [nM]		IC_{50} (or K_i) ratio	Ref.
		μ	δ	δ / μ	
bovine β-casomorphin	Tyr-Pro-Phe-Pro-Gly-Pro-Ile	1160	6500	5.6	134
morphiceptin	Tyr-Pro-Phe-Pro-NH_2	63	30000	476	115
PL 032	Tyr-Pro-Phe-D-Pro-NH_2	4.3	20000	4651	115
PL 017	Tyr-Pro-NMePhe-D-Pro-NH_2	5.5	10000	1818	115
dermorphin	Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2	1.7	158	90	135
DALDA	Tyr-D-Arg-Phe-Lys-NH_2	1.69	19200	11400	120
ADAMB	N–-amidino-Tyr-D-Arg-Phe-Me_Ala	12.9	>1000	>7.75	128
Tyr-MIF-1	Tyr-Pro-Leu-Gly-NH_2	987	>40000	>40.5	133
Tyr-W-MIF-1	Tyr-Pro-Trp-Gly-NH_2	71	>10000	>141	133
	Tyr-c[D-Lys-Trp-Gly]	1.3	60	46.2	133
	Tyr-c[D-Orn-Trp-Gly]	3.6	170	47.2	133
D-TIPP-NH_2	Tyr-D-Tic-Phe-Phe-NH_2	7.3	519	71.1	132
DAMGO	Tyr-D-Ala-Gly-MePhe-Gly-ol	1.22	1280	1050	120
Endomorphin-1	Tyr-Pro-Trp-Phe-NH_2	0.36	1506	4183	24
Endomorphin-2	Tyr-Pro-Phe-Phe-NH_2	0.69	9233	13381	24

Antagonists. It is generally accepted that while alkaloid antagonists such as naloxone or naltrexone have high affinity for the μ receptor, their selectivity is marginal. Peptide antagonists with high μ receptor selectivity were discovered not from structure-activity studies of the endogenous opioid peptides but through modifications of somatostatin, which was shown to bind weakly to CNS opioid receptors [144]. This finding led Hruby and co-workers [145] to examine the possibility of developing somatostatin-like analogs with potent and receptor specific opioid activity. For this purpose they utilized various structural considerations and conformational constraints to modify somatostatin analogs in a manner that enhanced their affinity for the μ opioid receptor, while at the same time decreasing their affinity for somatostatin receptors. Octapeptide analog CTP, which varied from a potent somatostatin analog RC-121 only in two positions (-Thr-Pen- instead of –Val-Cys-) was found to be a selective μ opioid receptor ligand [145, 146]. CTP was extremely stable to enzymatic degradation and did not cross the blood-brain barrier [147]. Further modifications of CTP resulted in the synthesis of CTAP and CTOP [148], being so far the two most selective and potent μ receptor antagonists of the following structure:

D—Phe—Cys—Tyr—D—Trp—Lys—Thr—Pen—Thr—NH

CTP

D—Phe—Cys—Tyr—D—Trp—Arg—Thr—Pen—Thr—NH_2

CTAP

D—Phe—Cys—Tyr—D—Trp—Om—Thr—Pen—Thr—NH_2

CTOP

Some novel [D-Arg^2]dermorphin(1-4) analogs with μ opioid receptor antagonist activity were reported recently [149].

2.6. κ *Receptor Ligands*

Agonists. The heptadecapeptide dynorphin A (Dyn A), Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln, obtained from porcine pituitary is the putative endogenous ligand for κ opioid receptor [150]. The isolation of other members of dynorphin family, such as the 13-residue dynorphin B and shorter α- and β-neoendorphins was also reported [151, 152] (Table **1**). The sequence of Dyn A is rather unusual, containing several charged residues along with the common N-terminal segment, which it shares with Leu-enkephalin. Dyn A is relatively non-selective, since it also binds to μ and δ opioid receptors with quite high affinity. Systematic truncation of Dyn A at the C-terminus revealed that the shorter Dyn A(1-13) and Dyn A(1-11) and their corresponding analogs with a C-terminal carboxamide function have a *in vitro* activity profile similar to that of the parent peptide [153-155]. Therefore, these shorter Dyn A peptides are often used as parent compounds for structure-activity studies of Dyn A. The attractiveness of utilizing the ligands for the κ receptor is that their pharmacology involves low abuse potential and a milder form of dependence in comparison to the typical μ opioid ligands, though they have undesirable side-effects and have been implicated in dysphoria and diuresis[156]. Numerous structure-activity studies have been performed during the last 20 years in an effort to develop Dyn A analogs with increased κ receptor selectivity, as reviewed by Naqvl *et al*. [157]. The most important findings revealed in these studies can be summarized as follows:

- The Tyr^1 and Phe^4 residues are indispensable for the opioid activity of Dyn A [158].
- The C-terminal address domain is responsible for imparting the selectivity toward the κ receptor, with $Arg^{6,7}$ and Lys^{11} making the greatest contribution [155].
- Arg^9 is the only one out of five basic residues at the C-terminus, which can be replaced by a non-basic residue without substantial loss in κ selectivity [159].
- Replacement of Gly^2 by various L-amino acids leads to analogs with weak affinities and potencies in the central and peripheral nervous systems [160].
- Replacement of Gly^2 by various D-amino acids leads to analogs fairly potent in the GPI assay, which exhibited high μ and κ affinities [160].
- N-Monoalkylations of Tyr^1 lead to analogs that are highly selective for the central κ vs μ and δ receptors [161, 162].

To complement the linear peptides several conformationally constrained Dyn A analogs have been reported [163-166]. The cyclization was performed either via an amide bond or via a disulfide bridge. Neither of the cyclic analogs was very selective.

Opioid receptor binding affinities of dynorphin most selective analogs are summarized in Table **10**.

Antagonists. While κ agonists show some promise as non-addictive analgesics, κ antagonists might be useful in the treatment of addiction. The development of Dyn A analogs with κ antagonist properties has met with only limited success so far. Several Dyn A-derived κ antagonists have been described [169-172], but none of them showed both, high κ antagonist potency and high κ receptor selectivity. Among various non-peptide κ antagonists reported to date, norbinaltorphimine (norBNI) and GNTI display high activity and κ selectivity. Recent studies of Schiller *et al*. (173) indicate that the positively charged N-terminal amino group may be essential for signal transduction, but not for receptor binding, and suggested that its deletion in agonist opioid peptides containing an N-terminal 2,6-dimethyltyrosine (Dmt) residue may represent a general way to convert them into antagonists. The [Dmt^1]Dyn A(1-11)-NH_2 analog in which the N-terminal amino group was replaced with a methyl group, [(2*S*)-Mdp^1]Dyn A(1-11)-NH_2, (where (2*S*)-Mdp is (2*S*)-2-methyl-3-(2,6-dimethyl-4-hydroxyphenyl)propanoic acid) showed subnanomolar κ antagonist potency against Dyn A(1-13) and very high κ selectivity {174] (Table **10**). This new potent κ antagonist was named dynantin.

Table 10. Opioid Receptor Binding Affinities of Dynorphin Analogues.

Peptide	IC_{50} (or K_i) [nM]			IC_{50} (or K_i) ratio	Ref.
	κ	μ	δ	κ/μ/δ	
Dyn A(1-13)	0.023	0.4	1.8	1/18/83	160
Dyn A (1-11)-NH_2	0.074	7.6	2.9	1/103/39	167
[Pro^3]Dyn A(1-11)-NH_2	2.7	5700	8800	1/2110/3260	167
[D-Ala^3]Dyn A(1-11)-NH_2	0.76	260	1000	1/342/1315	168
[D-Pro^{10}]Dyn A(1-11)	0.03	0.24	2.1	1/8/70	161
N-CPM[D-Pro^{10}] Dyn A(1-11)	0.02	9.6	558	1/480/27900	161
c[Pen^2,Cys^{11}]Dyn A(1-11)-NH_2	0.52	1.26	86.3	1/2,4/165	166
c[Cys^5,Cys^{10}]Dyn A(1-11)-NH_2	0.59	4.26	82.5	1/7,2/139	166
c[Cys^5,Cys^9]Dyn A(1-11)-NH_2	0.87	6.43	31.2	1/7,4/36	166
c[Cys^5,Pen^{11}]Dyn A(1-11)-NH_2	1.0	17	319	1/17/319	163
c[Cys^5,D-Pen^{11}]Dyn A(1-11)-NH_2	1.1	39	242	1/28,2/220	163
c[Pen^5,Pen^{11}]Dyn A(1-11)-NH_2	3.1	67.6	717	1/21,8/231	163
[(2*S*)-Mdp^1]Dyn A(1-11)-NH_2	0.823	213	163	1/259/198	174
[Dmt^1]Dyn A(1-11)-NH_2	0.322	0.435	1.18	1/1/4	174

CONCLUSIONS

Following the discovery of specific opioid binding sites in the mammalian brain much effort was directed to research focused on finding the endogenous ligands for opioid receptors. As a result, a large number of naturally occurring opioid peptides of different origin has been identified and characterized over the years. Analogs of the natural peptides with high selectivity and potency have been synthesized. The concept of conformational restriction has been successfully applied to the development of opioid peptide analogs with certain desired activity profiles. The incorporation of conformational constraints in opioid peptides has been shown to enhance selectivity for a distinct opioid receptor type and to increase stability against enzymatic degradation. The development of pharmacological agonists and antagonists that can distinguish the opioid receptors has facilitated investigations of their distinct functional roles.

The discovery of endogenous ligands for the opioid receptors rose the hope for rapid progress in understanding and treatment of various pain states. This however, has not proved true. Many pharmacologically interesting peptides have been developed that possess biological profiles with few of the side-effects associated with morphine and other plant opiates. However these potentially useful compounds have not as yet found their way to the clinics. Unquestionably morphine is still the most efficient analgesic drug known.

New approaches to drug design are needed to overcome serious limitations of the known peptide ligands, such as relative inability to cross the blood-brain barrier, long-term toxicity or low biostability. New generations of peptide or peptidomimetic drugs that may be able to control pain, addiction, immune response, gut transit and many other areas where opioid receptors are involved are yet to be discovered.

ACKNOWLEDGEMENT

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ABBREVIATIONS

CNS	=	Central nervous system
β-CM	=	β-casomorphin
GPI	=	Guinea pig ileum
MVD	=	Mouse vas deferens
A_2pr	=	α,β-diaminopropionic acid
A_2bu	=	α,γ-diaminobutyric acid
Pen	=	β,β-dimethylcysteine
Aib	=	α-aminoisobutyric acid
Ac_6c	=	1-aminocyclohexane carboxylic acid
Tic	=	1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid
Dmt	=	2,6-dimethyltyrosine
(2*S*)-Mdp	=	(2*S*)-2-methyl-3-(2,6-dimethyl-4-hydroxyphenyl)propionic acid

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